REVIEW

Histological assessment of non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is an important complication of the metabolic syndrome, which is becoming an increasingly common cause of chronic liver disease. Histological changes typically mainly affect perivenular regions of the liver parenchyma and include an overlapping spectrum of steatosis, steatohepatitis and perisinusoidal or pericellular fibrosis, in some cases leading to cirrhosis. Once cirrhosis has developed, typical hepatocellular changes are often no longer conspicuous, leading to such cases being mistakenly diagnosed as ‘cryptogenic’. Portal inflammation, ductular reaction and periporal fibrosis can also be seen as part of the morphological spectrum of NAFLD, particularly in the paediatric population.

Keywords: cirrhosis, fatty liver, grading and staging, insulin resistance, liver biopsy, metabolic syndrome, non-alcoholic, steatohepatitis, steatosis

Abbreviations: ALD, alcoholic liver disease; ASH, alcoholic steatohepatitis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IR, insulin receptor; LFT, liver function test; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ROS, reactive oxygen species; TNF, tumour necrosis factor

Introduction

Non-alcoholic fatty liver disease (NAFLD) comprises a morphological spectrum of liver lesions, closely resembling those seen in alcoholic liver disease (ALD), but developing in individuals who do not consume excessive amounts of alcohol. The great majority of NAFLD occurs in the setting of the metabolic syndrome in which insulin resistance plays a key role (primary NAFLD).1 Other much less common causes of NAFLD are summarized in Table 1.2,3

The metabolic syndrome is an increasingly common metabolic disorder related to the increasing worldwide prevalence of obesity.4,5 Diagnostic criteria vary, but important components are central or visceral obesity, glucose intolerance (Type 2 diabetes), systemic hypertension and dyslipidaemia (hypertriglyceridaemia and low high-density lipoprotein-cholesterol). Prevalence rates of the metabolic syndrome are as high as 50% in some western countries. Most of the adverse effects of the metabolic syndrome are associated with complications related to diabetes and cardiovascular disease. However, there is an increasing recognition that NAFLD is also a common component of the metabolic syndrome, with estimated prevalence rates in the region of 10–30% in countries where the metabolic syndrome is common.3,6–10

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Table 1. Classification and aetiology of fatty liver disease (from Reid 2001 and Ramesh 2005)²,³

<table>
<thead>
<tr>
<th>Classification</th>
<th>Aetiology</th>
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<tr>
<td>Alcoholic</td>
<td>Drug-induced fatty liver disease</td>
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<tr>
<td>Non-alcoholic</td>
<td>Primary (insulin resistance)</td>
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<tr>
<td></td>
<td>Obesity, diabetes, hyperlipidaemia</td>
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<td>Secondary (other causes)</td>
<td>Drugs</td>
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<td></td>
<td>Amiodarone</td>
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<td>Glucocorticoids</td>
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<td></td>
<td>Perhexiline maleate</td>
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<td>Synthetic oestrogens</td>
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<td></td>
<td>Tamoxifen</td>
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<td></td>
<td>4,4'-diethylaminoethoxyhexestrol</td>
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<tr>
<td></td>
<td>Isoniazid</td>
</tr>
<tr>
<td></td>
<td>Industrial exposure to petrochemicals</td>
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<tr>
<td>Surgical procedures</td>
<td>Jejunoileal bypass</td>
</tr>
<tr>
<td></td>
<td>Biliopancreatic diversion</td>
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<tr>
<td></td>
<td>Extensive small bowel resection</td>
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<tr>
<td></td>
<td>Gastroplasty for morbid obesity</td>
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<tr>
<td>Total parenteral nutrition</td>
<td></td>
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<tr>
<td>Inborn errors of metabolism</td>
<td></td>
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<td></td>
<td>Wilson disease</td>
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<td></td>
<td>Abetalipoproteinaemia</td>
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<td></td>
<td>Tyrosinaemia</td>
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<tr>
<td></td>
<td>Hypobetalipoproteinaemia</td>
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<tr>
<td>Lipodystrophy</td>
<td>Congenital</td>
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<tr>
<td></td>
<td>Acquired (HIV-related)</td>
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This article will review the histological changes occurring in NAFLD, highlighting areas in which liver biopsy is most important in the diagnosis and management of patients with this disease. Brief consideration will also be given to clinical aspects of NAFLD and some of the proposed pathogenic mechanisms.

Clinical impact of non-alcoholic fatty liver disease

Although a non-alcoholic form of fatty liver disease was first recognized some 25 years ago,¹¹ the clinical significance of NAFLD has started to be properly recognized only during the last few years. Several studies have shown that NAFLD is the commonest histological diagnosis in patients with otherwise unexplained persistently abnormal liver function tests (LFTs), being present in 40–70% of such cases.¹²–¹⁵ Perhaps of greater concern is the recognition that the full histological spectrum of fatty liver disease can also be seen in up to 70% of people with the metabolic syndrome who have normal LFTs.¹⁶,¹⁷ Epidemiological studies have suggested that NAFLD is the most important cause of so-called ‘cryptogenic’ cirrhosis¹⁸,¹⁹ and it has also been implicated in the pathogenesis of hepatocellular carcinoma.²⁰–²⁴ Based on these observations, it has been suggested that, in countries where risk factors for the metabolic syndrome are prevalent, NAFLD is likely to become the commonest cause of chronic liver disease.⁸

Pathogenic mechanisms

The presence and severity of NAFLD are closely related to risk factors for insulin resistance and the metabolic syndrome.⁶,²⁵,²⁶ The relationship between insulin resistance and NAFLD is complex and the pathogenic mechanisms involved in mediating liver damage in this setting are still not fully understood.¹–³,⁶,¹⁰,²⁷–³³ A ‘two hit’ process has been proposed. The first ‘hit’ involves accumulation of fat in hepatocytes. This is closely associated with metabolic derangements related to central obesity and insulin resistance. An increased delivery of free fatty acids to the liver is combined with impaired fatty acid metabolism in hepatocytes, leading to a net accumulation of triglyceride within the liver. It has also been recognized more recently that the accumulation of fat in the liver exacerbates insulin resistance by interfering with phosphorylation of insulin receptor substrates.³⁴ Increased hepatocellular expression of the microsomal cytochrome P450 2E1 (CYP2E1) may be important in mediating this process,³⁵ which leads to a potential vicious cycle where the metabolic syndrome causes fatty change in the liver and vice versa.⁶

The factors involved in determining the progression from steatosis to the more serious lesions of steatohepatitis and fibrosis (discussed further below) are more complex and less clearly established. Oxidative stress is
thought to play a key role in the second ‘hit’, which also involves peroxidation of lipid accumulated within steatotic hepatocytes. Factors promoting lipid peroxidation and oxidative stress include induction of hepatic CYP2E1 and mitochondrial dysfunction leading to the formation of reactive oxygen species (ROS). Immunohistochemical techniques have demonstrated increased staining for lipid oxidation products in livers with fatty change, with further increases in those showing steatohepatitis. Immune responses to lipid peroxidation products may also be involved in disease progression. Other changes occurring in the metabolic syndrome that could mediate hepatic inflammation include increased levels of proinflammatory cytokines such as tumour necrosis factor (TNF)-α, which may be released directly from adipocytes in visceral fat, and decreased levels of anti-inflammatory cytokines such as adiponectin, also produced by adipocytes. These observations suggest that the metabolic syndrome itself may be involved in mediating the second as well as the first ‘hit’ in NAFLD. Adiponectin is also produced within the liver, mainly by endothelial cells, whilst its receptors are expressed on hepatocytes. A significantly reduced intrahepatic expression of adiponectin and its receptors has been observed in biopsies showing non-alcoholic steatohepatitis (NASH) compared with simple steatosis.

Interactions between lipid-laden hepatocytes, Kupffer cells, inflammatory cells and hepatic stellate cells are important in the subsequent development of fibrosis and cirrhosis. Factors regulating this process include activation of profibrogenic cytokines, such as interleukin-10 and transforming growth factor-β, which in turn are regulated by other factors including leptin and neurotransmitters such as noradrenaline. Lipid released from damaged hepatocytes may also result in mechanical and/or inflammatory cell-mediated occlusion of hepatic venules, leading to parenchymal collapse and fibrosis.

**Liver lesions in non-alcoholic fatty liver disease**

Similar to the changes seen in ALD, the histological changes occurring in NAFLD typically predominantly affect the liver parenchyma, where they are mainly present in perivenular regions (acinar zone 3). However, there is an increasing recognition that portal and periportal lesions can also be seen as part of the spectrum of fatty liver disease.

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**Figure 1.** Histological spectrum and estimated prevalence of liver lesions in non-alcoholic fatty liver disease (NAFLD). The majority of people with risk factors for NAFLD will develop fatty change, which is reversible. A smaller proportion progress to more severe liver disease, which is less readily reversible.

**Parenchymal lesions in non-alcoholic fatty liver disease**

These can be divided into three main categories: non-alcoholic fatty liver (NAFL), NASH and cirrhosis. As with ALD, areas of overlap exist between these three main patterns of liver injury and they are probably best regarded as different parts of a broad histological spectrum. Histological changes are often reversible, particularly during the early stages of the disease. However, with progression to fibrosis and cirrhosis, the potential for reversibility diminishes.

Fatty change is predominantly macrovesicular in type. The severity of fatty change is generally determined by estimating the proportion of hepatocytes containing fat droplets: < $1/3$ = mild, $1/3$– $2/3$ = moderate, > $2/3$ = severe. Two studies have suggested lower thresholds for grading the severity of steatosis (< $10\%$ = mild, $10$–$30\%$ = moderate, > $30\%$ = severe), although these were both related to steatosis occurring in the setting of chronic hepatitis C virus (HCV) infection. It has been suggested that very mild degrees of steatosis, involving < $5\%$ of hepatocytes, may not actually represent a true pathological abnormality. However, in the absence of definite evidence to the contrary, it is still appropriate to document any degree of steatosis present.
in the liver biopsy report. Although fatty change tends mainly to involve perivenular regions, in severe cases it can extend to a panacinar distribution.

Features of steatohepatitis include hepatocellular injury (beyond simple fatty change), inflammation and fibrosis (Table 2) (Figure 3). These changes also predominantly involve acinar zone 3. Hepatocyte ballooning is the most characteristic feature of steatohepatitis and is typically associated with formation of Mallory’s hyaline. Mallory bodies in NAFLD are often small and poorly formed and may be difficult to detect in routinely stained sections.\textsuperscript{45} Immunohistochemical techniques can be used to demonstrate antigens associated with Mallory’s hyaline. These include ubiquitin, p62 and cytokeratins 8 and 18\textsuperscript{31,46,47} (Figure 4). Death of hepatocytes may occur by apoptosis or necrosis, probably mainly the former.\textsuperscript{48–50}

Table 2. Typical lobular changes occurring in steatohepatitis

<table>
<thead>
<tr>
<th>Hepatocellular injury</th>
<th>Ballooning</th>
<th>Apoptosis/necrosis</th>
<th>Mallory’s hyaline</th>
<th>Giant mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>Neutrophil polymorphs</td>
<td>Other cells (e.g. T lymphocytes, macrophages)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Perisinusoidal</td>
<td>Pericellular</td>
<td></td>
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</tbody>
</table>

although apoptotic bodies are not usually conspicuous histologically. Fatty liver disease is associated with increased hepatocellular expression of the membrane receptor Fas, rendering these cells more susceptible to apoptosis by Fas-ligand-expressing inflammatory cells.\textsuperscript{51} The severity of steatosis appears to be important in determining the extent of apoptosis.\textsuperscript{52} Furthermore, formation of ROS is associated with Fas-ligand expression on hepatocytes, thus producing a pathway for
fratricidal apoptosis. Megamitochondria, which are more typically present in ALD, have also been recognized in non-alcoholic steatohepatitis. One study showed that mitochondrial defects in the form of loss of cristae and paracrystalline inclusions were present in patients with NASH but not in those with fatty change alone, supporting the suggestion that mitochondrial abnormalities play an important role in the pathogenesis of progressive liver injury in NAFLD. Parenchymal inflammation is typically mild and comprises a mixed population including neutrophils, lymphocytes (mainly CD3+ T cells) and macrophages/Kupffer cells. Neutrophils typically predominate (Figure 3). Natural killer cells may also be involved.

The parenchymal fibrosis that occurs in fatty liver disease typically has a perisinusoidal and/or pericellular distribution (Figure 5) and, in a proportion of cases, eventually progresses to cirrhosis. This initially has a micronodular pattern, but larger nodules may subsequently evolve. Typical features of steatohepatitis often become less conspicuous once cirrhosis has developed and may disappear altogether. This is the reason why many cases of NAFLD-related cirrhosis were previously classified as ‘cryptogenic’. Vascular changes occurring in cirrhosis have been postulated as possible reasons for this phenomenon—these include portosystemic shunting, resulting in a lower exposure of hepatocytes to insulin, and sinusoidal capillarization, which may impair the trans-sinusoidal passage of lipoproteins carried to the liver in the portal circulation.

Hepatocellular carcinoma (HCC) is recognized to occur as a complication of cirrhosis related to NAFLD, although the relative risk of HCC in NASH-associated cirrhosis compared with other forms of chronic liver disease is uncertain. HCC has also been noted as a rare complication of precirrhotic NAFLD. In addition to the carcinogenic factors associated with cirrhosis in general, there may also be risk factors specifically associated with fatty liver disease. Amongst patients with ‘cryptogenic’ (presumed NAFLD-related) cirrhosis, the presence of obesity and diabetes is significantly associated with the development of HCC. In another population-based case–control study, diabetes was found to be associated with an increased risk of HCC, regardless of the presence of other major HCC risk factors. The insulin resistance syndrome is emerging as a risk factor for a wide variety of other cancers, including colon, breast and endometrium. Hepatic steatosis is associated with replicative senescence and apoptosis of hepatocytes, stimuli for progenitor cell proliferation, which has been identified as a potentially important step in hepatocellular carcinogenesis. Furthermore, the ROS and lipid peroxidation products that are formed with progression from steatosis to steatohepatitis cause DNA damage and gene mutations.

PORTAL/PERIPORTAL LESIONS IN NON-ALCOHOLIC FATTY LIVER DISEASE

These include inflammatory changes similar to those seen in chronic hepatitis and biliary features resembling those occurring in low-grade biliary obstruction (Figure 6). Both of these patterns of damage provide a mechanism for the development of progressive periportal fibrosis (Figure 7).

Chronic hepatitis-like changes

Minor degrees of portal inflammation associated with interface hepatitis and periportal fibrosis are commonly present and can produce changes resembling those seen in various forms of chronic hepatitis. They usually occur in combination with typical parenchymal features of steatohepatitis, but can sometimes occur as an isolated phenomenon (isolated portal fibrosis). The latter particularly applies to fatty liver disease occurring in the paediatric population. Portal inflammation and fibrosis may become more marked during the later stages of NASH. In some instances these changes can be attributed to another concurrent disease. This possibility should be considered particularly if the severity of portal inflammation is disproportionate to the more typical lobular features of steatohepatitis or if there are atypical features such as lymphoid follicles (suggestive of hepatitis C) or a prominent plasma cell component (suggestive of autoimmune hepatitis). The relationship between

Figure 5. Pericellular fibrosis in non-alcoholic steatohepatitis. Delicate strands of collagen surround individual hepatocytes in acinar zone 3. (Haematoxylin van Gieson.)
hepatitis C and NAFLD will be considered further later. Autoantibodies have been found in up to 50% of individuals with NAFLD, although their pathogenic significance in this setting is uncertain. Autoantibodies are also commonly present in low titre in other chronic liver diseases, where they are considered to be a non-specific response to hepatocellular injury. One study has shown that NAFLD patients who were autoantibody positive had significantly higher inflammatory grades and more advanced fibrosis than those who were autoantibody negative, suggesting that host immune mechanisms may interact with other factors causing liver injury in NAFLD. However, another study failed to identify any significant clinical or histological differences in NASH patients who had autoantibodies compared with those in whom they were absent.

Biliary features
Ductular reaction is commonly seen and may produce a picture resembling that seen in various types of biliary tract disease. It is usually present to a minor degree, but may become more severe, particularly in late-stage disease. The ductular reaction which occurs in NAFLD is thought to be related to a progenitor cell response occurring as a consequence of steatosis-induced impaired hepatocellular replication. The severity of steatosis in the setting of HCV-associated NAFLD has been shown to correlate with the extent of ductular reaction and this in turn provides a mechanism for the development of progressive periportal fibrosis. A cholestatic variant of NAFLD with bile duct inflammation and duct loss has recently been described. However, bile duct damage and duct loss are not typical features of NAFLD and the presence of these additional findings should raise the possibility of another cause of chronic biliary disease such as primary biliary cirrhosis or sclerosing cholangitis. Likewise, the presence of unusually prominent ductular reaction or abundant periportal deposits of copper-associated protein should also prompt a search for an additional biliary tract pathology.

Isolated portal fibrosis
The term ‘isolated portal fibrosis’ has been used to describe cases of fatty liver disease developing fibrous portal expansion without typical lobular features of steatohepatitis. In the adult population, this pattern of damage is particularly seen amongst patients with morbid obesity, where it occurs in 18–33% of cases. Fatty change is typically mild or moderate in severity and inflammation is also predominantly portal in distribution. Portal fibrosis may persist following treatment, despite improvement in other histological features, suggesting that different pathogenic mechanisms are involved in mediating portal changes in fatty liver disease.

OTHER HISTOLOGICAL FINDINGS IN NON-ALCOHOLIC FATTY LIVER DISEASE

Minor degrees of siderosis are commonly present. The significance of iron overload in potentiating liver
damage in NAFLD is uncertain, but the overall evidence suggests that it is unlikely to be of major importance. The presence of more than mild siderosis should raise the possibility of other causes of hepatic iron overload.

Liver biopsy occasionally reveals previously unsuspected abnormalities (e.g. α₁-antitrypsin globules) indicating an alternative or additional diagnosis to fatty liver disease.

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Establishing a morphological diagnosis

Although fatty liver disease has been identified as the most common diagnosis in people with otherwise unexplained persistently abnormal alanine aminotransferase values, in at least 20–30% of such cases liver biopsy reveals an alternative diagnosis. Liver biopsy may therefore be helpful to confirm the presence of fatty liver disease, particularly in those cases where risk factors for the metabolic syndrome are lacking.

Distinction between steatosis and steatohepatitis

Although fatty change can be reliably diagnosed by non-invasive methods, the distinction between pure fatty change (NAFL) and steatohepatitis (NASH) can only be made histologically. Likewise, non-invasive methods are unreliable in diagnosing mild degrees of fibrosis. Because of the generally poor correlation between clinical, biochemical and histological findings in NAFLD, it could be argued that liver biopsy should be carried out on all people with a suspected diagnosis of fatty liver disease. However, for logistic and safety reasons the use of liver biopsy is generally restricted to those patients with risk factors for more severe forms of fatty liver disease in which there is a likelihood of progression to fibrosis. These risk factors include age, obesity, hypertension and the presence of diabetes mellitus.

What are the minimum criteria required to make a diagnosis of steatohepatitis?

An important problem has been the lack of universally accepted criteria for making the histological diagnosis of steatohepatitis. Some studies have defined steatohepatitis as fatty change and inflammation of any type. This approach lacks specificity and may result in other unrelated diseases, including systemic illnesses associated with ‘non-specific reactive hepatitis’, being mistakenly classified as fatty liver disease. The use of more rigid diagnostic criteria including the presence of hepatocyte ballooning with Mallory’s hyaline and/or pericellular/perisinusoidal fibrosis improves diagnostic specificity but may lack sensitivity in detecting cases with mild disease or those where portal/peribiliary changes predominate. In an attempt to resolve this issue a working party organized by the American Association for the Study of Liver Diseases has produced a consensus document in which a number of essential and non-essential features of steatohepatitis have been identified. These are summarized in Table 3.

Role of liver biopsy in fatty liver disease

There are three main areas in which liver biopsy is used in the diagnosis and management of fatty liver disease: 1 Establishing a morphological diagnosis, including the distinction between simple steatosis and steatohepatitis. 2 Providing pointers to the aetiology, including cases in which a dual pathology appears to be present. 3 Assessing disease severity using histological grading and staging.

In addition to establishing an initial morphological diagnosis, repeat liver biopsies may be useful in monitoring responses to treatment, particularly in the context of clinical trials testing novel therapeutic approaches.
The most important diagnostic criterion for distinguishing steatohepatitis from simple steatosis is the presence of hepatocyte ballooning. The classical ballooned hepatocyte in fatty liver disease is associated with a partly cleared cytoplasm in which residual cytoplasmic fragments condense to form Mallory’s hyaline. Ballooned hepatocytes are typically perivenular in location and are closely associated with other histological components of fatty liver disease including inflammation and pericellular fibrosis. However, less severe forms of hepatocyte ballooning frequently lack typical Mallory bodies and may be more difficult to diagnose reliably. They may also be difficult to distinguish from the minor degrees of hepatocyte swelling, which can sometimes arise from processing artefacts. Not surprisingly, therefore, the histological diagnosis of hepatocyte ballooning is associated with more observer variability than other features of NAFLD such as steatosis and fibrosis.99,100 Important pointers to genuine hepatocyte ballooning are its perivenular location and close association with steatotic hepatocytes.62,101,102 In cases where there is

| Table 3. Histological abnormalities which may be present in non-alcoholic steatohepatitis/fibrosis (NASH) (based on American Association for the Study of Liver Diseases Single Topic Conference on NASH, Atlanta, GA, September 2002)31

1 Necessary components
- Steatosis, macro > micro, mainly zone 3
- Mixed, mild lobular inflammation—polymorphs as well as mononuclear cells
- Hepatocyte ballooning, most apparent near steatotic cells

2 Usually present, but not necessary for diagnosis
- Perisinusoidal fibrosis (zone 3)
- Glycogenated nuclei (zone 1)
- Lipogranulomas (usually small)
- Occasional acidophil bodies or periodic acid–Schiff-stained Kupffer cells

3 May be present, but not necessary for diagnosis
- Mallory’s hyaline in ballooned hepatocytes, usually zone 3 (typically poorly formed—may require immunostaining for ubiquitin, p62, cytokeratins 7, 8, 19 to confirm)
- Mild siderosis (hepatocytes and/or sinusoidal cells)
- Megamitochondria

4 Unusual for NASH, consider other causes of liver disease
- Macrovesicular steatosis (< 30% of parenchyma involved or non-zonal distribution)
- Pure or predominant microvesicular steatosis
- Sclerosing hyaline necrosis, veno-occlusive lesions, perivenular fibrosis, phlebosclerosis
- Portal inflammation > lobular inflammation, lymphoid aggregates, plasma cells
- Significant eosinophils in portal or lobular inflammation, epithelioid granulomas
- Portal/periportal fibrosis in the absence of, or markedly greater than, zone 3 perisinusoidal fibrosis
- Lobular disarray, marked inflammation, confluent or bridging necrosis, endophlebitis
- Acute cholestasis, bile plugs
- Chronic cholestasis +/- florid bile duct lesions, bile duct loss, ductular proliferation, copper granules in periportal hepatocytes
- Periodic acid–Schiff diastase-resistant globules (α1-antitrypsin) in periportal hepatocytes
- Significant granular iron in hepatocytes +/- zone 1 to zone 3 gradient

doubt about the presence of ballooning, immuno-histochemical staining may also help to demonstrate small amounts of Mallory’s hyaline-like material that cannot be reliably identified in conventionally stained sections.

Is the distinction between steatosis and steatohepatitis clinically important?

A number of studies have indicated that pure fatty liver is a non-progressive reversible lesion, whereas the presence of features of steatohepatitis indicates the potential for progressive liver damage, in some cases leading to cirrhosis.9,103,104 In two studies with a combined population of 149 patients with a pure fatty liver, none developed clinical or histological features of advanced liver disease over median follow-up periods of >11 years.9,103 In a third study of 132 patients undergoing liver biopsy for NAFLD who were followed up for a median period of 8.3 years, only two of 59 (3%) with fatty change alone or fatty change and lobular inflammation progressed to cirrhosis, whereas 18 of 73 patients (25%) whose biopsies showed additional features of steatohepatitis (ballooning, Mallory’s hyaline, fibrosis) became cirrhotic.104 This study also emphasizes the importance of using more rigid criteria than fatty change and inflammation alone for the diagnosis of steatohepatitis.

The suggestion that pure fatty change (NAFL) is associated with a favourable outcome has recently been challenged. Three studies have shown that up to 10% of patients with pure fatty liver progress to steatohepatitis with fibrosis or cirrhosis.105–107 However, in one of these studies the risk of progression to cirrhosis was significantly greater in patients whose initial diagnosis was NASH (20%) than in those with steatosis alone (3%).107 Other recent studies have suggested that other factors such as diabetes, body mass index and obesity are more reliable predictors of fibrosis progression than baseline biopsy findings.108,109 Furthermore, many of these cases develop fibrosis progression despite improvement in other histological abnormalities including steatosis, hepatocyte ballooning and Mallory’s hyaline. Biochemical abnormalities including serum transaminase levels may also improve despite progression of fibrosis.109 These observations suggest that repeat liver biopsies may have a role in monitoring disease progression even in cases where the initial biopsy shows simple steatosis and in those where there appears to be an improvement in liver biochemistry. They also confirm previous observations that features of fatty liver disease are frequently lacking in cases that have progressed to cirrhosis.

Aetiological considerations

ALCOHOLIC VERSUS NON-ALCOHOLIC STEATOHEPATITIS

A generally accepted diagnostic criterion for NAFLD is the exclusion of significant alcohol consumption (no more than 20–40 g/day).3 Moderate alcohol consumption has been associated with a lower risk of developing NASH, possibly by reducing insulin resistance.63 However, in people with heavy alcohol consumption, obesity has been implicated as a risk factor for the development of acute alcoholic hepatitis and cirrhosis, suggesting that there are common pathways of liver damage in alcoholic and non-alcoholic fatty liver disease.80,110

Faced with a liver biopsy showing features of fatty liver disease, are there any particular features that suggest a non-alcoholic rather than an alcoholic aetiology? In the great majority of such cases a distinction between these two processes cannot be made on histological grounds alone and the final diagnosis is based on clinicopathological correlation. In general, NAFLD tends to be associated with relatively more severe fatty change, whereas features of steatohepatitis such as ballooning, Mallory body formation, neutrophilic infiltration and pericellular fibrosis are generally less severe than in ALD.31,45,111,112 Nuclear vacuolation of hepatocytes is seen in 70–80% of cases of NAFLD compared with only 5–10% of cases of ALD and may be a useful pointer to glycogen accumulation occurring in the setting of insulin resistance111,112 (Figure 8). Nuclear vacuolation of hepatocytes can also be seen in NAFLD-related cirrhosis, even when other features of steatohepatitis are no longer conspicuous.

Figure 8. Nuclear vacuolation of hepatocytes in non-alcoholic fatty liver disease. Many hepatocytes show nuclear vacuolation, a feature much more commonly seen when fatty liver has a non-alcoholic rather than alcoholic aetiology.
zone 3 changes falling into the spectrum of severe alcoholic hepatitis or central sclerosing hyaline necrosis are rarely seen in NASH and point to an alcoholic cause for liver damage. These changes include abundant deposits of Mallory’s hyaline, dense infiltration by neutrophils, severe bilirubinostasis, extensive zone 3 fibrosis associated with sinusoidal obliteration and hepatic veno-occlusive lesions. However, occasional cases of florid NASH with subacute liver failure have been documented. Other recent studies have suggested that NASH can be distinguished from alcoholic steatohepatitis (ASH) on the basis of different patterns of fibrosis (lattice pattern in NAFLD, solid in ALD) or by different immunohistochemical staining patterns for the protein tyrosine phosphatase 1B (PTP1B) and insulin receptor on hepatocytes. PTP1B negatively regulates the insulin receptor (IR) through dephosphorylation and was found to be up-regulated in the cytoplasm of hepatocytes in biopsies obtained from patients with NASH compared with those from cases of ASH. This was accompanied by loss of membranous staining for IR in hepatocytes from NASH biopsies, compared with ASH biopsies where it was still preserved.

"PRIMARY" VERSUS "SECONDARY" CAUSES OF NAFLD

The great majority of NAFLD occurs in the setting of the metabolic syndrome (primary NAFLD) (Table 1). In cases where risk factors for primary NAFLD are lacking and a history of excess alcohol consumption can be confidently excluded, a number of less common causes of fatty liver disease can be considered in the differential diagnosis. Although there are many potential causes of steatosis, too numerous to discuss in detail here, relatively few of these are associated with additional features of steatohepatitis (Table 1). In the adult population, drug-induced NASH is the main differential diagnosis. In drug-related causes of NASH, Mallory’s hyaline is often present in large amounts out of proportion to only mild fatty change and may have a perportal rather than perivenular distribution. In children, the diagnosis of NASH requires exclusion of inherited metabolic causes of fatty liver disease. The hepatocyte ballooning and Mallory’s hyaline occurring in Wilson’s disease tend to have a predominantly perportal (zone 1) rather than perivenular distribution and pericellular fibrosis is rarely seen.

NAFLD CO-EXISTING WITH OTHER CHRONIC LIVER DISEASES

With the increasing prevalence of fatty liver disease, histological features of NAFLD are now seen with increasing frequency in association with other causes of chronic liver disease, particularly HCV infection. The relationship between NAFLD and HCV infection is complex. Fatty change is a common finding in chronic HCV infection. This may in part be a direct cytopathic effect of the virus itself, possibly related to viral proteins interfering with fatty acid oxidation or other pathways of lipid metabolism. Chronic HCV infection is a risk factor for the development of insulin resistance, thus also predisposing to the development of NAFLD. The development of insulin resistance in HCV-infected individuals may relate to viral-induced production of TNF-α, which interferes with insulin signalling. In cases infected with genotype 3 the severity of steatosis correlates with levels of intrahepatic viral replication. Genotype 3 has also been implicated as a risk factor for progression to steatohepatitis. The severity of steatosis in HCV genotype 3-infected patients correlates with fibrosis development. In contrast, in HCV+ individuals infected with other genotypes, the severity of steatosis and the risk of progression to steatohepatitis and fibrosis are associated with risk factors for the metabolic syndrome. Overall, approximately 5–20% of patients with chronic HCV infection have superimposed features of steatohepatitis.

Recognizing the presence and severity of fatty liver disease in HCV-infected individuals is potentially important, both as a prognostic factor for the development of liver fibrosis and in determining the likelihood of poor response to antiviral therapy. Steatosis predisposes to the development of both the perisinusoidal and periportal pathways of fibrosis in chronic HCV infection, apparently independently of HCV-associated necroinflammation. Portal fibrosis appears to be related to the activation and proliferation of portal myofibroblasts, possibly via a paracrine pathway. Alternatively, as discussed earlier, steatosis is associated with impaired hepatocyte regeneration, predisposing to progenitor cell activation and bile ductular reaction and the extent of ductular reaction has been shown to correlate with fibrosis severity.

Risk factors for NAFLD have been implicated in the pathogenesis of fatty change occurring in some individuals with chronic hepatitis B virus infection. Fatty change is generally mild in severity and, in contrast to steatosis occurring in the setting of chronic HCV infection, is not associated with progression to steatohepatitis or fibrosis. Other diseases in which NAFLD has been implicated as a cofactor include ALD (discussed earlier), drug toxicity (e.g. methotrexate,
Assessing disease severity

The prevalence and natural history of the different liver lesions seen in NAFLD are difficult to determine accurately as many of these patients are not biopsied and few studies have investigated disease progression in serial biopsies. It has been estimated that amongst a population of obese/diabetic individuals approximately 50–90% will have fatty change, 20–30% will progress to steatohepatitis/fibrosis and 2–5% will eventually become cirrhotic (Figure 1). Cross-sectional studies have suggested a higher prevalence of cirrhosis (in the region of 10–30%) amongst patients undergoing liver biopsy for NAFLD, perhaps reflecting a degree of selection bias. In a review of four previously published series describing 30 patients with NAFLD, who had paired liver biopsies at intervals ranging from 1.0 to 9.0 years, 14 showed progression in fibrosis, six cases resulting in cirrhosis. Amongst the other 16 cases, one showed improvement in liver histology, whereas the other 15 showed no change. Other studies have suggested that fibrosis may remain stable or regress in up to 60–70% of cases.

Table 4. A proposed scheme for histological grading and staging of non-alcoholic steatohepatitis/fibrosis (NASH) (based on Brunt 1999 and Neuschwander-Tetri and Caldwell 2003)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Steatosis</th>
<th>Ballooning (zone 3)</th>
<th>Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>1–2</td>
<td>Minimal</td>
<td>Lobular 1–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Portal 0–1</td>
</tr>
<tr>
<td>Moderate</td>
<td>2–3</td>
<td>Present</td>
<td>Lobular 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Portal 1–2</td>
</tr>
<tr>
<td>Severe</td>
<td>3</td>
<td>Marked</td>
<td>Lobular 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Portal 1–2</td>
</tr>
</tbody>
</table>

Individual features graded semiquantitatively on a scale of 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Severity of steatosis based on proportion of hepatocytes involved: 1 = < 33%, 2 = 33–66%, 3 = > 66%.

Severity of lobular inflammation based on inflammatory foci per × 200 field: 1 = 1–2, 2 = up to 4, 3 = >4.

b. Staging of NASH

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>Zone 3 perivenular, perisinusoidal or pericellular fibrosis; focal or extensive</td>
</tr>
<tr>
<td>Stage 2</td>
<td>As for stage 1 plus focal or extensive portal fibrosis</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Bridging fibrosis, focal or extensive</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

applies only to NASH (and does not thus encompass the entire spectrum of NAFLD) and to cases with classical lobular features of steatohepatitis. An alternative approach is thus required for cases which have a predominantly portal-based disease process; this particularly applies to fatty liver disease occurring in children. In an attempt to address some of these problems a modified histological scoring system has been devised by a group of North American pathologists working under the auspices of the Non-alcoholic Steatohepatitis Clinical Research Network (Table 5).

This system evaluates the same three main features of disease activity that were used in the original system devised by Brunt et al., but instead of being incorporated into an overall grade they are scored separately and the individual scores then added to produce an overall ‘NAFLD Activity Score’ (NAS). There is also recognition of portal fibrosis as a separate pathway for disease progression. The main purpose of the system proposed by Kleiner et al. was to determine robust criteria for establishing a histological diagnosis of

GRADING AND STAGING OF FATTY LIVER DISEASE

Histological grading and staging are widely used in the assessment of liver biopsies from patients with chronic viral hepatitis, particularly hepatitis C. A similar approach has been applied to the assessment of disease severity in fatty liver disease. Features which are graded are fatty change, ballooning and inflammation (parenchymal and portal). Staging involves an assessment of the severity of fibrosis.

A number of systems have been proposed for assessing the severity of fatty liver disease. The main features of a scheme devised by Brunt and colleagues, which has been most widely used for assessing disease severity in non-alcoholic steatohepatitis, are summarized in Table 4. One criticism of this approach is the incorporation of fatty change, ballooning and inflammation into an overall grade. This implies that these three histological features increase in parallel to each other, which is not necessarily the case. For example, a biopsy may show severe (grade 3) fatty change but only mild (grade 1) ballooning and inflammation and it is not clear what overall grade should be applied to such a specimen. Other limitations of this system relate to the fact that it

Problems inherent to all histological scoring systems that have been used in assessment of liver disease relate to observer reproducibility and sampling variation. Amongst the features which have been assessed in fatty liver disease, observer agreement is generally good for steatosis, moderate for ballooning and less good for lobular inflammation and Mallory’s hyaline. Studies of paired liver biopsies have suggested that sampling variability presents a greater problem. Although fatty change has a reasonably uniform distribution, there is considerable variation in the severity of other histological features, particularly fibrosis. Heterogeneity of fibrosis scores is increased in small biopsies (< 16 mm long).

Conclusion and future developments

It is likely that histological assessments will continue to play an important role in the diagnosis and management of people with NAFLD. In those cases where the diagnosis of NAFLD has already been made clinically, liver biopsy is required to make the distinction between simple steatosis and steatohepatitis and, where appropriate, to determine the severity of liver damage within the broad spectrum of steatohepatitis/fibrosis. In some cases where NAFLD is suspected clinically, liver biopsy may reveal an additional or alternative cause for liver damage. It is also becoming increasingly apparent that NAFLD is an important cofactor in other chronic liver diseases, particularly hepatitis C. In addition to establishing that a dual pathology is present, liver biopsy may help to determine which of the two diseases is the predominant cause of liver damage. Further studies are still required to determine the natural history of NAFLD and the role of liver biopsy in monitoring therapeutic responses. The utility of recently devised systems for grading and staging NAFLD also requires further evaluation.

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