Liver biopsy in alcoholic and non-alcoholic steatohepatitis patients

Biopsie hépatique pour stéatopathie alcoolique et métabolique

D.G. Tiniakos

Laboratory of Histology & Embryology, Medical School, University of Athens, 75, M. Asias street, Goudi, 11527 Athens, Greece

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Summary  Alcoholic liver disease and non-alcoholic liver disease share a similar histological spectrum that starts with 'simple' steatosis, and may be accompanied by inflammation. Alcoholic steatohepatitis and non-alcoholic steatohepatitis (NASH) are progressive forms of alcoholic liver disease and non-alcoholic liver disease, respectively, and can evolve into cirrhosis. The currently accepted minimum diagnostic criteria for steatohepatitis include steatosis, lobular inflammation and hepatocellular injury, but not fibrosis. Steatosis involving more than 5% of hepatocytes is required for the diagnosis of non-alcoholic fatty liver disease, but is not necessary for the diagnosis of alcoholic liver disease. Lobular inflammation is usually mild and frequently consists of a mixed, acute and chronic, inflammatory cell infiltrate composed of neutrophils and mononuclear cells. The presence of large numbers of neutrophils favors an alcoholic etiology. Hepatocellular injury in fatty liver disease usually occurs in the form of ballooning, but it can also present as apoptotic (acidophilic) bodies and lytic necrosis. The characteristic pattern of fibrosis in non-cirrhotic steatohepatitis is pericellular/perisinusoidal and is the result of deposition of collagen in the space of Disse. In both alcoholic steatohepatitis and NASH, sinusoidal collagen formation is the result of hepatic stellate cell activation that, in NASH, has been correlated with the grade of steatosis and fibrosis.

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Résumé  La maladie alcoolique du foie et la stéatopathie métabolique ont des lésions histologiques semblables débutant de la «simple» stéatose et pouvant être accompagnées d’inflammation. La stéatohépatite alcoolique et la stéatohépatite métabolique (NASH) sont les formes évolutives de respectivement la maladie alcoolique et la stéatopathie métabolique qui peuvent aboutir à une cirrhose. Les critères diagnostiques minimaux de stéatohépatite associent stéatose, inflammation lobulaire, et lésions hépatocellulaires. La fibrose n’est pas nécessaire au diagnostic de stéatohépatite. Une stéatose de plus de 5 % des hépatocytes est nécessaire au diagnostic de stéatopathie métabolique mais n’est pas nécessaire au diagnostic de maladie alcoolique du foie. L'inflammation lobulaire est généralement minime et associe
fréquemment un infiltrat inflammatoire mixte, aigu et chronique, composé de neutrophiles et de cellules mononucléées. La présence d’un grand nombre de neutrophiles est en faveur d’une origine alcoolique. Au cours des stéatopathies, les lésions hépatocellulaires apparaissent sous forme de ballonisation mais peuvent aussi apparaître sous forme de corps apoptotiques (acidophiles) et de nécrose cellulaire. Au cours de la stéatohépatite, la fibrose est péricellulaire et périsinusoïdale et résulte de la déposition de collagène dans l’espace de Disse. Au cours de la stéatohépatite alcoolique et métabolique, la formation de collagène sinusoidial résulte de l’activation des cellules étoilées. Au cours de la stéatohépatite métabolique, l’activation des cellules étoilées est correlative au grade de stéatose et au stade de fibrose.

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Introduction

Fatty liver disease is generally classified into two main clinical categories, each with a different etiology — namely, alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD). Both lead to pathological triglyceride accumulation within hepatocytes (steatosis), and progressive necroinflammatory liver disease (steatohepatitis). ALD refers to the entire spectrum of liver disease due to alcohol abuse [1], while NAFLD is a complex metabolic liver disease that is mainly related to the consequences of insulin resistance and obesity [2]. NAFLD is diagnosed when, after meticulous clinical and laboratory investigations, it is confirmed that patients are not consuming significant amounts of alcohol (typically greater than 10g/day for women and greater than 20g/day for men) [3]. Other associations of NAFLD include jejunoileal bypass/gastoplasty surgery for severe obesity, total parenteral nutrition, kwashiorkor, dietary disorders, bacterial contamination of the small bowel, inherited metabolic disorders, and a wide range of drug and environmental toxins [4].

ALD and NAFLD share a similar histological spectrum, which starts with ‘simple’ steatosis and may be accompanied by inflammation (Table 1) [4]. Steatosis is present in 90% of ALD patients and in 100% of patients with NAFLD [5]. Alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) are the progressive forms of ALD and NAFLD, respectively, that evolve into cirrhosis [6,7]. Alcohol abuse is thought to be responsible for up to 45% of hepatocellular carcinoma in Western countries and, although most cases arise within a context of alcoholic cirrhosis [8], hepatocellular carcinoma can be seen in non-cirrhotic ALD [9]. It may also arise in NAFLD-related cirrhosis and cryptogenic cirrhosis, although not as frequently as in ALD- or viral-hepatitis-related cirrhosis [10,11]. Recently, hepatocellular carcinoma development has been reported in non-cirrhotic patients with either NAFLD/NASH [12] or the metabolic syndrome [13].

An increasing number of non-invasive tests for diagnosing NASH have emerged in recent years [14]. However, liver biopsy is still considered the gold standard for confirming or excluding the diagnosis of NASH in a patient with chronically elevated liver enzymes, imaging-detected steatosis and other relevant clinical features that are mainly related to the presence of the metabolic syndrome [4,14].

In ALD, as treatment decisions are not usually based on histology, liver biopsy is not generally necessary for patient management [15]. However, it is required for confirmation of the diagnosis of steatohepatitis before inclusion into therapeutic protocols and for evaluation of concurrent liver disease. In addition, liver biopsy in ALD can provide prognostic information that is not only related to the severity of the histopathological lesions, but is also — given the presence of histological features such as mixed steatosis, perivenular fibrosis and megamitochondria — associated with progression to advanced liver disease and an increased rate of cirrhosis development [1,15].

Histopathology of adult fatty liver disease

The histological lesions that characterize non-cirrhotic fatty liver disease of alcoholic or non-alcoholic etiology in the adult are accentuated in zone 3 of the acinus (centrilobular region). Steatosis may be accompanied by lobular and/or portal inflammation in early fatty liver disease. The currently accepted minimum diagnostic criteria of steatohepatitis include steatosis, lobular inflammation and hepatocellular injury (Fig. 1). Fibrosis is not required for such a diagnosis, as it is not necessary for the diagnosis of chronic hepatitis of other etiology [4].

Figure 1 Constellation of steatohepatitis histological features in zone 3: macrovesicular steatosis (thin black arrow); lobular inflammation (white arrowhead); and hepatocellular ballooning (black arrowheads). The ballooned hepatocyte on the left contains a Mallory–Denk body. THV: terminal hepatic vein (H&E stain, × 200).
Hepatocytes have a foamy appearance due to the abundant fat present. In microvesicular steatosis, the affected steatosis is a combination of micro- and macrovesicular. The latter is commonly seen in both ALD and NAFLD. In macrovesicular steatosis, a single large fat droplet displaces the nucleus and cytoplasm to the periphery of affected hepatocytes. Mixed steatosis is a combination of micro- and macrovesicular steatosis, when both large- and small-droplet intracellular fat is present. In microvesicular steatosis, the affected hepatocytes have a foamy appearance due to the accumulation of tiny fat droplets surrounding a centrally placed nucleus; special stains for lipids, such as oil red O, may be needed to identify the intracytoplasmic material as fat [5]. In ALD, microvesicular steatosis arising in zone 3, and occasionally extending into zone 2, is known as 'alcoholic foamy degeneration' and is an indication of more severe liver disease [1,18]. In NAFLD, small patches of microvesicular steatosis may be seen at non-zonal locations [4].

The accumulation of fat in fatty liver disease usually starts in zone 3 and, in more severe cases, may even occupy the whole acinus. Histological assessment of steatosis is usually semiquantitative and based on the percentage of parenchymal involvement. The easiest method follows the acinar architecture of the liver parenchyma and describes the involvement by steatosis in thirds — < 33% (or 0—5%), 33—66% and > 66% — or as mild, moderate or severe [19,20].

Interestingly, steatosis may not persist during the progression of fatty liver disease and, for this reason, may not be reliably identified in cirrhotic specimens. In ALD-related cirrhosis, steatosis may be absent even when alcohol intake continues [1]. Clinical studies can associate 'cryptogenic' cirrhosis (no distinct morphological findings) with underlying clinical correlates of NAFLD [21,22], but the pathologist can only speculate as to the etiology.

### Table 1 Overlapping histological features in alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), and histological lesions seen so far only in alcoholic steatohepatitis (ASH) and not in non-alcoholic steatohepatitis (NASH).

<table>
<thead>
<tr>
<th>Histological lesions seen in both ALD and NAFLD</th>
<th>Histological lesions seen in ASH, but not in NASH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis (macrovesicular &gt; mixed)</td>
<td>Sclerosing hyaline necrosis</td>
</tr>
<tr>
<td>Required for the diagnosis of NAFLD</td>
<td></td>
</tr>
<tr>
<td>May not be present in ALD</td>
<td></td>
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<tr>
<td>Lobular inflammation</td>
<td></td>
</tr>
<tr>
<td>Satellitosis common in ASH</td>
<td></td>
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<tr>
<td>Portal inflammation</td>
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<tr>
<td>Lipogranulomas</td>
<td></td>
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<tr>
<td>Hepatocellular ballooning</td>
<td></td>
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<tr>
<td>Acidophil (apoptotic) bodies</td>
<td></td>
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<tr>
<td>Zone 3 periportal fibrosis</td>
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<tr>
<td>Portal fibrosis</td>
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<tr>
<td>Bridging fibrosis leading to Cirrhosis</td>
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<tr>
<td>Mallory—Denk bodies</td>
<td></td>
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<tr>
<td>Rope-like and sometimes</td>
<td></td>
</tr>
<tr>
<td>abundant in ASH</td>
<td></td>
</tr>
<tr>
<td>Thin, wispy and less</td>
<td></td>
</tr>
<tr>
<td>numerous in NASH</td>
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<tr>
<td>Megami tochondria</td>
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<tr>
<td>Glycogenated nuclei</td>
<td></td>
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<tr>
<td>Infrequent in ASH</td>
<td></td>
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<tr>
<td>Frequent in NASH</td>
<td></td>
</tr>
<tr>
<td>Iron (in hepatocytes and/or)</td>
<td></td>
</tr>
<tr>
<td>sinusoidal lining cells</td>
<td></td>
</tr>
<tr>
<td>Ductular reaction</td>
<td></td>
</tr>
</tbody>
</table>

### Steatosis

Steatosis in small amounts is a frequent finding in liver biopsies [16] and is the earliest pathological finding in ALD [17]. Steatosis involving more than 5% of hepatocytes is required for the diagnosis of NAFLD, but is not necessary for the diagnosis of ALD [4]. The accumulated intracytoplasmic fat in hepatocytes may be macrovesicular, microvesicular or mixed. The latter is commonly seen in both ALD and NAFLD. In macrovesicular steatosis, a single large fat droplet displaces the nucleus and cytoplasm to the periphery of affected hepatocytes. Mixed steatosis is a combination of micro- and macrovesicular steatosis, when both large- and small-droplet intracellular fat is present. In microvesicular steatosis, the affected hepatocytes have a foamy appearance due to the abundance of tiny fat droplets surrounding a centrally placed nucleus; special stains for lipids, such as oil red O, may be needed to identify the intracytoplasmic material as fat [5]. In ALD, microvesicular steatosis arising in zone 3, and occasionally extending into zone 2, is known as 'alcoholic foamy degeneration' and is an indication of more severe liver disease [1,18]. In NAFLD, small patches of microvesicular steatosis may be seen at non-zonal locations [4].

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Interestingly, steatosis may not persist during the progression of fatty liver disease and, for this reason, may not be reliably identified in cirrhotic specimens. In ALD-related cirrhosis, steatosis may be absent even when alcohol intake continues [1]. Clinical studies can associate 'cryptogenic' cirrhosis (no distinct morphological findings) with underlying clinical correlates of NAFLD [21,22], but the pathologist can only speculate as to the etiology.

### Lobular and portal inflammation

Lobular inflammation is usually mild and frequently consists of a mixed, acute and chronic, inflammatory cell infiltrate composed of neutrophils and mononuclear cells. The presence of large numbers of neutrophils favors an alcoholic etiology [23]. Satellitosis — where neutrophils surround ballooned hepatocytes containing Mallory—Denk bodies — can occur in steatohepatitis [1,18,23,24]. Lipogranulomas, comprising chronic inflammatory cells, Kupffer cells and rare eosinophils surrounding a steatotic hepatocyte or a large lipid droplet, may be found scattered in the acini or portal tracts. Microgranulomas, composed of clusters of Kupffer cells, and PAS—diastase-positive pigmented Kupffer cells on their own may also be seen, and represent prior necroinflammatory activity [1,5,24].

Mild chronic mononuclear inflammation may be observed in the acini and portal tracts during both the active and resolving phases of steatohepatitis [25]. However, the presence of marked mononuclear cell infiltration and/or disproportionate portal inflammation in relation to the acini should raise the possibility of a superimposed chronic liver disease such as chronic viral hepatitis C [25—27]. Nevertheless, a predominantly portal lymphocytic infiltrate can occur in ALD in the absence of serological markers of chronic viral infection, and has been shown to correlate with fibrosis [28]. It is suggested that a predominantly lymphocytic inflammatory infiltrate in ALD is a reflection of the autoimmune component of the underlying liver disease [29].

Portal inflammation in NAFLD is usually lymphocytic whereas, in ALD, polymorphs may be seen in portal and periportal areas as well [18,30]. In untreated patients with NAFLD, increased portal inflammation has been correlated with the diagnosis of definite steatohepatitis and...
with advanced fibrosis, indicating that it may be a marker of advanced disease [31]. In treated NASH, portal chronic inflammation is a feature that is indicative of disease resolution [25].

Hepatocellular injury

Hepatocellular injury in fatty liver disease usually presents as cellular ballooning, but it can also be demonstrated by apoptotic (acidophilic) bodies and lytic necrosis.

Balloonated hepatocytes are enlarged and have a rarefied, edematous cytoplasm. They are seen among steatotic hepatocytes predominantly in acinar zone 3 and usually in areas of perisinusoidal fibrosis (Fig. 1) [18,24]. Balloonated hepatocytes are not exclusive to fatty liver disease, but can also be seen in other liver diseases such as viral hepatitis and chronic cholestatic disorders [32]. In ALD, they have been considered the result of microtubular disruption and cytoskeletal disturbances that arise in severe cell injury leading to lytic necrosis [16]. The large cell volume of balloonated hepatocytes may be the result of increased fluid in the cytosol [1]. However, in NAFLD, recent ultrastructural evidence suggests that some balloonated hepatocytes contain small fat droplets and may represent an adaptation rather than a degenerative phenomenon [33]. The assessment of hepatocellular ballooning shows significant interobserver variation in both ALD [1] and NAFLD [34]. Loss of keratin 8/18 (K8/18) immunoreactivity has recently been proposed as an objective marker in the histological identification of balloonated hepatocytes in ASH and NASH [35].

Apoptotic hepatocytes are seen in steatohepatitis and their numbers correlate with disease activity [36—38]. Also, recent studies have shown that markers for apoptosis are more common in NASH than in ASH [39] and, in NAFLD, serum levels of apoptotic markers, such as K18 fragments, have been shown to predict disease severity [40].

Confluent or bridging necrosis, although uncommon, can occur in fatty liver disease and is more frequently described in ASH [5]. Rare cases of severe steatohepatitis with extensive necrosis associated with liver failure have been reported in obese patients undergoing gastric bypass surgery [41].

Fibrosis

The characteristic pattern of fibrosis in non-cirrhotic steatohepatitis is pericellular/perisinusoidal and is the result of collagen deposition in the space of Disse. The appearance has been described as a ‘chickenwire’ pattern, as the collagen fibers resemble the latticework seen in wire fencing. This type of fibrosis is initially observed in zone 3 of the acinus (Fig. 2) and is usually associated with active steatohepatitic lesions [4,16,41,42]. In the absence of active lesions, this finding may indicate prior episodes of steatohepatitis. In ASH, there is frequently a distinctive perivenular fibrosis with thickening of the hepatic vein walls; more severe cases may present with venous occlusion [43].

With progression of the disease, portal fibrosis may be evident. Portal-based fibrosis in association with zone 3 fibrosis is more common in ASH than in NASH. Bridging fibrosis with central–central, central–portal and portal–portal fibrous septa may follow, leading to cirrhosis. Micronodular cirrhosis is especially common in ALD [1,44]. At the cirrhotic stage, perisinusoidal fibrosis and active lesions of steatohepatitis may be absent [4].

In ASH and NASH, as in other chronic liver diseases, sinusoidal collagen formation is the result of hepatic stellate cell activation [45,46]. Such activation in NASH has been correlated with the degree of both steatosis and fibrosis [45]. Recently, the hepatic stellate cell activation score, as measured by alpha-smooth muscle actin immunohistochemistry, has been shown to predict progression to fibrosis in patients with NAFLD [47].

Other histological lesions in non-cirrhotic fatty liver disease

Mallory—Denk bodies (Mallory’s hyaline)

Mallory—Denk bodies are eosinophilic intracytoplasmic inclusions that are commonly found near or surrounding the nucleus of balloonated hepatocytes in steatohepatitis of alcoholic, metabolic or drug (amiodarone, perhexiline maleate) etiology, copper storage diseases, glycogenosis, chronic cholestatic diseases, proliferative lesions such as focal nodular hyperplasia, and benign and malignant hepatocellular neoplasms [48,49]. In ASH, Mallory—Denk bodies may also be observed in shrunken, acidophilic hepatocytes, and hepatocellular death may cause their transfer to the extracellular space [23]. In ASH and NASH, hepatocytes with Mallory—Denk bodies are detected in acinar zone 3 and are more common in areas of perisinusoidal fibrosis. In ASH, Mallory—Denk bodies are dense and rope-like, and may be found in abundance whereas, in NASH, they are thin, wispy and less frequently seen, and may not be easily identified with hematoxylin-and-eosin (H&E) staining [4]. In NASH, Mallory—Denk bodies are related to more necroinflammatory activity and more severe disease [19]. Mallory—Denk bodies, although helpful for the diagnosis of steatohepatitis when present, are not a required histological feature.

Mallory—Denk bodies comprise misfolded ubiquitin-tagged keratin 8/18 intermediate filaments, the stress-induced and ubiquitin-binding protein p62, heat-shock proteins 70 and

Figure 2 Pericellular/perisinusoidal fibrosis in zone 3. THV: terminal hepatic vein (Masson trichrome stain, × 200).
Megamitochondria

Megamitochondria (giant mitochondria) are round or cylindrical intracellular eosiophilic structures with ultrastructural abnormalities such as intramitochondrial paracrystalline inclusions, loss of cristae and multilamellar membranes [52]. The presence of megamitochondria in hepatocytes is usually associated with chronic alcohol abuse [1], but they are also observed in NASH [53,54], acute fatty liver of pregnancy and Wilson’s disease. In ALD, megamitochondria are more frequent in milder cases but, when associated with mixed steatosis in the absence of inflammation, they can be considered markers of progression to fibrosis [55]. In NAFLD, the presence of megamitochondria is not related to histological severity and their significance is not clear. In NASH, hepatocytes containing megamitochondria show a non-zonal distribution [56], indicating that mitochondrial structural abnormalities represent a generalized — and most probably adaptive — response to oxidative stress rather than being a secondary result of cell injury [57].

Iron deposition

In ALD, mildly increased iron stores are common. Iron deposition (grade 1+ or 2+) is detected in hepatocytes and Kupffer cells with a non-zonal distribution [1,23]. When higher grades of siderosis (3+, 4+) with a predominantly periportal hepatocellular distribution are observed in non-cirrhotic ALD, the possibility of hereditary hemochromatosis should be excluded. In ALD, stainable hepatic iron is positively correlated with fibrosis [58].

In NAFLD, iron deposition — when present — is mild (1+ or 2+), and is usually observed in periportal/periseptal hepatocytes and/or reticuloendothelial cells (sinusoidal lining cells, portal macrophages and endothelium of larger vessels) [4,59]. Studies of the relationship of abnormal iron indices, iron genetics and iron tissue deposition with the development of liver fibrosis and pathogenesis of NAFLD have shown conflicting results [4,60,61].

Glycogenated nuclei

Glycogenated nuclei are hepatocellular nuclei characterized by vacuolation. They are a frequent finding in NAFLD, and are most commonly seen in periportal areas, but also in clusters with a non-zonal distribution [62]. Glycogenated nuclei are of uncertain significance beyond a possible association with diabetes and obesity [42,63]. They are more often observed in NASH than in ASH [18,30,63].

Ductular reaction

The appearance of hyperplastic ductular structures at the portal tract interface arising from hepatic progenitor cells is known as a ‘ductular reaction’. The proliferating ductules, which may be highlighted with immunohistochemical stains for keratin 7/19, are often accompanied by neutrophils and stromal changes [64].

In ALD, a ductular reaction may be observed in the later stages usually as a secondary cholestatic phenomenon [1]. However, the possibility that it may be the result of an impaired regenerative activity of hepatocytes due to the depressive effect of alcohol cannot be excluded [65,66]. In ALD with bridging fibrosis, newly formed ductular structures can be seen along and within the fibrous septa [23].

In NAFLD, a mild ductular reaction is commonly seen in fibrosed portal tracts, but it may become more extensive in late-stage disease [67]. As in ALD, a ductular reaction in NAFLD most likely reflects a steatosis-induced impairment of hepatocellular regeneration [65,68]. In NASH, a ductular reaction can also be seen extending into the acini and correlates with advanced stages of fibrosis [69]. As a ductular reaction stimulates fibrogenesis, it may provide a pathway for progressive fibrosis in both ALD and NAFLD [70].

Adaptive hepatocellular changes

In advanced ALD, the cytoplasm of some hepatocytes may have a ‘ground-glass’ appearance due to smooth endoplasmic reticulum proliferation, or it may be deeply eosinophilic — the so-called ‘oxyphilic’ or ‘oncocytic’ change — due to increased numbers of mitochondria. These changes are adaptive and, although in the past they were considered indicative of continued drinking, it is now believed that they may also be observed after prolonged periods of abstinence [1]. In NAFLD, adaptive changes in hepatocytes have so far not been recorded.

Histological features representing resolution of steatohepatitis

One of the goals of modern therapeutic trials in NASH is resolution of the disease at the tissue level. One interesting observation from treatment trials is that spontaneous regression of steatosis may occur in some cases of NASH [71].

Treatment trials using different interventions, including the PPAR-gamma agonists, metformin, vitamins E and C, dietary regimens and weight-reduction surgery, have shown various degrees of improvement of steatosis, lobular inflammation, ballooning, steatohepatitis and, in some cases, fibrosis in post-treatment biopsies [72]. Noteworthy features related to resolution were a shift towards increased portal inflammation, and a change in the quality of zone 3 periportal fibrosis from dense to delicate in some cases [21].

In ALD, the treatment involves mainly abstinence from alcohol [6]. In simple steatosis, fat may completely disappear from the liver within four weeks of stopping alcohol consumption [1]. In steatohepatitis, however, other characteristic lesions — including lobular inflammation, ballooning and Mallory–Denk bodies — may persist even six months after stopping alcohol, albeit with decreased severity [73]. This means that the presence of such features in a liver biopsy should not always be interpreted as continued alcohol intake. It is worth noting, though, that abstinence is related to increased portal lymphocytic infiltration [73], a feature
that is now emerging as a resolution phenomenon in treated NASH, as discussed above.

**Pediatric fatty liver disease**

Pediatric NAFLD/NASH is usually characterized by portal-based chronic inflammation and fibrosis, more extensive steatosis, infrequent occurrence of ballooned hepatocytes and Mallory—Denk bodies, absence of lobular inflammation and absence of an acinar zone 3 predominance of involvement on histology. The portal (zone 1) accentuated pattern is identified as ‘type 2’ pediatric NASH, while a less commonly observed pattern resembles that of adult NAFLD and is referred to as ‘type 1’ [74,75]. Type 2 NASH is more frequently seen in the liver biopsies of male patients who are younger and of Asian, Hispanic or Native American descent [74]. Recent studies have revealed that the majority of pediatric NAFLD/NASH liver biopsies show characteristics of both histological types [76,77]. The possibility that alcohol may have been implicated in the pathogenesis of liver lesions in some cases of teenage NAFLD cannot be excluded with certainty.

Portal-based fibrosis characterizes the initial stage of fibrosis in most cases of pediatric NAFLD, while zone 3 perisinusoidal fibrosis is a less frequent finding [74,77], and progressive fibrosis leading to cirrhosis rarely occurs in children with NASH [78,79]. Recently, the use of the enhanced liver fibrosis (ELF) test (an algorithm based on serum levels of hyaluronic acid, aminoterminal propeptide of type III collagen and tissue inhibitor of metalloproteinase-1) in pediatric NAFLD patients has shown high sensitivity and high specificity in the non-invasive assessment of liver fibrosis [80].

**Differential diagnosis of alcoholic and non-alcoholic steatohepatitis**

Clinicopathological correlation is of the utmost importance in helping to define the exact etiology of steatohepatitis from a liver biopsy, as the histology of NASH is similar to that of ASH (Table 1) [5]. Indeed, steatohepatitis can occur as a result of a number of conditions, including drug toxicity (for example, tamoxifen, glucocorticoids and amiodarone), jejunoileal bypass, total parenteral nutrition, metabolic diseases (Wilson’s disease, tyrosinemia), lipodystrophy and malnutrition [4].

In general, it is accepted that the overall histopathological appearances of NASH are usually milder than those observed in ASH [1]. In Table 1, the histological features observed in non-cirrhotic ALD that have not yet been described in NAFLD are summarized. These distinct histological lesions include:

- alcoholic foamy degeneration;
- acute cholestasis [1,23].

In ASH, the Mallory—Denk bodies are usually well formed and may be seen in abundance whereas, in NASH, they are thin, wispy and not easily identified in H&E-stained sections. It is believed that, when Mallory—Denk bodies are numerous, the disease process is more likely to be of alcoholic than metabolic origin [16,23]. Qualitative differences in fibrosis related to the etiology of steatohepatitis have also been identified. Lattice fibrosis, mostly composed of type I collagen, is more common in NASH than in ASH. In the latter, a solid fibrosis composed mostly of type III collagen is observed [81].

Although extensive steatosis, periporal glycogenated nuclei and lipogranulomas are more commonly seen in NAFLD than in ALD, the usefulness of these features in the differential diagnosis of fatty liver disease is limited [1,4].

Immunohistochemistry for insulin receptor and protein tyrosine phosphatase 1B (PTP1B), a protein that acts as a negative regulator of insulin-receptor expression, can help in the differential diagnosis between ASH and NASH. Decreased insulin receptor and increased PTP1B expression has been proposed as characteristic of NASH, in contrast to the normal insulin receptor and low-level PTP1B expression usually seen in ASH [82]. A recent non-invasive clinical model of the differential diagnosis between ALD and NAFLD incorporates the mean corpuscular volume, aspartate aminotransferase/alanine aminotransferase ratio, body mass index and gender. An ALD/NAFLD index (ANI) greater than 0 favors ALD, while an ANI less than 0 is diagnostic of NAFLD [83].

**Grading and staging of fatty liver disease**

Histological scoring systems for grading necroinflammatory activity and staging fibrosis in NAFLD are mainly used in treatment trials, to assess the efficacy of therapeutic interventions, and in natural history studies.

Currently, the most widely used histological scoring system intended for use in both adult and pediatric NAFLD was that published in 2005 by the NASH Clinical Research Network, sponsored by the National Institute of Diabetes, Digestive and Kidney Diseases [20]. This additive scoring system for disease activity is based on the major histological lesions of NAFLD. The NAFLD activity score (NAS) is the summation of the individual scores for steatosis, lobular inflammation and hepatocellular ballooning, and ranges from 0 to 8 (Table 2) [20]. An NAS of 1 or 2 indicates ‘definitely not NASH’ cases, while an NAS score of 5 to 8 correlates with a diagnosis of ‘definite NASH’. NAS scores of 3 and 4 are borderline cases that fulfill some, but not all, of the criteria for definite NASH [20]. An alternative system for grading necroinflammatory activity in NASH, still popular among pathologists, was published by Brunt et al. in 1999 [19]. This semiquantitative method of deriving a global activity grade is based on steatosis, lobular and portal inflammation, and hepatocellular ballooning. NASH is graded as ‘mild’, ‘moderate’ or ‘severe’ based on differences in inflammation and ballooning (Table 3) [19].
Table 2. NASH Clinical Research Network system for scoring activity and staging fibrosis in NAFLD\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Steatosis grade\textsuperscript{b} (S)</th>
<th>Lobular inflammation (L)</th>
<th>Hepatocellular ballooning (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0: &lt; 5%</td>
<td>0: none</td>
<td>0: none</td>
</tr>
<tr>
<td>1: 5—33%</td>
<td>1: &lt; 2 foci/20 × o.f.</td>
<td>1: mild, few ballooned cells</td>
</tr>
<tr>
<td>2: 34—66%</td>
<td>2: 2—4 foci/20 × o.f.</td>
<td>2: moderate-to-marked, many ballooned cells</td>
</tr>
<tr>
<td>3: &gt; 66%</td>
<td>3: &gt; 4 foci/20 × o.f.</td>
<td></td>
</tr>
</tbody>
</table>

NAFLD Activity Score (NAS): S + L + B, range 0—8

Stage of fibrosis

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>1a, mild (delicate) zone 3 perisinusoidal fibrosis</td>
</tr>
<tr>
<td>1b</td>
<td>moderate (dense) zone 3 perisinusoidal fibrosis</td>
</tr>
<tr>
<td>1c</td>
<td>portal/periportal fibrosis only</td>
</tr>
<tr>
<td>2</td>
<td>Zone 3 perisinusoidal fibrosis with portal/periportal fibrosis</td>
</tr>
<tr>
<td>3</td>
<td>Bridging fibrosis</td>
</tr>
<tr>
<td>4</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

o.f.: optical field.

\textsuperscript{a} Adapted from Kleiner et al. [20].

\textsuperscript{b} Percentage of liver parenchyma involved by steatosis.

The NASH Clinical Research Network scoring system uses a five-tier (0—4) staging method for fibrosis based on the prototype Brunt et al. staging system [19], which recognized four stages of fibrosis in NASH, starting from zone 3 perisinusoidal fibrosis (stage 1) and ending in cirrhosis (stage 4; Table 3). In this system, stage 1 is further subdivided into delicate perisinusoidal zone 3 fibrosis (stage 1a), dense perisinusoidal zone 3 fibrosis (stage 1b) and portal fibrosis only (stage 1c; Table 2) [20]. The latter refers to a pattern of fibrosis sometimes seen in severely obese patients and in pediatric NASH. The system can also be applied to the entire histological spectrum of NAFLD, and can even be used to score pediatric NAFLD biopsies [20]. It is mainly used for treatment trials, and validation studies for its application in routine settings are necessary.

Reproducibility studies assessing interobserver variability in adult NAFLD have shown excellent-to-good agreement in the extent of steatosis, presence of perisinusoidal fibrosis and stage of fibrosis. The feature with the least agreement is lobular inflammation [20,84,85]. In pediatric NAFLD, interobserver concordance is less than that seen in adult cases for all features except steatosis [20], which probably reflects the different histological patterns identified in pediatric NAFLD. Intraobserver agreement is generally better than interobserver agreement for all histological features and diagnostic categories.

Sampling variability is an important issue in NAFLD — as it is in other chronic liver diseases — and has to be considered in the design of treatment trials and natural history studies [86]. Paired biopsies from the same lobe [87,88] or from different lobes [89—91] have shown high rates of concordance in the extent of steatosis and diagnosis of definite NASH, and excellent-to-moderate agreement in NAFLD activity scores. However, agreement was only fair in necroinflammation and moderate in fibrosis, indicating that, in NAFLD, these lesions are irregularly distributed throughout the liver. A recent study has shown that the size (length) of the liver biopsy correlates with the percentage of patients with definite NASH,

Table 3. Grading activity and staging fibrosis in NASH\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Steatosis\textsuperscript{b}</th>
<th>Ballooning\textsuperscript{c}</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 (mild)</td>
<td>1—2</td>
<td>Minimal</td>
<td>L = 1—2, P = 0—1</td>
</tr>
<tr>
<td>Grade 2 (moderate)</td>
<td>2—3</td>
<td>Present</td>
<td>L = 1—2, P = 1—2</td>
</tr>
<tr>
<td>Grade 3 (severe)</td>
<td>2—3</td>
<td>Marked</td>
<td>L = 3, P = 1—2</td>
</tr>
</tbody>
</table>

Stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>Zone 3 perisinusoidal fibrosis</th>
<th>Periportal fibrosis</th>
<th>Bridging fibrosis</th>
<th>Cirrhosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Focal or extensive</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Focal or extensive</td>
<td>Focal or extensive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>+/—</td>
<td>+/—</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>+/—</td>
<td>+/—</td>
<td>Extensive</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Adapted from Brunt et al. [19].

\textsuperscript{b} 1 = < 33%; 2 = 33—66%; 3 = > 66% of liver parenchyma affected by steatosis.

\textsuperscript{c} Zonal location recorded.

L: lobular inflammation: 0 = absent; 1 = < 2 foci/20 × optical field (o.f.); 2 = 2—4 foci/20 × o.f., 3 = > 4 foci/20 × o.f.

P: portal inflammation: 0 = absent; 1 = mild; 2 = moderate; 3 = marked.
suggesting that, in NALFD, larger biopsies might increase the diagnostic histological yield [92].

In ALD, the use of grading and staging systems is limited, and no formal semiquantitative scoring system has been accepted by consensus and validated [23]. It is assumed, based on the histopathological similarities between ALD and NALFD, that one of the widely used systems for the assessment of severity in NALFD could be easily adapted for use in ALD [23]. Yip and Burt have recently proposed a detailed semiquantitative scoring system for the assessment of disease severity in ALD [1]. However, reproducibility studies and validation of emerging scoring systems in ALD are, as yet, still lacking.

References

[23] Liver biopsy in ASH and NASH 937
Liver biopsy in ASH and NASH


