

# Pathology of fatty liver: differential diagnosis of non-alcoholic fatty liver disease

Matthew M Yeh

Elizabeth M Brunt

## Abstract

Non-alcoholic fatty liver disease (NAFLD) has emerged as a worldwide epidemic in the 21st century. The spectrum of its histopathology ranges from simple steatosis to steatohepatitis, with risk for progressive fibrosis that may lead to cirrhosis and hepatocellular carcinoma. This review is an update of the histopathology of NAFLD and its differential diagnosis, in particular alcoholic liver disease (ALD), encountered by practising pathologists. It also discusses the pathology of NAFLD in various 'non-traditional' settings: in children, after therapeutic intervention, and with concomitant liver diseases. The current grading and staging systems of NAFLD and ALD are also discussed.

**Keywords** alcoholic liver disease; fatty liver; non-alcoholic; steatohepatitis

## Introduction

Varying amounts of steatosis are commonly encountered in liver biopsies, particularly in biopsies from adult patients. In order to assess the significance of steatosis, the pathologist must first determine the type (macro- or micro-vesicular), amount, location and associated histological lesions, and then correlate these with the known clinical setting of the biopsy. As there are a variety of potential causes of hepatic steatosis, such as non-alcoholic fatty liver disease (NAFLD), alcoholic fatty liver disease, hepatitis C, some medications and/or combinations of all of these, the diagnostic conclusion may remain open-ended and descriptive if the available clinical information does not provide specific a underlying process(es).

## Terminology

The implications of the broad categorization of steatosis into macro- and micro-vesicular are quite significant, thus definitions are important. Purely microvesicular steatosis is not only uncommon, but it arises in very specific and typically clinically urgent situations: acute fatty liver of pregnancy, Reye syndrome, alcoholic foamy degeneration and certain drug toxicities, such as

valproic acid. This type of steatosis is a manifestation of severe mitochondrial dysfunction and commonly is associated with markedly elevated liver tests, with or without hepatic encephalopathy. In a minority of cases of NAFLD, non-zonal clusters of hepatocytes may contain microvesicular steatosis. The histological features of true microvesicular steatosis include hepatocyte enlargement, flocculent alteration of the cytoplasm and central retention of the nucleus (Figure 1a). Whether droplets of steatosis are visible by routine stains or not can be quite variable, but this is not diagnostic. True microvesicular steatosis often requires the application of Oil red O on frozen section of a liver biopsy for confirmation (Figure 1b).

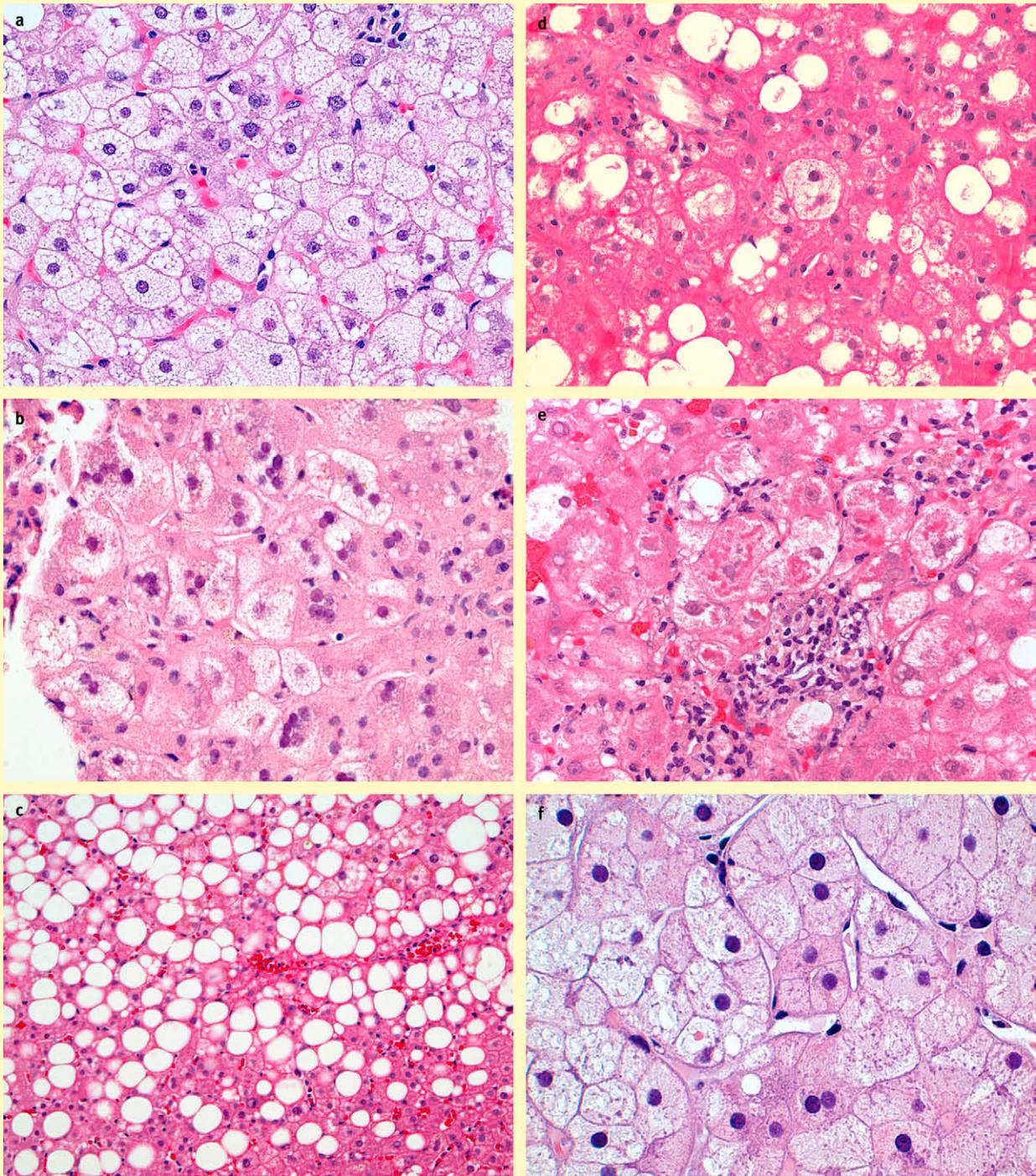
In contrast, macrovesicular steatosis is visible by routine stains as clear, rounded, well-defined spaces within the cytoplasm of normal-sized or enlarged hepatocytes. The droplets can be of varying sizes, and can occupy the cytoplasm of a hepatocyte as a single large droplet that causes the nucleus and remaining cytoplasm to be eccentrically displaced, or may comprise several small clustered droplets, each of which can be well defined (Figure 1c). Some hepatocytes will contain both large and small droplet macrovesicular steatosis. At the current time, no clinical significance is attributed to the differing morphological aspects of large and small droplet macrovesicular steatosis.

## Fatty liver disease: non-alcoholic fatty liver disease and alcoholic liver disease

Interestingly, the exact distinction(s) between alcoholic liver disease (ALD) and NAFLD are not as uniformly applied as would be anticipated. However, in general, there is a common guideline of less than 40 g alcohol/day for men and less than 20 g alcohol/day for women as acceptable for *non*-alcoholic. This general guideline, of course, does not take into account the well-known and important factors associated with ALD, such as genetic predispositions to the various pathways involved, types of alcohol consumed or pattern of alcohol consumption, and thus, should only be used as a very general guideline. In reality only complete abstinence from alcohol is truly *non*-alcoholic, and even that raises problems as there are known by-products of ethanol produced in the gut that do affect the liver both directly via the enteroportal circulation and indirectly via endotoxin production and stimulation of intrahepatic tumour necrosis factor (TNF)- $\alpha$ . Similarly, an obese individual who consumes 'excess' amounts of alcohol may be affected by both NAFLD and ALD. Thus, for the liver pathologist, there often are overlapping histological features noted between ALD and NAFLD, particularly at the milder end of the spectrum with steatosis or steatosis and inflammation. However, it is relatively safe to say that marked steatohepatitis (SH) with features of severe damage and dense perisinusoidal fibrosis, as will be described, are very uncommon in uncomplicated NAFLD and should raise a clinical concern of ALD. Furthermore, the overlap is not complete between the two processes: there are entities in the 'spectrum' of ALD that have yet to be described in NAFLD; these include cholestasis, veno-occlusive disease, sclerosing hyaline necrosis and alcoholic hepatitis (without steatosis). Both NAFLD and ALD may result in cirrhosis with or without active lesions of SH, and it may not be possible for the pathologist to discern the underlying aetiology.

**Matthew M Yeh MD** is at the Department of Pathology, University of Washington School of Medicine, Seattle, Washington, USA.

**Elizabeth M Brunt MD** is at the Department of Pathology and Immunology, Washington University, St Louis, Missouri, USA.



Microvesicular steatosis **a** is characterized by variably enlarged hepatocytes with flocculant cytoplasm due to very fine fat droplets; nuclei remain centrally located (400X); **b** in this example of acute fatty liver of pregnancy, an Oil Red O stain was confirmatory that the cytoplasmic alterations were due to steatosis and not viral cytopathic effect (400X). **c** Macrovesicular steatosis, on the other hand, shows clear, rounded, and well-defined fat droplets (often single) within the cytoplasm and the nuclei are peripherally displaced by the fat droplets (200X). **d** Steatohepatitis (SH) with a ballooned hepatocyte in the centre, surrounded by macrovesicular steatosis and inflammatory infiltrates (400X). **e** Mallory-Denk bodies (Mallory's hyaline) can be seen as ropey and eosinophilic inclusions in the ballooned hepatocytes in either non-alcoholic SH (NASH) or alcoholic liver disease (ALD) (400X). **f** Megamitochondria can also be seen as round or oblong and eosinophilic inclusions in the hepatocytes in NASH or ALD (600X).

**Figure 1**

## Epidemiology

It is challenging to obtain exact data for the prevalence of either ALD or NAFLD, as both require significant input from patient sources and there is reticence to convey truthful caloric intake whether from ethanol or diet. Neither disease has serum markers or imaging tests that are diagnostic; even sophisticated equipment cannot assess steatosis less than 30%. However, the presence of features of metabolic syndrome (which depend on the specific definition, the WHO or Adult Treatment Panel,<sup>1</sup> and include central obesity, hypertension, dyslipidaemia as characterized by low high-density triglycerides and elevated low-density triglycerides, and either diabetes or glucose intolerance), and specifically the presence of insulin resistance, validates the concern of 'NAFLD' as a component, if not the sole, cause of the steatosis or SH in a liver biopsy. Based on studies utilizing surrogate markers of NAFLD (elevated liver tests, presence of metabolic syndrome, prevalence of obesity, imaging studies) and biopsy series,<sup>2</sup> it is estimated that up to 20–30% of the population of the USA has fatty liver, with a notable ethnic variability favouring less involvement of African-Americans in every study.<sup>2,3</sup> Cirrhosis due to NAFLD may be the most common indication for liver transplant, having now surpassed hepatitis C and alcohol. Up to 17% of children and adolescents have been documented to be affected.<sup>4</sup> With the global epidemic of obesity now recognized, this condition and the related concern of type 2 diabetes are no longer seen as strictly problems of affluence. The projected *percentage change* for obesity-related diabetes mellitus worldwide ranges from 32% in Europe to 72% in the USA, and greater than 150% in India, sub-Saharan Africa and the Middle East.<sup>5</sup>

ALD is also a worldwide phenomenon, but is less of a paediatric problem. There are widely differing geographical prevalence figures for ALD, some presumably related to the influence of religious and cultural dictums. Estimates of *per capita* consumption L/year range from 13.9 in Russia and 9.3 in North America, to 0.6 in Afghanistan and Pakistan.<sup>6</sup> In the USA, ALD remains a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma, and death.

## Role of liver biopsy

Clinical evaluation and current imaging modalities, and serological and laboratory tests can be strongly suggestive of the presence of hepatic steatosis, but none of these is capable of distinguishing SH from uncomplicated steatosis; likewise, these evaluations can generally detect advanced liver disease (i.e. portal hypertension), but none can truly assess the degree of liver necroinflammatory injury, lesser stages of fibrosis and architectural remodelling. Liver biopsy evaluation, therefore, remains the 'gold standard' to unequivocally diagnose SH and to document the severity of hepatic injury and fibrosis.<sup>7–9</sup> Importantly, biopsy evaluation has also shown that not all individuals with unexplained liver test elevations have fatty liver,<sup>10</sup> not all phenotypic 'non-alcoholic SH (NASH)' patients have steatosis<sup>11</sup> and that the full spectrum of fatty liver disease may be present when laboratory values are normal.<sup>12</sup> It is important to realize, however, that biopsy findings of steatosis and/or SH usually cannot differentiate between alcoholic and non-alcoholic fatty liver. Finally, evaluation of liver biopsy is useful for detecting possible coexistent processes.<sup>13–15</sup> As NAFLD is closely linked to underlying consequences of being overweight, and as overweight and obesity are present in over

two-thirds of adults in the USA, it is not unexpected that other 'common' liver diseases may coexist in individuals with NAFLD. Clinicopathological studies have documented the constellation of histological features of NASH in biopsies from patients with another serologically and/or clinically defined chronic liver disease.<sup>16,17</sup>

It is somewhat controversial whether a liver biopsy should be performed in patients clinically suspected for ALD. In general, a liver biopsy may confirm cirrhosis as well as alcoholic hepatitis. However, common 'scores' (the Maddrey score and Glasgow score) derived from clinical tests are more often utilized before medication such as corticosteroids is administered. Liver biopsy can be useful, however, to exclude other liver diseases and to provide prognostic information. For instance, in patients with concurrent ALD and viral hepatitis, a liver biopsy may help determine the degree of necroinflammatory lesions related to either the ALD or the viral hepatitis. However, a percutaneous liver biopsy is usually contraindicated in cases with severe portal hypertension with coagulopathy, thrombocytopenia and ascites. Transjugular liver biopsy may be an alternative option, if the operator is sufficiently skilled, as assessment of fibrosis in an inadequate transjugular biopsy is not reliable.<sup>18</sup> Finally, as there are recognizable histopathological features in severe ALD, liver biopsy evaluation in individuals with clinically 'uncertain' alcohol use can be helpful if these features are found.

## Histopathological features of non-alcoholic liver disease and alcoholic liver disease

### Primary lesions and differential diagnostic considerations

Although many American and European liver pathologists had observed the features we now know as NAFLD and NASH in non-drinking patients, it was Ludwig et al who first coined the term 'NASH' in their 1980 manuscript based on the study of 20 biopsies.<sup>19</sup> The primary lesions described in that study, namely steatosis, liver cell injury and the unique centri-zonal 'chicken-wire' fibrosis, remain central in establishing the diagnosis.<sup>2</sup> The entity was initially recognized by several pathologists as one that closely resembled alcoholic hepatitis, but in a clinically determined 'non-alcoholic' setting. After almost three decades, the current pathological diagnoses of NASH can still be traced to the clinicopathological criteria derived from Ludwig's seminal manuscript. Further clinical studies showed the linkage of NAFLD to insulin resistance<sup>20</sup>; Marchesini et al established that NAFLD is the hepatic component of the metabolic syndrome.<sup>21</sup>

It has been generally conceptualized that steatosis is a necessary but not sufficient component to constitute a diagnosis of SH, and features of liver cell injury and inflammation are also required before this diagnosis can be established. Hepatocytic ballooning is perhaps the most intriguing yet crucial form of liver cell injury for pathologists to recognize in NASH. It is characterized primarily by cytoplasmic alterations, as well as swelling and enlargement of hepatocytes, resulting in loss of its normal hexagonal shape. The cytoplasm is rarefied or finely reticulated; this may be the result of accumulation of intracellular fluid, ultrastructural level of minute vacuoles of steatosis and/or other acute or chronic toxic cell injuries (Figure 1d).<sup>22</sup> Affected cells' nuclei may be enlarged and/or hyperchromatic. A study a decade ago provided the groundwork that has led to

the recognition that hepatocytic ballooning is a significant histological feature for distinguishing progressive NAFLD from the less aggressive forms.<sup>23</sup> In the same study, other features such as Mallory–Denk bodies (Mallory’s hyaline, Figure 1e) or fibrosis observed in the biopsies were also found to be associated with a higher incidence for cirrhosis and liver-related death. Subsequent studies further strengthened the notion that hepatocytic ballooning is not only a unique morphological change reflecting liver cell injury in NASH, but it is also a distinctive histological feature that experienced liver pathologists commonly use to arrive at a diagnosis of SH.<sup>8,24</sup> In a clinicopathological correlative study based on blinded review of entry biopsies for a treatment trial, hepatocytic ballooning was associated with higher serum cholesterol level; there was also a trend towards the presence of ballooning in biopsies from patients with abnormal glycaemic control, greater insulin resistance and increased serum markers of necroinflammation compared with patients without ballooning.<sup>25</sup> Thus, the link between hepatocytic ballooning and liver cell injury in NASH and the insulin-resistant metabolic syndrome has been strongly suggested.

In Younossi et al’s study of interobserver variability in histological evaluation, histological features that had significant inter- and intra-observer concordance among both junior and experienced liver pathologists were steatosis, fibrosis, ballooning degeneration and vacuolated (glycogenated) nuclei,<sup>24</sup> suggesting these histological features might be useful for the development of a standardized and reliable pathological scoring scheme for NAFLD and its various clinical outcomes. Almost simultaneously, as a result of a detailed review of biopsies from clinically characterized NASH, Brunt et al ushered in a semiquantitative grading and staging scheme for NASH in which steatosis was a ‘given’ and the grading system was based on incremental increase in a combination of lesions: ballooning, and lobular and portal inflammation.<sup>25</sup> Fibrosis stage was proposed to reflect the typical fibrosis pattern of NASH, which distinguishes it from other forms of portal-based chronic liver disease: the unique zone 3 perisinusoidal/pericellular fibrosis, through portal fibrosis, bridging fibrosis to cirrhosis.<sup>8</sup>

Taken together, steatosis, ballooning and lobular inflammation are considered the common constellation of minimal criteria for the diagnosis of NASH.<sup>26</sup> This has been supported by a multiple regression analysis that showed the diagnosis of NASH is not dependent on a single histological feature, but rather involves assessment of multiple independent features.<sup>27</sup> The results of the NIDDK-sponsored NASH Clinical Research Network (CRN) study to validate a scoring system also confirmed, in multivariate analysis, that several histological features are significantly associated with the diagnosis of NASH, including lobular inflammation, ballooning degeneration, fibrosis and steatosis.<sup>9</sup>

Studies in human liver and in animal models have confirmed increased apoptotic indices or enhanced signals of apoptosis in NASH, as in ALD.<sup>28–32</sup> A recent microarray study in NAFLD has demonstrated that genes related to liver apoptosis were differentially expressed.<sup>33</sup> Whether the presence and/or quantity of apoptotic bodies is significant for distinguishing NASH from milder forms of NAFLD awaits further investigation.

Microvesicular steatosis may occur in NAFLD/NASH biopsies, as well as active ALD. Typically, this finding will be noted in clusters of hepatocytes<sup>9</sup> in NAFLD. In ALD, microvesicular steatosis

may be a prognostic indication of progressive disease.<sup>34</sup> In certain situations, microvesicular steatosis accumulation may resemble ballooning degeneration in that the hepatocytes are enlarged and have flocculent cytoplasm; however, the nuclei remain centrally located and may appear to be indented by the small fat droplets in microvesicular steatosis (Figure 1a). Another more subtle difference between ballooned hepatocytes and those with microvesicular steatosis is the presence of larger and hyperchromatic nuclei in the former. As mentioned above, predominant microvesicular steatosis is a histological manifestation of severe liver disease caused by mitochondrial dysfunction,<sup>22,35,36</sup> and should not be seen as the primary type of steatosis in individuals with NAFLD.

Occasionally in NAFLD there may be hepatocytes with glycogenosis. As with the finding of microvesicular steatosis, this feature is not a dominant finding in hepatocytes. The notably large, swollen, glycogen-rich hepatocytes of glycogenosis may occur in glycogen storage diseases or in glycogenic hepatopathy; the latter entity is associated with type 1 diabetes with poor glycaemic control.<sup>37</sup> Of note, biopsies from patients with glycogenic hepatopathy typically lack significant steatosis, inflammation, lobular spotty necrosis and the characteristic perisinusoidal fibrosis of NAFLD. Further, the clinical setting is quite different.

Swollen hepatocytes that may resemble ballooning may also occur due to hepatocellular cholestasis; affected hepatocytes appear enlarged and have clear, flocculent cytoplasm, so-called feathery degeneration. This lesion is noted in the perivenular region in acute cholestasis and is accompanied by canalicular bile stasis. The clinical setting of biliary obstruction or drug/toxin injury, the lack of the typical perisinusoidal/pericellular fibrosis, and the presence of canalicular cholestasis differentiates this from ballooning of NAFLD. *Cholate stasis*, the histopathological term used to describe the alteration in periportal hepatocytes in chronic cholestatic conditions, also includes cellular swelling and cytoplasmic material clumped around the nucleus, and large lucent peripheral areas of the cell. Mallory–Denk bodies (Mallory’s hyaline) may also be present.<sup>38</sup> These findings can be distinguished from ballooning in NASH by the clinical setting and periportal, zone 1 location in the non-cirrhotic liver. It is challenging to differentiate these, however, in advanced fibrosis, as the remodelled parenchyma usually no longer has a distinctly zonal architecture. In this situation, a copper stain can be of assistance to document cholate stasis.

Megamitochondria, or giant mitochondria, may occur in NASH; these structures are round or needle-shaped intracytoplasmic inclusions (Figure 1f). With electron microscopy, they have been shown to have multilamellar membranes, paracrystalline inclusions, with loss of cristae. Megamitochondria may reflect injury or adaptive change.<sup>39,40</sup>

Lobular inflammation in both NAFLD and ALD may be mixed, predominantly mononuclear or have foci of ‘satellitosis’. The latter is noted by clusters of polymorphonuclear leucocytes surrounding a hepatocyte that contains a Mallory–Denk body (Mallory’s hyaline). Microgranulomas and small lipogranulomas may also be noted within the lobules of both NAFLD and ALD.<sup>22,26</sup>

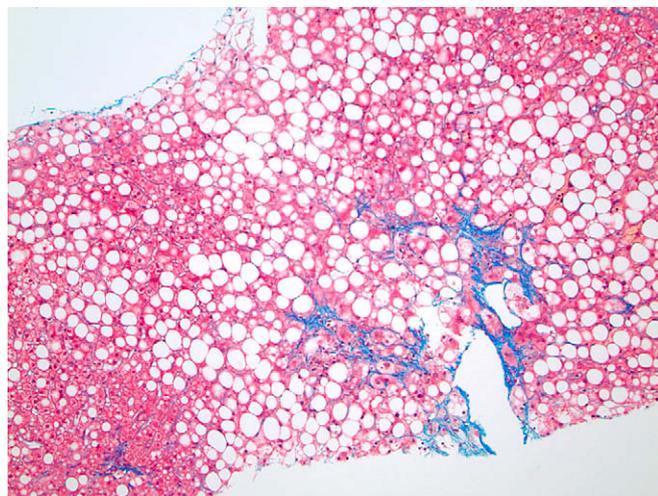
Portal chronic inflammation is commonly ‘mild’ in NAFLD; but is noted commonly in paediatric NAFLD (discussed below) and may increase with clinical and histological severity in adult

NAFLD.<sup>8,41</sup> As will be discussed, however, it is important to note that disproportionately increased portal inflammation in a biopsy of NAFLD or NASH may be an indication of another, concurrent chronic liver disease. In ALD, portal inflammation may be mixed acute and chronic with associated features of ductular reaction and periportal fibrosis<sup>42</sup>; these changes may be related to concurrent pancreatitis and are not seen in NAFLD.

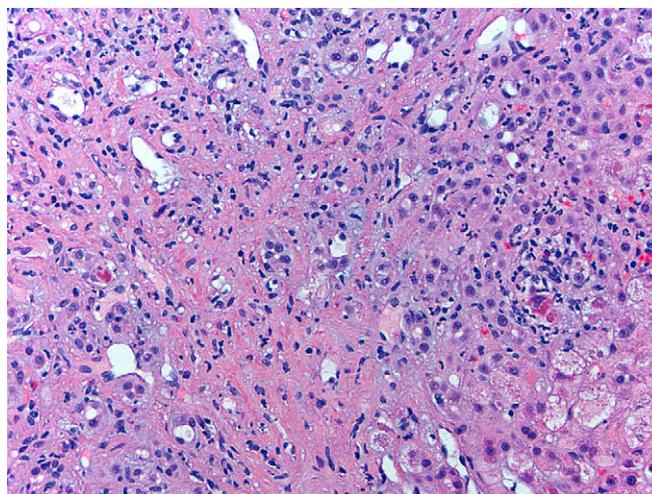
The pattern(s) of fibrosis in NAFLD and ALD can be quite similar. Although in the early stages of the disease, fibrosis is seen in zone 3 as perisinusoidal, pericellular collagen (Figure 2), with progression, portal and bridging fibrosis occur, and ultimately cirrhosis. Recently, the significance of the 'ductular reaction' (ductular proliferation accompanied by inflammation and fibrosis) as related to portal fibrosis in NAFLD was reported.<sup>43</sup> The authors noted that the ductular reaction correlates with degrees of insulin resistance as well as impaired hepatocyte replication in the setting of NAFLD. Active lesions of SH may or may not be retained in cirrhosis. Both of these processes may 'burn out' and both may be underlying causes of otherwise cryptogenic cirrhosis. Finally, isolated perivenular fibrosis is described in ALD, but has not been critically evaluated in NAFLD.

#### Distinguishing non-alcoholic fatty liver disease and alcoholic liver disease

Many histological features, including steatosis, hepatocytic ballooning, apoptosis, mixed lobular inflammation, Mallory–Denk bodies (Mallory's hyaline), megamitochondria, portal lipogranulomas and the unique pattern of perisinusoidal/pericellular fibrosis are shared by alcoholic SH (ASH) and NASH.<sup>44</sup> In part, both diseases also share many pathogenetic alterations that result in hepatic steatosis, activation of cytochrome P450 2E1 (CYP2E1)<sup>45,46</sup> and altered cytokine expression. However, canalicular cholestasis, cholangiolitis (marked ductular reaction and acute inflammatory infiltrate in the portal regions, Figure 3) and lymphocytic phlebitis, obliterative fibrosis of the terminal hepatic venule and the unique lesion of sclerosing hyaline necrosis (Figure 4) are features in ASH not noted in NASH.<sup>44</sup> Histological changes more commonly seen



**Figure 2** The characteristic fibrosis pattern in nonalcoholic steatohepatitis or alcoholic liver disease is collagen deposition within the perisinusoidal spaces in zone 3 region (perivenular/pericellular fibrosis, Masson trichrome stain, 100X).

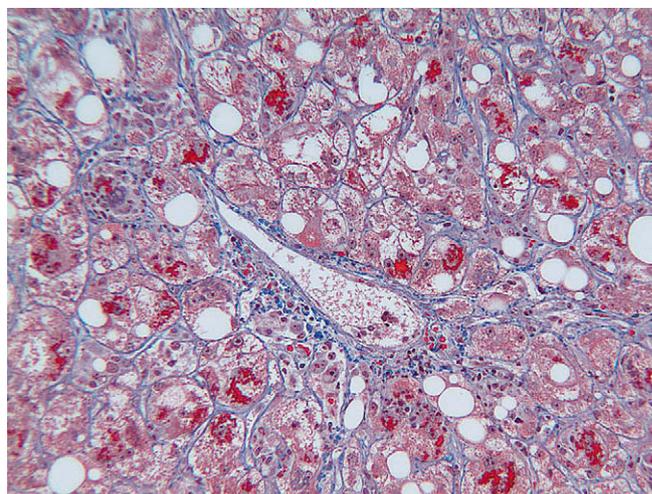


**Figure 3** Marked bile ductular reaction with accompanying neutrophilic infiltrates in the portal tract in alcoholic hepatitis (200X). Mallory–Denk bodies (Mallory's hyalines) are present in the adjacent hepatocytes.

in NASH than ASH are more severe steatosis and nuclear vacuolization (glycogenated nuclei).<sup>42</sup> Necroinflammatory activity is generally milder in NASH than in ASH,<sup>19,22,44,47,48</sup> while large, dense Mallory–Denk bodies (Mallory's hyaline) and periportal fibrosis are more common in ASH.<sup>44,49</sup> Interestingly, steatosis is not always present in alcoholic hepatitis, yet steatosis is a necessary component to arrive at a diagnosis of NASH, especially when the liver is not cirrhotic. Finally, alcoholic foamy degeneration does not have a known counterpart in NAFLD.

#### Grading and staging non-alcoholic fatty liver disease

Until a proposal was published in 1999, there were no standardized methods of histological evaluation for NAFLD, and no systematically agreed-upon criteria of separating NASH from



**Figure 4** High power magnification shows sclerosing hyaline necrosis with obliterative fibrosis of the terminal hepatic venule in alcoholic hepatitis (Masson trichrome stain). There is a focus of hepatocytic dropout adjacent to the terminal hepatic venule and Mallory–Denk bodies (Mallory's hyalines) are prominent.

steatosis. The proposal in the *American Journal of Gastroenterology*<sup>8</sup> was based on a review of 52 biopsies from 51 clinically well-characterized patients; in addition to noting a semiquantitative 'score' for each of the lesions already well-described in SH [steatosis, ballooning, lobular and portal inflammation, Mallory–Denk bodies (Mallory's hyaline), pigmented Kupffer cells, acidophil bodies, glycogenated nuclei and fibrosis], there was a 'gestalt' score of severity of the lesions. Then, it was determined which of the lesions appeared to be most contributory to this global severity. Also, just as the 'fathers' of modern hepatopathology noted in chronic hepatitis that the potentially reversible necroinflammatory lesions could be scored separately as 'grade' from potentially irreversible lesions of fibrosis and remodelling ('stage') (reviewed in<sup>13</sup>), this model was followed in the proposal. However, dissimilar to other forms of chronic hepatic and biliary liver disease, the fibrosis of SH was noted to occur first in perisinusoidal spaces in centrilobular regions (zone 3) prior to involvement of portal/periportal regions.<sup>19</sup> In other forms of chronic liver disease, portal fibrosis is the initial finding, and perisinusoidal fibrosis is actually atypical. Thus, the grading and staging for NASH was proposed, as illustrated in Table 1.

In 2005, a system was published<sup>9</sup> in order to: (1) address the broader spectrum of NAFLD; (2) propose a system validated by a panel of pathologists based on blinded review of paediatric and adult biopsies; and (3) provide more numerical assessment of specific lesions for comparisons in intervention trials. The system maintained features of the prior proposal, but added modifications to meet the above criteria. It is referred to by the group that validated it as the 'NASH CRN' and is now widely utilized. It should be reiterated that the numeric score of the NAFLD Activity Score, the NAS, is not intended to and *cannot* replace a diagnosis, but rather the two are separate exercises performed for different purposes. The NAS and fibrosis scores are shown in Table 2. As can be seen, the fibrosis score expanded stage 1 in order to take into account delicate and dense perisinusoidal fibrosis, as well as the pattern of then-recognized fibrosis of paediatric NAFLD<sup>50</sup> that predominates in portal/periportal regions.

### Grading and staging alcoholic liver disease

As yet, there is no widely accepted system for semi-quantitative scoring of ALD. A proposal based on modifications of the NASH CRN system was recently published (Table 3).<sup>51</sup> A difficulty in deriving a system for ALD is the much broader spectrum of lesions for this entity than encountered in NAFLD.

### Histopathological differential diagnosis of fatty liver disease

#### Hepatitis C virus

Steatosis is not a specific feature for NAFLD/NASH or ALD. Approximately 70% of individuals chronically infected with hepatitis C virus (HCV) show at least a mild degree of macrovesicular steatosis.<sup>52</sup> Different from NASH/NAFLD and ALD, the distribution of fat in chronic hepatitis C is often not accentuated in the perivenular region. The underlying causes of steatosis in hepatitis C are under intense investigation. It is well known that genotype 3a is specifically associated with steatosis in the liver.<sup>53–55</sup> The lesion, therefore, is likely to be a viral cytopathic effect. On the

### Proposed grading and staging for histopathological lesions of NASH (modified from<sup>8</sup>).

<i>Steatosis:</i>	Graded as 1 = 0–33%, 2 = 33–66%, 3 >66%.
<i>Ballooning:</i>	Zonal location noted and severity (mild or marked) recorded according to estimate of numbers of hepatocytes involved
<i>Lobular inflammation:</i>	0–3 based on observations of foci per 20x field; 1 = 1–2 foci, 2 = up to 4 foci, 3 = > 4 foci. In addition, cell types (acute or chronic) and location were noted
<i>Portal inflammation:</i>	0–3, 1 = mild, 2 = moderate, 3 = severe
<b>Grade 1, mild</b>	
<i>Steatosis:</i>	Predominantly macrovesicular, involves <33% up to 66% of the lobules
<i>Ballooning:</i>	Occasionally observed; zone 3 hepatocytes
<i>Lobular inflammation:</i>	Scattered and mild acute (polymorphs) inflammation and occasional chronic inflammation (mononuclear cells)
<i>Portal inflammation:</i>	None or mild
<b>Grade 2, moderate</b>	
<i>Steatosis:</i>	Any degree and usually mixed macrovesicular and microvesicular
<i>Ballooning:</i>	Obvious and present in zone 3
<i>Lobular inflammation:</i>	Polymorphs may be noted associated with ballooned hepatocytes, pericellular fibrosis; mild chronic inflammation may be seen
<i>Portal inflammation:</i>	Mild to moderate
<b>Grade 3, severe</b>	
<i>Steatosis:</i>	Typically >66% (panacinar); commonly mixed steatosis
<i>Ballooning:</i>	Predominantly zone 3; marked,
<i>Lobular inflammation:</i>	Scattered acute and chronic inflammation; polymorphs may appear concentrated in zone 3 areas of ballooning and perisinusoidal fibrosis
<i>Portal inflammation:</i>	Mild or moderate
<b>Staging fibrosis in nonalcoholic steatohepatitis (NASH)</b>	
<i>Stage 1:</i>	Zone 3 perivenular perisinusoidal/pericellular fibrosis, focal or extensive
<i>Stage 2:</i>	As above with focal or extensive periportal fibrosis
<i>Stage 3:</i>	Bridging fibrosis, focal or extensive
<i>Stage 4:</i>	Cirrhosis, probable or definite

**Table 1**

other hand, non-genotype 3 steatosis may be induced by underlying obesity-associated insulin resistance.<sup>27</sup> Finally, HCV core protein has been shown to induce hepatic steatosis in transgenic mice.<sup>56</sup> Clinically, hepatitis C can be diagnosed by serology and HCV RNA. Histologically, inflammation in hepatitis C tends to be

### NAFLD activity score (NAS) and fibrosis scoring from NIDDK NASH CRN (modified from<sup>9</sup>).

#### NAS (0–8) (only to be applied in cases of NAFLD/NASH)

<i>Steatosis:</i>	0 (< 5%); 1 (5–33%); 2 (33–66%); 3 (> 66%)
<i>Lobular inflammation:</i>	Counted in 20x fields 0, 1 (< 2 foci), 2 (2–4 foci), 3 (> 4 foci)
<i>Ballooning:</i>	0, 1 (few), 2 (many)

#### Fibrosis/staging: 0–4

0: None
1a: Mild, zone 3 perisinusoidal fibrosis (requires trichrome stain to see)
1b: Moderate zone 3 perisinusoidal fibrosis
1c: Portal fibrosis only
2: Zone 3 perisinusoidal AND periportal fibrosis
3: Bridging fibrosis
4: Probable or definite cirrhosis

**Table 2**

accentuated in the portal tracts; in addition, chronic hepatitis C typically does not have ballooned hepatocytes or the distinct perivenular/pericellular fibrosis. The presence of the latter in biopsies with hepatitis C is strongly suggestive of concurrent SH.<sup>16,17</sup>

#### Autoimmune liver disease

Antinuclear antibody (ANA) titres can be elevated in up to 20% of patients diagnosed with NASH by biopsy.<sup>57</sup> Other non-organ-specific antibodies such as antismooth muscle antibody and even antimitochondrial antibody (AMA) have been documented in 2–40% of cohorts of NAFLD patients.<sup>58</sup> Thus, it is important to correlate the histological, clinical and serological findings before establishing a diagnosis as the therapy for NASH and autoimmune hepatitis differ significantly. On the other hand, given many of the overlapping demographic features in these two entities, it is not unexpected that, in some situations, autoimmune hepatitis and NASH may coexist.<sup>16,59,60</sup>

#### Iron-overload diseases

Ferritin, a known acute-phase reactant protein, may be ‘non-specifically’ elevated in NAFLD, or may be a manifestation of aberrant *HFE* genetics<sup>61</sup> or insulin resistance.<sup>62,63</sup> Iron deposition in NAFLD may be hepatocellular, reticuloendothelial or both.<sup>8</sup> Whether aberrant genetics and/or iron deposition in NAFLD relates to progression of disease is an area of active investigation.

#### Wilson disease

Wilson disease has been known as a great mimicker in liver diseases. Histological features in Wilson disease may also be characterized by steatosis and glycogenated nuclei; additional features noted in varying stages of the disease include atypical lipofuscin, acidophil bodies, bile, increased iron, anisonucleosis, moderate portal chronic inflammation, Mallory–Denk bodies (Mallory’s hyaline) and swollen hepatocytes, some with megamitochondria. Clearly, if Wilson disease is a concern, tissue should be sent for copper quantitation.

### Proposed scoring system for alcoholic liver disease (ALD) (modified from<sup>51</sup>).

#### Grade: (0–11)

*Steatosis* (0–3), as in NIDDK NASH CRN System:

0: <5%
1: 5–33%
2: 34–66%
3: >66%

*Lobular inflammation* (0–3), as in NIDDK NASH CRN System:

0: None
1: <2/20X
2: 2–4/20X
3: >4/20X

*Cell Death* (0–3):

0: None
1: Focal apoptosis (acidophil bodies)
2: Many acidophil bodies
3: Confluent necrosis

*Ballooning* (0–2), as in NIDDK NASH CRN System

0: None
1: Few
2: Many; prominent

#### Stage: (0–6)

0: None
1: Mild zone 3; focal zone 3; pericellular and/or perivenular
2: Marked zone 3 fibrosis (most zones 3)
3: Fibrous linkage: hepatic veins with septa
4: Fibrous linkage: hepatic veins and portal tracts with septa
5: Incomplete cirrhosis
6: Probable or definite cirrhosis

#### In addition the following are recorded:

<i>Steatosis:</i>	Macrovesicular, microvesicular, mixed
<i>Lobular inflammation:</i>	Piecemeal necrosis, chronic, mixed
<i>Iron:</i>	0–4 and note distribution
<i>Mallory–Denk bodies</i>	
<i>Lipogranulomas</i>	
<i>Portal inflammation</i>	
<i>Isolated portal fibrosis</i>	

**Table 3**

### Non-alcoholic fatty liver disease/alcoholic liver disease with concomitant liver diseases

Brunt et al described the not so uncommon occurrence of concomitant histological SH in 1–7% of non-allograft liver biopsies from patients with other serologically diagnosed liver diseases, including hepatitis C, autoimmune hepatitis, primary biliary cirrhosis, *HFE* mutation,  $\alpha$ -1-AT deficiency and hepatitis B. Given that more stringent criteria for SH were applied in the study (i.e. presence of zone 3 perisinusoidal fibrosis) so as not to overestimate NASH, the prevalence of the concurrence may actually have been underestimated.<sup>16</sup> Other studies have also documented a similar rate of concurrence with hepatitis C<sup>17,64</sup> or other forms of chronic liver disease.<sup>17</sup> Such frequent concurrence may reflect

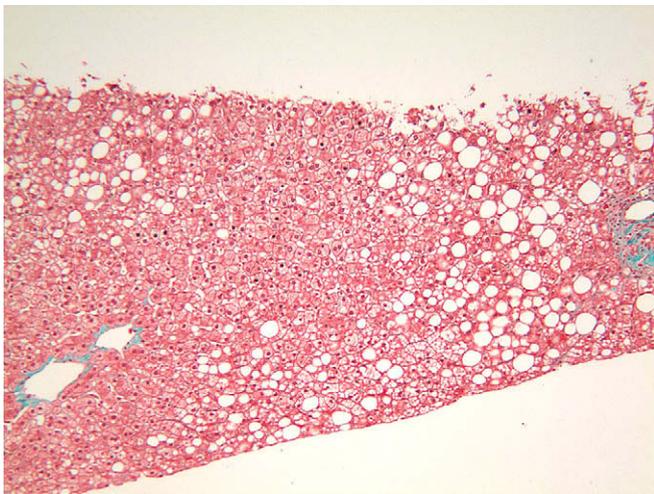
the common presence of NASH in the general population, and reiterates the importance for pathologists to exclude NASH in liver biopsies clinically diagnosed with other liver diseases.

As previously indicated, some form of SH, NAFLD or ALD can be suggested in biopsies with HCV when perisinusoidal and/or perivenular fibrosis are present. The figures for concurrent HCV and ALD are not known, but it is commonly recognized that ALD potentiates fibrosis in HCV.

### Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis in the paediatric population

The prevalence of NAFLD in children and adolescents is estimated at 17%, and is likely to reach epidemic proportions worldwide,<sup>65-67</sup> as the prevalence of obesity and insulin resistance among children has increased over the past decades.<sup>68,69</sup> NAFLD may be more common in boys than girls based on several studies.<sup>69-72</sup> Ethnic variation in children is noted, just as in adults. In Schwimmer et al's study, boys and children of Asian, Native American and Hispanic ethnicity were more likely than girls and children of white race, respectively, to have the clinical features and characteristic histological finding of portal-based injury pattern.<sup>50</sup> Whether this observation represents bias due to the study of a selected patient population from Southern California awaits further investigation; however, similar findings have been noted in adult studies.<sup>3,5</sup>

In contrast to the typical zone 3 injury pattern seen in adult NAFLD/NASH, inflammation and fibrosis are accentuated in the portal region in paediatric NAFLD, and ballooning degeneration and perisinusoidal fibrosis are not typically obvious. This portal-based injury and fibrosis pattern without ballooned hepatocytes and perisinusoidal fibrosis (Figure 5) was described in over half (51%) of a cohort of 100 children or adolescents with NAFLD, and thus was the basis for the designation of the type 2 pattern of NASH. In this study, only 17% showed the typical distinct histological features of NASH seen in the adult population (type 1 pattern), i.e. ballooning degeneration and/or perisinusoidal fibrosis,



**Figure 5** In paediatric non-alcoholic steatohepatitis, ballooning degeneration and zone 3 perivenular/pericellular fibrosis are not typically apparent and steatosis is not accentuated in zone 3 (Masson trichrome stain).

besides steatosis.<sup>50</sup> In the same study, children with paediatric, 'type 2' NASH were more often boys, were significantly younger and had more severe obesity than children with the adult, 'type 1' NASH histological pattern. These observations underscored the necessity of using a different set of definitions and criteria for paediatric NASH.

Challenges remain in paediatric NAFLD, however. Schwimmer et al showed that 16% of these 100 paediatric patients had features that overlap with both type 1 and type 2 NASH, whereas a recent study from Italy showed overlap of both patterns in the majority of biopsies.<sup>67</sup> It is likely that just as in adults, a spectrum of patterns of disease exists in paediatric NAFLD, and further studies may elucidate the underlying associations with age, gender and hormonal alterations that correlate with the pathological features in the paediatric and adolescent populations.

### Liver histology following intervention: non-alcoholic fatty liver disease and alcoholic liver disease

To date, the standard treatment of NAFLD/NASH remains diet and physical exercise, as well as management of associated metabolic conditions. Resolution of the histological lesions as assessed by liver biopsy remains the gold standard for successful treatment outcomes. Interestingly, randomized control trials<sup>73</sup> and studies of series of liver biopsies<sup>74,75</sup> have confirmed that apparent resolution or improvement of liver injury in NASH may be seen in biopsies in subjects without any therapeutic intervention. A possible explanation for this is that patients may have lifestyle changes after realizing the significance of their medical condition.<sup>76</sup> Clinical trials,<sup>73,75,77,78</sup> including those using insulin sensitizing agents, have shown histological improvements of NASH (steatosis, inflammation, ballooning and fibrosis), some even with complete resolution of NASH. Of interest, it was noted in one successful trial that after a year, liver test elevations recurred<sup>75</sup> and a recent biopsy study showed that the initial improvement at the end of defined therapy periods was not sustained after discontinuation of the medication.<sup>79</sup> These studies suggest that long-term therapy will be required to maintain improvements in NASH activity. On the other hand, surgical intervention (various alimentary tract by-pass procedures) have been shown to provide long-term improvement.<sup>80</sup>

Of note, several treatment studies, including those from surgical intervention, with follow-up biopsies have shown a shift in proportion of lobular inflammation to portal inflammation.<sup>75,77,80</sup> Thus, pathologists should be aware that increased portal inflammation in subjects with NAFLD may be a reflection of resolution.<sup>81</sup> The recently characterized ductular reaction and accompanying portal fibrosis in NASH have shown improvement following treatment.<sup>43,81</sup>

Steatosis in ALD usually rapidly improves with abstinence.<sup>18,34,82</sup> However, a minority of patients with alcoholic hepatitis may show similar histological lesions or progression to cirrhosis, even if they remained sober and underwent surveillance liver biopsy.<sup>83</sup> While abstinence appears to be the foundation of treatment for ALD and the use of corticosteroids is hampered by scepticism and underestimation of its benefits, potential alternative therapies include cytokine-directed therapy such as pentoxifylline and anti-TNF agents; however, the histopathology after these therapies is largely unknown due to lack of biopsy data.<sup>18</sup>

### Recurrence of non-alcoholic fatty liver disease and alcoholic liver disease following liver transplantation

Several studies have reported the recurrence/occurrence of NAFLD/NASH after orthotopic liver transplantation (OLT).<sup>84–88</sup> Since the distinct histological features of NAFLD/NASH may no longer be identifiable in end-stage cirrhotic liver, diagnosis of NAFLD/NASH pre-OLT in those patients with cryptogenic cirrhosis has relied on clinical characteristics of the metabolic syndrome and exclusion of other liver diseases. Some of the studies suggested that in a time-dependent manner, fatty liver almost universally occurs after OLT in these patients. One confounding factor is that cumulative steroid exposure correlates with the development of fatty liver in the allograft.<sup>88</sup> Another is the clinical development of features of metabolic syndrome due to OLT-related medications. Thus, while the observations that NAFLD/NASH occurs/recurs after OLT further serves as a line of evidence that the disease is a manifestation of the metabolic syndrome,<sup>74,89</sup> caution is still necessary prior to attribution of cases of ‘cryptogenic cirrhosis’ to ‘burned-out’ NASH solely based on occurrence of the pathology post-OLT.

In the western world, liver cirrhosis due to alcohol is the second most common indication for liver transplantation<sup>44</sup> in that approximately 17–25% of transplants are performed for ALD. Most transplant programmes in the USA require a minimum of 6 months’ abstinence before transplantation. A recidivism rate of 20–30% after OLT has been reported; higher rates are suggested when the pre-OLT abstinence period is less than 6 months.<sup>90</sup> Histologically, steatosis, fibrosis, particularly perivenular/pericellular fibrosis, and rapid fibrosis progression are more common in post-OLT heavy alcohol use versus non-drinkers.<sup>91–95</sup>

### Conclusion

With the emergence of the NAFLD epidemic, pathologists are frequently encountering liver biopsies showing a spectrum of fatty changes. Pathologists need to be aware of the clinical scenario of the biopsy specimens, and that the diagnosis of non-alcoholic fatty liver disease or non-alcoholic SH remains clinicopathological. Significant overlap occurs with features of ALD in varying stages; on the other hand, there are lesions that are more suggestive of ALD than of NAFLD, and the pathologist should be aware of these. Concurrent liver diseases are not uncommon, and have to be considered. Importantly, the histopathology of NASH post intervention and NASH in the paediatric population often does not mimic the classical morphological descriptions of the constellation of lesions of NASH. The shift of lobular- to portal- based injury pattern may be seen in both of these settings. In addition, the presence of findings of other forms of liver disease needs to be excluded. Finally, it is worth emphasizing that no scoring system is meant to replace a diagnosis; in particular, the NAS score was designed to compare the histological changes after treatment of NASH, but it is not intended as a means of deriving a diagnosis from numeric sums. ◆

### REFERENCES

- Panel. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486–2497.
- Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387–1395.
- Weston SR, Leyden W, Murphy R, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005; **41**: 372–379.
- Patton HM, Sirlin C, Behling C, Middleton MS, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. *J Pediatr Gastroenterol Nutr* 2006; **43**: 413–427.
- Hossain P, Kavar B, El Nahas M. Obesity and diabetes in the developing world - a growing challenge. *N Engl J Med* 2007; **356**: 213–215.
- Mandayam S, Jamal MM, Morgan TR. Epidemiology of alcoholic liver disease. *Semin Liver Dis* 2004; **24**: 217–232.
- Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**(2 Suppl 1): S99–S112.
- Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467–2474.
- Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313–1321.
- Skelly MM, James PD, Ryder SD. Findings on liver biopsy to investigate abnormal liver function tests in the absence of diagnostic serology. *J Hepatol* 2001; **35**: 195–199.
- Merriman RB, Ferrell LD, Patti MG, et al. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 874–880.
- Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. *Hepatology* 2003; **37**: 1286–1292.
- Brunt EM. Grading and staging the histopathological lesions of chronic hepatitis: the Knodell histology activity index and beyond. *Hepatology* 2000; **31**: 241–246.
- Brunt EM. Liver biopsy interpretation for the gastroenterologist. *Curr Gastroenterol Rep* 2000; **2**: 27–32.
- Ishak KG. Chronic hepatitis: morphology and nomenclature. *Mod Pathol* 1994; **7**: 690–713.
- Brunt EM, Ramrakhiani S, Cordes BG, et al. Concurrence of histologic features of steatohepatitis with other forms of chronic liver disease. *Mod Pathol* 2003; **16**: 49–56.
- Sanyal AJ, Contos MJ, Sterling RK, et al. Nonalcoholic fatty liver disease in patients with hepatitis C is associated with features of the metabolic syndrome. *Am J Gastroenterol* 2003; **98**: 2064–2071.
- Levitsky J, Mailliard ME. Diagnosis and therapy of alcoholic liver disease. *Semin Liver Dis* 2004; **24**: 233–247.
- Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434–438.
- Chitturi S, Abeygunasekera S, Farrell GC, et al. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373–379.
- Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917–923.
- Burt AD, Mutton A, Day CP. Diagnosis and interpretation of steatosis and steatohepatitis. *Semin Diagn Pathol* 1998; **15**: 246–258.

- 23 Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413–1419.
- 24 Younossi ZM, Gramlich T, Liu YC, et al. Nonalcoholic fatty liver disease: assessment of variability in pathologic interpretations. *Mod Pathol* 1998; **11**: 560–565.
- 25 Brunt EM, Neuschwander-Tetri BA, Oliver D, Wehmeier KR, Bacon BR. Nonalcoholic steatohepatitis: histologic features and clinical correlations with 30 blinded biopsy specimens. *Hum Pathol* 2004; **35**: 1070–1082.
- 26 Brunt EM. Nonalcoholic steatohepatitis. *Semin Liver Dis* 2004; **24**: 3–20.
- 27 Hui JM, Sud A, Farrell GC, et al. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. [corrected] *Gastroenterology* 2003; **125**: 1695–1704.
- 28 Kashireddy PR, Rao MS. Sex differences in choline-deficient diet-induced steatohepatitis in mice. *Exp Biol Med (Maywood)* 2004; **229**: 158–162.
- 29 Svegliati-Baroni G, Candelaresi C, Saccomanno S, et al. A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor- $\alpha$  and n-3 polyunsaturated fatty acid treatment on liver injury. *Am J Pathol* 2006; **169**: 846–860.
- 30 Feldstein AE, Canbay A, Angulo P, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology* 2003; **125**: 437–443.
- 31 Ramalho RM, Cortez-Pinto H, Castro RE, et al. Apoptosis and Bcl-2 expression in the livers of patients with steatohepatitis. *Eur J Gastroenterol Hepatol* 2006; **18**: 21–29.
- 32 Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 27–33.
- 33 Younossi ZM, Gorreta F, Ong JP, et al. Hepatic gene expression in patients with obesity-related non-alcoholic steatohepatitis. *Liver Int* 2005; **25**: 760–771.
- 34 Teli MR, Day CP, Burt AD, Bennett MK, James OF. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. *Lancet* 1995; **346**: 987–990.
- 35 Fromenty B, Grimbert S, Mansouri A, et al. Hepatic mitochondrial DNA deletion in alcoholics: association with microvesicular steatosis. *Gastroenterology* 1995; **108**: 193–200.
- 36 Pessayre D, Berson A, Fromenty B, Mansouri A. Mitochondria in steatohepatitis. *Semin Liver Dis* 2001; **21**: 57–69.
- 37 Torbenson M, Chen YY, Brunt E, et al. Glycogenic hepatopathy: an underrecognized hepatic complication of diabetes mellitus. *Am J Surg Pathol* 2006; **30**: 508–513.
- 38 Li MK, Crawford JM. The pathology of cholestasis. *Semin Liver Dis* 2004; **24**: 21–42.
- 39 Caldwell SH, Swerdlow RH, Khan EM, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 430–434.
- 40 Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183–1192.
- 41 Brunt EM, Kleiner DE, Behling C, Wilson L, Lavine JE, Neuschwander-Tetri BA, Nonalcoholic Steatohepatitis Clinical Research Network Group. Portal inflammation in NAFLD: correlations from the NASH CRN database. Presented, AASLD, 2006. *Hepatology* 2006; **44**: 655A.
- 42 Itoh S, Yougel T, Kawagoe K. Comparison between nonalcoholic steatohepatitis and alcoholic hepatitis. *Am J Gastroenterol* 1987; **82**: 650–654.
- 43 Richardson MM, Jonsson JR, Powell EE, et al. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. *Gastroenterology* 2007; **133**: 80–90.
- 44 Brunt EM. Alcoholic and nonalcoholic steatohepatitis. *Clin Liver Dis* 2002; **6**: 399–420, vii.
- 45 Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, Liddle C. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 1998; **27**: 128–133.
- 46 Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology* 1996; **111**: 1645–1653.
- 47 Pinto HC, Baptista A, Camilo ME, Valente A, Saragoca A, de Moura MC. Nonalcoholic steatohepatitis. Clinicopathological comparison with alcoholic hepatitis in ambulatory and hospitalized patients. *Dig Dis Sci* 1996; **41**: 172–179.
- 48 Thung SNGM. Alcoholic hepatitis vs. nonalcoholic steatohepatitis. In: Thung SN, ed. *Differential diagnosis in pathology: liver disorders*. New York: Igaku-Shoin, 1995, p. 24–26.
- 49 Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. *Semin Liver Dis* 2001; **21**: 17–26.
- 50 Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641–649.
- 51 Yip WW, Burt AD. Alcoholic liver disease. *Semin Diagn Pathol* 2006; **23**: 149–160.
- 52 Goodman ZD, Ishak KG. Histopathology of hepatitis C virus infection. *Semin Liver Dis* 1995; **15**: 70–81.
- 53 Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 2001; **33**: 1358–1364.
- 54 Castera L, Hezode C, Roudot-Thoraval F, et al. Worsening of steatosis is an independent factor of fibrosis progression in untreated patients with chronic hepatitis C and paired liver biopsies. *Gut* 2003; **52**: 288–292.
- 55 Monto A, Alonzo J, Watson JJ, Grunfeld C, Wright TL. Steatosis in chronic hepatitis C: relative contributions of obesity, diabetes mellitus, and alcohol. *Hepatology* 2002; **36**: 729–736.
- 56 Moriya K, Yotsuyanagi H, Shintani Y, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. *J Gen Virol* 1997; **78**: 1527–1531.
- 57 Cotler SJ, Kanji K, Keshavarzian A, Jensen DM, Jakate S. Prevalence and significance of autoantibodies in patients with non-alcoholic steatohepatitis. *J Clin Gastroenterol* 2004; **38**: 801–804.
- 58 Loria P, Lonardo A, Leonardi F, et al. Non-organ-specific autoantibodies in nonalcoholic fatty liver disease: prevalence and correlates. *Dig Dis Sci* 2003; **48**: 2173–2181.
- 59 McFarlane IG. Autoimmune hepatitis: diagnostic criteria, subclassifications, and clinical features. *Clin Liver Dis* 2002; **6**: 605–621.
- 60 Reid AE. Nonalcoholic steatohepatitis. *Gastroenterology* 2001; **121**: 710–723.
- 61 Bonkovsky HL, Jawaid Q, Tortorelli K, et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 421–429.

- 62 Bugianesi E, Manzini P, D'Antico S, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179–187.
- 63 Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, Oren R. NAFLD and hyperinsulinemia are major determinants of serum ferritin levels. *J Hepatol* 2007; **46**: 700–707.
- 64 Ong JP, Younossi ZM, Speer C, Olano A, Gramlich T, Boparai N. Chronic hepatitis C and superimposed nonalcoholic fatty liver disease. *Liver* 2001; **21**: 266–271.
- 65 Franzese A, Vajro P, Argenziano A, et al. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 1997; **42**: 1428–1432.
- 66 Mager DR, Roberts EA. Nonalcoholic fatty liver disease in children. *Clin Liver Dis* 2006; **10**: 109–131, vi-vii.
- 67 Nobili V, Marcellini M, Devito R, et al. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 2006; **44**: 458–465.
- 68 Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA* 2004; **291**: 2847–2850.
- 69 Schwimmer JB, Deutsch R, Rauch JB, Behling C, Newbury R, Lavine JE. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. *J Pediatr* 2003; **143**: 500–505.
- 70 Moran JR, Ghishan FK, Halter SA, Greene HL. Steatohepatitis in obese children: a cause of chronic liver dysfunction. *Am J Gastroenterol* 1983; **78**: 374–377.
- 71 Baldrige AD, Perez-Atayde AR, Graeme-Cook F, Higgins L, Lavine JE. Idiopathic steatohepatitis in childhood: a multicenter retrospective study. *J Pediatr* 1995; **127**: 700–704.
- 72 Manton ND, Lipsett J, Moore DJ, Davidson GP, Bourne AJ, Couper RT. Non-alcoholic steatohepatitis in children and adolescents. *Med J Aust* 2000; **173**: 476–479.
- 73 Lindor KD, Kowdley KV, Heathcote EJ, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. *Hepatology* 2004; **39**: 770–778.
- 74 Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221–1231.
- 75 Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 2003; **38**: 1008–1017.
- 76 Brunt EM. Nonalcoholic steatohepatitis: pathologic features and differential diagnosis. *Semin Diagn Pathol* 2005; **22**: 330–338.
- 77 Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004; **39**: 188–196.
- 78 Huang MA, Greenon JK, Chao C, et al. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. *Am J Gastroenterol* 2005; **100**: 1072–1081.
- 79 Lutchman G, Modi A, Kleiner DE, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. *Hepatology* 2007; **46**: 424–429.
- 80 Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease: improvement in liver histological analysis with weight loss. *Hepatology* 2004; **39**: 1647–1654.
- 81 Brunt EM, Clouston AD. Histologic features of fatty liver disease. In: Bataller R, ed. *International hepatology updates: nonalcoholic steatohepatitis*. Barcelona: Permanyer Press, 2007, p. 95–110.
- 82 Leevy CM. Fatty liver: a study of 270 patients with biopsy proven fatty liver and review of the literature. *Medicine (Balt)* 1962; **41**: 249–276.
- 83 Galambos JT. Natural history of alcoholic hepatitis. 3. Histological changes. *Gastroenterology* 1972; **63**: 1026–1035.
- 84 Czaja AJ. Recurrence of nonalcoholic steatohepatitis after liver transplantation. *Liver Transpl Surg* 1997; **3**: 185–186.
- 85 Molloy RM, Komorowski R, Varma RR. Recurrent nonalcoholic steatohepatitis and cirrhosis after liver transplantation. *Liver Transpl Surg* 1997; **3**: 177–178.
- 86 Carson K, Washington MK, Treem WR, Clavien PA, Hunt CM. Recurrence of nonalcoholic steatohepatitis in a liver transplant recipient. *Liver Transpl Surg* 1997; **3**: 174–176.
- 87 Kim WR, Poterucha JJ, Porayko MK, Dickson ER, Steers JL, Wiesner RH. Recurrence of nonalcoholic steatohepatitis following liver transplantation. *Transplantation* 1996; **62**: 1802–1805.
- 88 Contos MJ, Cales W, Sterling RK, et al. Development of nonalcoholic fatty liver disease after orthotopic liver transplantation for cryptogenic cirrhosis. *Liver Transpl* 2001; **7**: 363–373.
- 89 Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202–1219.
- 90 Watt KD, McCashland TM. Transplantation in the alcoholic patient. *Semin Liver Dis* 2004; **24**: 249–255.
- 91 Burra P, Mioni D, Cecchetto A, et al. Histological features after liver transplantation in alcoholic cirrhotics. *J Hepatol* 2001; **34**: 716–722.
- 92 Everson G, Bharadhwaj G, House R, et al. Long-term follow-up of patients with alcoholic liver disease who underwent hepatic transplantation. *Liver Transpl Surg* 1997; **3**: 263–274.
- 93 Pageaux GP, Bismuth M, Perney P, et al. Alcohol relapse after liver transplantation for alcoholic liver disease: does it matter? *J Hepatol* 2003; **38**: 629–634.
- 94 Conjeevaram HS, Hart J, Lisssoos TW, et al. Rapidly progressive liver injury and fatal alcoholic hepatitis occurring after liver transplantation in alcoholic patients. *Transplantation* 1999; **67**: 1562–1568.
- 95 Savolainen V, Perola M, Lalu K, Penttila A, Virtanen I, Karhunen PJ. Early perivenular fibrogenesis - precirrhotic lesions among moderate alcohol consumers and chronic alcoholics. *J Hepatol* 1995; **23**: 524–531.

### Practice points

- Given the epidemiology of both alcohol use and obesity in the Western World, many times the pathologist cannot distinguish between the two in liver biopsies with either steatosis or with Steatohepatitis
- Nonalcoholic Steatohepatitis is a diagnosis that can only be made with clinical correlation. Steatohepatitis is a histologic diagnosis, based on the presence of steatosis, lobular inflammation and ballooning in a characteristic zone 3 location. Fibrosis may or may not be present. The amount of steatosis can vary from around 5% to greater than 66%, but the “severity” of the lesions depends on all of the features

- More than one liver disease may occur and may be diagnosable on liver biopsy. HCV and NAFLD/NASH may co-exist in obese, insulin-resistant patients with viral infection. Likewise, AIH, PBC and other forms of chronic liver disease may co-exist. The criteria for making the diagnosis are discussed
- Biopsies from pediatric patients may or may not show the same histopathologic features as those from adults. In some pediatric biopsies, the steatosis may be preferentially around zone 1 and ballooning is not seen. Steatosis may also occur in a panacinar distribution
- True microvesicular steatosis may occur in NASH; most often, it occurs in small “patches” and may have megamitochondria. The most common type(s) of steatosis, however, in NAFLD/NASH is mixed large and small droplet macrovesicular steatosis

### Research directions

- Development of biomarker for the diagnosis of NASH
- Investigation of iron overload in NAFLD
- Morphology and pathogenesis of NAFLD/NASH in the paediatric population
- Evaluation of ballooned hepatocytes in NASH: do they reflect oxidative damage and/or represent small droplet steatosis?; and the use of immunohistochemistry for diagnosing ballooning degeneration
- Which comes first: insulin resistance or steatosis?
- Role of the apoptotic pathway in NASH
- Role of the innate immune system in NASH