Fatty Liver Disease: NASH and Related Disorders

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Non-alcoholic fatty liver disease (NAFLD), like hepatitis C and HIV, is a disease of our generation. Mostly unrecognized prior to 1980 and seldom taken seriously until the past few years, NAFLD has seemingly been thrust upon us unexpectedly like an orphaned child left at our clinical bedside. In fact, NAFLD was conceived during the industrial revolution, which caused food to be processed differently, provided that food more abundantly and made physical work less demanding. In the 1980’s, information technology and virtual reality have enhanced sedentary lifestyles and the decline of physical activity, key factors in exacerbating lifestyle disorders. NAFLD shares these roots with its older siblings—obesity and diabetes mellitus—but is only now being accepted into the full family of the metabolic syndrome.

The clinical importance of this disease is introduced in the first chapter of this book, which is the first devoted exclusively to NAFLD and its more serious form—non-alcoholic steatohepatitis (NASH). In Chapter 1, the editors have introduced the seminal issues related to NAFLD; its definition, epidemiology, pathophysiology and treatment. The nascent yet expanding knowledge of these issues served not only as the basis for developing this book, but also for the selection of its topics and contributing authors.

This disease currently impacts virtually all fields of clinical medicine and will continue to do so with increasing prevalence and adversity to patients. The misconception that NAFLD is benign is fading, but there remain some lingering doubts. This book should dispel those doubts, convince the reader that NAFLD and especially NASH is important, and clarify which affected persons are the ones we need to worry most about. NAFLD/NASH is common, expensive to society, adversely affects quality of life and causes liver-related death in a significant, but still imprecisely known percentage of patients. Certainly important questions remain. Why does only a subset of NAFLD patients develop NASH? What is the interaction between genetic and environmental factors in NAFLD? Is our current knowledge of the natural history and pathophysiology of NAFLD sufficient to recommend management algorithms (including the indications for liver biopsy) or treatments that are cost effective with an acceptable risk benefit ratio? Hopefully, this book will serve as a platform from which these questions can be answered and from which clinicians can gain some confidence in the management of this disease that remains a mosaic of evolving complex issues. Interactions between the determinants of metabolic disease and other disorders, especially hepatitis C and alcoholic liver disease, is one very important advance in understanding with practical implications for patient care.

The editors would like to thank each of the authors for their efforts in this work. We also want to acknowledge the pathologists who have contributed such high quality histologic micrographs. We appreciate the authors’ patience and gracious tolerance to the time-lines, deadlines and urgent e-mails that are inevitably associated with this type of work. Their expertise and ability to share their knowledge have made this book a very informative and readable text. Our colleagues at Blackwell Publishing have been extremely helpful in guiding us through the editorial process and we appreciate their professional input. Finally, we wish to thank both the patients with NAFLD and the clinicians who care for them. These are the people for whom this book was written and without whom it would not have been achieved.

The Editors
Abstract

This chapter introduces the history, definitional and semantic issues, spectrum and general importance of non-alcoholic fatty liver diseases (NAFLD). Non-alcoholic steatohepatitis (NASH) is a form of metabolic liver disease in which fatty change (steatosis) is associated with lobular inflammation, hepatocytic injury, polymorphs and/or hepatic fibrosis. It comprises a pathogenic link in the chain of non-alcoholic fatty liver diseases (NAFLD) that extends from bland steatosis to some cases of ‘cryptogenic cirrhosis’. NAFLD and NASH are usually hepatic manifestations of the insulin resistance syndrome, but the factors that transform steatosis to NASH remain unclear. In 20–25% of cases, NASH may progress to advanced stages of hepatic fibrosis and cirrhosis; liver failure then becomes the most common cause of death. Clinicians should consider NAFLD/NASH as a primary diagnosis by its metabolic associations with obesity, insulin resistance and type 2 diabetes, rather than simply as a disease of exclusion. Correction of insulin resistance by lifestyle modification (dietary measures and increased physical activity) is a logical approach to prevent or reverse NAFLD/NASH.

Key learning points

1. Non-alcoholic steatohepatitis (NASH) is a form of metabolic liver disease in which fatty change (steatosis) is associated with lobular inflammation, hepatocytic injury, and polymorphs and/or hepatic fibrosis.
2. NASH comprises a pathogenic link in the chain of non-alcoholic fatty liver diseases (NAFLD) that extends from bland steatosis to some cases of ‘cryptogenic cirrhosis’.
3. NAFLD and NASH are usually hepatic manifestations of the insulin resistance syndrome, but the factors that transform steatosis to NASH remain unclear.
4. In 20–25% of cases, NASH may progress to advanced stages of hepatic fibrosis and cirrhosis; liver failure then becomes the most common cause of death.
5. Clinicians should consider NAFLD/NASH as a primary diagnosis by its metabolic associations with obesity, insulin resistance and type 2 diabetes, rather than simply as a disease of exclusion.
6. Correction of insulin resistance by lifestyle modification (dietary measures and increased physical activity) is a logical approach to prevent or reverse NAFLD/NASH.
providing ‘hepatocellular protection’ has been shown to improve liver tests in short-term small studies, but larger randomized controlled trials are needed to establish whether any of these approaches arrest progression of hepatic fibrosis and prevent liver complications, and at what stage interventions are cost-effective.

**History of NASH**

In 1980, Ludwig et al. [1] described a series of patients who lacked a history of ‘significant’ alcohol intake but in whom the liver histology resembled that of alcoholic liver disease. They were the first to use the term ‘non-alcoholic steatohepatitis’ for this condition, the principal features of which were hepatic steatosis (fatty change), inflammation and exclusion of alcohol as an aetiological factor. Further small case series were published during the next 15 years [2–10]. After much debate, the entity of NASH became accepted, but it is only in the last 10 years that NASH and other forms of metabolic (non-alcoholic) fatty liver diseases (NAFLD) have been widely recognized and diagnosed in clinical practice. The pace of research into the pathogenesis, natural history and treatment of NAFLD/NASH has accelerated in the last 5 years (Fig. 1.1). Thus, Marchesini and Forlani [11] were able to locate only 161 articles which addressed this topic between 1980 and 1999 (approximately 8/year) but 122 in 2000–01 (approximately 60/year). These advances have been reviewed elsewhere [11–19].

**What is NASH?**

**Terminology and definitions**

The spectrum of fatty liver disease associated with metabolic determinants and not resulting from alcohol (NAFLD) extends from hepatic steatosis through steatohepatitis to cirrhosis (Table 1.1). As described in Chapter 2, NASH can be defined pathologically as significant steatohepatitis not resulting from alcohol, drugs, toxins, infectious agents or other identifiable exogenous causes (Table 1.2). However, standardized definitions are lacking, particularly of what pathology is encompassed by ‘significant steatohepatitis’ (such as types 3 or 4 NAFLD; see Table 1.1). Outstanding challenges confronting pathological definition include the following.

1. Agreement on the importance, validity and concordance between observers of histological features of hepatocellular injury, especially ballooning degeneration.
2. Categorizing the grade and diagnostic reliability of patterns of hepatic fibrosis.
3. Interpretation of what cases of ‘cryptogenic cirrhosis’ can be attributed to NASH.

This book adopts general recommendations on nomenclature for what comprises NASH that are similar to those suggested by Brunt et al. [20] and

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**Fig. 1.1** Chronology of the pace of research into pathogenesis, natural history and treatment of NAFLD/NASH.
discussed at a single topic conference of the American Association for Study of Liver Diseases (AASLD), September 2002, Atlanta, Georgia (see Chapter 2) [19,20].

When one particular cause of steatohepatitis is evident, the term steatohepatitis is qualified (e.g. alcoholic steatohepatitis, drug-induced steatohepatitis, experimental [dietary] steatohepatitis). Such cases are often referred to as ‘secondary NASH’ (Table 2.2; see Chapters 13, 20 and 21). Because of its strong association with ‘metabolic’ determinants (obesity, insulin resistance, type 2 diabetes, hyperlipidaemia), the acronym ‘MeSH’ has been suggested as an alternative for ‘idiopathic’ (or ‘primary’) NASH, but seems unlikely to gain widespread acceptance.

Non-alcoholic fatty liver diseases

The term NAFLD is gaining acceptance and is useful because it is more comprehensive than NASH (Table 1.1) [15–17]. NAFLD includes less significant forms of steatosis either alone (type 1 NAFLD) or with inflammation but no hepatocyte ballooning or fibrosis (type 2). The term NAFLD will be used here when the pathology of metabolic liver disease is not known, or when specifically referring to the fuller spectrum. This now includes some cases of cryptogenic cirrhosis in which steatohepatitis and steatosis are no longer conspicuous.

Table 1.1 Categories of non-alcoholic fatty liver diseases (NAFLD): relationship to NASH. (After Matteoni et al. [15].)

<table>
<thead>
<tr>
<th>Category</th>
<th>Pathology</th>
<th>Clinicopathological correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Simple steatosis</td>
<td>Known to be non-progressive</td>
</tr>
<tr>
<td>Type 2</td>
<td>Steatosis plus lobular inflammation</td>
<td>Probably benign (not regarded as NASH)</td>
</tr>
<tr>
<td>Type 3</td>
<td>Steatosis, lobular inflammation and ballooning degeneration</td>
<td>NASH without fibrosis—may progress to cirrhosis</td>
</tr>
<tr>
<td>Type 4</td>
<td>Steatosis, ballooning degeneration and Mallory bodies, and/or fibrosis</td>
<td>NASH with fibrosis—may progress to cirrhosis and liver failure</td>
</tr>
</tbody>
</table>

Table 1.2 Causes of secondary steatohepatitis.

- Alcohol (alcoholic hepatitis)
- Drugs (tamoxifen, amiodarone, methotrexate)
- Copper toxicity (Wilson’s disease, Indian childhood cirrhosis)
- Jejuno-ileal bypass (see Chapter 20)
- Other causes of rapid profound weight loss (massive intestinal resection, cachexia, bulimia, starvation)
- Hypernutrition in adults (parenteral nutrition, intravenous glucose)
- A-betalipoproteinaemia
- Jejunal diverticulosis (contaminated bowel syndrome)
- Insulin resistance syndromes (familial and acquired lipodystrophies, polycystic ovary syndrome)

Primary and secondary steatohepatitis: the importance of alcohol

A key definitional issue is potential overlap between ‘primary’ (metabolic) NAFLD/NASH and pathologically similar fatty liver diseases associated with a single causative factor (Table 1.2). The most important consideration is the level of alcohol consumption considered unlikely to have any causal role in liver disease. Early publications describing ‘alcoholic hepatitis-like lesions’ were in non-drinkers or those with minimal intake (less than one drink a week in the Ludwig series). Since then, reports of NAFLD/NASH have used a variety of thresholds for alcohol intake. Some have required rigorous alcohol restriction, particularly for cases of ‘cryptogenic cirrhosis’ attributable to
NASH (e.g. none, or less than 40 g/week) [21,22]. Conversely, other authors have allowed alcohol intake to be as high as 210 g/week [23].

It is noted that 30 g/day is close to the level of 40 g/day associated with an increased risk of cirrhosis in women [24]. Safe levels of alcohol intake have also been difficult to define for other liver diseases, such as hepatitis C for which less than 10 g/day was recommended by the first National Institutes of Health (NIH) Consensus Conference in 1997 [25], but up to 30 g/day for men and 20 g/day for women by the second NIH Consensus Conference [26]. In this book, the definition of NASH requires alcohol intake to have never been greater than 140 g/week (ideally, ≤20 g/day for men and ≤10 g/day for women). However, it is acknowledged that there may be potential for even these low levels of alcohol intake levels to contribute to cell injury, fibrogenesis and hepatocarcinogenesis in steatohepatitis. Conversely, it remains possible that low levels of alcohol intake confer health benefits in obese persons with liver disease [27]. The implications for recommending optimal levels of alcohol intake for people with NAFLD/NASH are considered in Chapter 15.

### Interaction between steatohepatitis and other liver disorders

Another challenge is when the metabolic determinants of NASH (Table 1.3) coexist with known causes of liver disease. The latter include ‘moderate’ levels of alcohol intake (30–60 g/day in men, 20–40 g/day in women), hepatitis C and potentially hepatotoxic drugs (methotrexate, tamoxifen, calcium-channel blockers, highly active antiretroviral therapy) [28]. The likelihood that steatosis or the metabolic determinants that result in NASH contribute to liver injury and fibrotic severity of other liver diseases is canvassed in Chapter 23.

### Importance of NASH

Reasons why NASH is an important form of liver disease are summarized in Table 1.4.

---

**Table 1.3** Metabolic associations of NASH.

<table>
<thead>
<tr>
<th>Type 2 diabetes mellitus</th>
<th>Family history of type 2 diabetes</th>
<th>Insulin resistance, with or without glucose intolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central obesity (waist : hip ≥ 0.85 in women, ≥ 0.90 in men; waist &gt; 85 cm in women, &gt; 97 cm in men*)</td>
<td>Obesity (BMI ≥ 30 kg/m² in white people, ≥ 27 kg/m² in Asians)</td>
<td>Hypertriglyceridaemia</td>
</tr>
<tr>
<td>Rapid and massive weight loss in overweight subjects</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values vary between countries; 90 cm for women and 102 cm for men often used in USA.

**Table 1.4** Reasons why NAFLD/NASH is important.

| High prevalence of fatty liver disorders in urbanized communities with affluent (‘Western’) economies throughout the world | Most common cause of abnormal liver tests in community—92–8% of population have NAFLD |
| NASH now rivals alcoholic liver disease and chronic hepatitis C as reason for referral to gastroenterologist or liver clinic | NASH is a potential cause of cirrhosis, which may be ‘cryptogenic’, and lead to end-stage liver disease |
| Liver failure is most common cause of death in patients with cirrhosis resulting from NASH | Standardized mortality of liver disease in type 2 diabetes greatly exceeds vascular disease |
| NASH recurs after liver transplantation | Hepatic steatosis as a cause of primary graft non-function after liver transplantation |
| Role of metabolic determinants of NASH in pathogenesis of other liver diseases, particularly hepatitis C and alcoholic cirrhosis | Possible role of NASH/hepatic steatosis in hepatocarcinogenesis |
INTRODUCTION TO NASH AND RELATED DISORDERS

The NASH epidemic

In much of the world, abnormal liver tests attributable to hepatic steatosis or NASH have become the most common liver disease in the community. Depending on how an abnormal value for aminotransferase is defined in studies, such as the Third National Health and Nutritional Examination Survey (NHANES III), between 3 and 23% of the adult population may have NAFLD/NASH [29–31]. In studies that have employed hepatic imaging, autopsy or biopsy approaches, approximately 70% of obese people have hepatic steatosis and/or raised alanine aminotransferase (ALT) [12,21, 27,31–37]; NASH is present in approximately 20% of these [7,27]. In old autopsy studies, ~ 10% of diabetics had cirrhosis, but other factors (hepatitis B and C) were possible confounding variables. In more recent studies, both the prevalence and severity of NASH appear to be increased considerably in patients with type 2 diabetes [11,21,36,38–40].

The epidemiology of NAFLD/NASH is discussed in Chapter 3. Based on the continuing epidemic of obesity and type 2 diabetes through much of the world, it is likely that the prevalence of NASH will increase further during the next decade. In the USA and Australia, up to 60% of men and 45% of women are overweight, and about one-third of these are obese [41,42]. Similar increases have been noted in societies that until the last one or two generations were participating in physically active (‘hunter gatherer’) lifestyles (see Chapter 18). The prevalence of type 2 diabetes has doubled, trebled or increased 10- to 20-fold (as in Japanese youth) during the last decade, rates reaching 40% or more of the adult population in some communities [43–45]. Childhood cases of NASH are also clearly related to obesity and type 2 diabetes (see Chapter 19) [46,47]. Some possible reasons for high rates of obesity and type 2 diabetes in contemporary affluent societies (‘east’ and ‘west’, ‘north’ and ‘south’), and the implications for prevention and interruption of NASH are discussed in Chapters 3–5 and 18.

NAFLD/NASH varies in severity and clinical outcome

Steatosis alone has an excellent prognosis. It seems probable that most cases of steatosis with lobular inflammation but without conspicuous hepatocyte injury or fibrosis (NAFLD type 2) behaves in the same way, with very low rates of fibrotic progression (see Chapter 3). However, 20–25% of cases with NASH have or will progress to cirrhosis [15,16,19,21,22,39]. There is mounting evidence that a proportion of cases of ‘cryptogenic cirrhosis’ may be attributable to NASH, in which the histological features of steatohepatitis have resolved (see Chapter 14) [15,21,31,35,48]. Rare cases of subacute hepatic failure have also been attributed to possible NASH [49].

Earlier studies of NAFLD/NASH emphasized the good overall prognosis [8,10]. More recent studies that have defined cases according to fibrotic severity indicate that those with significant fibrosis may progress to liver failure [15,22,50]. Among cases of cirrhosis, the risk of death or liver transplantation may be as high as cirrhosis resulting from hepatitis C (both ~ 30% at 7 years) [15,16,22,50]. If this indolent progressive course is confirmed in larger prospective studies, NASH will cause a formidable disease burden in forthcoming decades.

A few well-documented cases of cirrhosis resulting from NASH have presented with, or less commonly have terminated in HCC [16,51]. HCC was recently noted to be a cause of death among obese patients with cryptogenic cirrhosis [52,53]. However, it is not clear that all such cases were caused by NASH [22], and several were diagnosed within 9 months of presentation. Others have suggested that steatosis could increase the risk of HCC associated with other liver diseases [54,55], but conflicting data have been noted (see Chapter 22).

Metabolic risk factors for NASH may worsen other liver diseases

As well as providing the setting for NASH, insulin resistance, obesity, type 2 diabetes and hepatic steatosis are now recognized as factors that favour fibrotic progression in hepatitis C [56,57]. Obesity is also an independent risk factor for alcoholic cirrhosis [58]. Thus, ‘NASH determinants’ may contribute to the overall burden of cirrhosis directly as the hepatic complication of obesity, insulin resistance and diabetes, and indirectly as factors that favour cirrhosis among people with chronic viral hepatitis or alcoholism (see Chapter 23).

When should the clinician think of NASH?

Clinicians need to consider that NAFLD/NASH is the most likely cause of liver test abnormalities in the
Laboratory tests, such as a raised serum urate, triglyceride, low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol are pointers to insulin resistance. The genetic factors that could predispose to NASH are considered in Chapter 6, and the insulin resistance syndrome is discussed in Chapter 5.

A raised serum ferritin level is a common ‘founder’ in cases of NAFLD/NASH [60–62]. As in alcoholic liver disease, this most often reflects increased hepatic release of ferritin as an ‘acute phase reactant’, reflecting the hepatic inflammatory response and increased permeability of steatotic and injured hepatocytes. If a persistently raised serum transferrin saturation suggests increased body iron stores, haemochromatosis gene testing should be conducted in those with a northern European or Celtic background. The proposed role of hepatic iron in worsening fibrotic severity in NASH is controversial (see Chapter 7) [60–62].

Confirming the diagnosis is NASH

Liver biochemical function tests, serum lipids and other laboratory results

Abnormal biochemical results (liver function tests) typically comprise minor (1.5- to 5-fold) elevations of ALT and gamma-glutamyl transpeptidase (GGT). The following laboratory tests may provide clues to the presence of cirrhosis: low platelet count, raised aspartate aminotransferase (AST) that is higher than ALT, and subtle changes in serum albumin or bilirubin that are not attributable to other causes (see Chapter 14).
Fasting hypertriglyceridaemia is present in 25–40% of patients with NASH [8,9,10,16,39]. It may be associated with hypercholesterolaemia (increased LDL cholesterol, particularly with low levels of HDL and a high LDL : HDL ratio). This pattern of lipid disorders is a feature of the insulin resistance syndrome.

Anthropometric measurements

Because nearly all patients with NASH have central obesity, anthropometric measurements should be routinely recorded at liver clinic visits (see Chapter 15). Height and weight are used to calculate body mass index (BMI), while girth (circumference at umbilicus), or waist : hip ratio form simple pointers to central obesity (see Chapters 5 and 15 for details). Some nutritionists recommend waist circumference as more useful than body weight for monitoring benefits of lifestyle change in overweight people.

Determination of insulin resistance

The near universal association of NASH with insulin resistance means that tests to document this pathophysiological state should form part of the approach to diagnosis. Fasting serum insulin and blood glucose levels can be used to construct the relatively crude (but practically useful) homoeostasis model assessment of insulin resistance (HOMA-IR). Values for HOMA-IR differ between population subgroups. Thus, application of this method requires reference to a local group of normal age-matched controls.

As discussed in Chapter 4, diabetologists prefer an ‘active’ measure of insulin sensitivity as opposed to a fasting one; the latter will be misleading when there is secondary failure of insulin secretion by pancreatic β cells. A simplified 75-g oral glucose tolerance test with 1 and 2 h blood glucose and serum insulin levels can be very informative. Fasting serum C-peptide level is an excellent measure of insulin production. It therefore appears to be a sensitive indicator of insulin resistance that can be used in hepatological practice.

Hepatic imaging

Hepatic imaging performed as part of investigations into abdominal pain, abnormal liver tests or suspected hepatic malignancy may be the first clue to the presence of steatosis [63]. The sensitivity of hepatic ultrasound for steatosis (increased echogenicity, or ‘bright liver’) appears fairly high, particularly when extensive steatosis (involving at least 33% hepatocytes) is present [63]. CT also appears to be relatively sensitive for hepatic steatosis, and has the advantage that nodularity resulting from cirrhosis may sometimes be appreciated. Careful attention should be given to features of portal hypertension (portal vein dilatation, splenomegaly, retroperitoneal varices). Otherwise, both ultrasonography and computerized tomography (CT) have low positive predictive value for detecting features of cirrhosis.

Neither ultrasonography nor CT is able to distinguish NASH from other forms of NAFLD (see Chapter 13). Thus, while hepatic imaging is useful for providing supportive evidence in favour of hepatic steatosis, it cannot substitute for liver biopsy for elucidating the fibrotic severity of NASH.

Newer imaging techniques (dual-energy X-ray absorptiometry [DEXA], magnetic resonance imaging [MRI]) are also valuable in determining body composition. Total body fat can be estimated accurately with DEXA, but greater interest will come from studies attempting to discern patterns of adipose tissue distribution (visceral versus subcutaneous or ectopic); these patterns are likely to correlate more closely with insulin resistance (see Chapter 4).

Liver biopsy

Clinical guidelines for when liver biopsy is indicated for suspected NASH are not yet standardized [16,18], with views ranging from the nihilistic to the enthusiastic! In considering whether a liver biopsy is indicated, one approach is to assess risk factors for fibrotic severity (obesity, diabetes, age over 45 years, and AST : ALT > 1) and to seek ‘warning signs’ of cirrhosis (see Chapter 14) [15,16,18]. One approach is not to recommend biopsy at first referral (see Chapter 15). If lifestyle intervention aimed at correcting insulin resistance and central obesity fails to normalize liver tests, and particularly if there are warning signs for cirrhosis or the patient expresses a strong desire to know the severity of their liver disease, the physician should proceed to liver biopsy (see Chapters 13 and 15). Liver biopsy interpretation is described in Chapter 2.

In following any paradigm for liver biopsy, it should be noted that liver test abnormalities in NASH are poorly related to fibrotic severity. Some patients...
with NASH cirrhosis may have normal ALT levels. A nihilistic approach to liver biopsy for NASH therefore raises the concern that some patients with advanced hepatic fibrosis and/or cirrhosis would not be counselled and monitored appropriately. Further, liver biopsy can sometimes produce unexpected findings indicative of another liver disease, thereby changing management.

**Why does NASH happen?**

The recurrence of NASH after orthotopic liver transplantation (see Chapter 17) is a dramatic demonstration of the importance of extrahepatic (metabolic) factors in its pathogenesis. Among these, genetic and acquired abnormalities of fatty acid turnover and oxidation are likely to be crucial in causing steatohepatitis [16,17,19,64]; some facilitate accumulation of free fatty acids (FFA), others favour the operation of oxidative stress. Factors that facilitate recruitment of an hepatic inflammatory (or innate immune) response, or determine the tissue response to liver injury are other potentially relevant variables.

Human and animal studies have started to address key issues in NASH pathogenesis, such as the nature of insulin resistance—why it occurs, whether it is responsible for inflammation and liver cell injury as well as FFA accumulation, the mechanisms for inflammatory recruitment and perpetuation, the biochemical basis and significance of oxidative stress, the cell biological basis of hepatocyte injury and the pathogenesis of fibrosis (see Chapters 4, 7, 8 and 10–12). It seems likely that many such factors are genetically determined (see Chapter 6). In this way, NASH, like type 2 diabetes, atherosclerosis and some cancers, is the outcome of an interplay between several genetic and environmental factors.

Lipid accumulation also favours increased concentrations of FFA that may be directly toxic to hepatocytes. It has recently been proposed that such ‘lipotoxicity’ in NASH results from failure of leptin or other hormones that modulate insulin sensitivity to correct for insulin resistance [65]. The humoral and dietary modulation of insulin receptor signalling that underlies this new concept is discussed in Chapter 4. The fatty liver also provides an excess of unsaturated FFA, oxidation of which results in the autopropagative process of lipid peroxidation. It is now clear that the steatotic liver is more susceptible to oxidative stress, as well as to injury after injection of endotoxin [16,18,64].

The liver normally responds to the chronic presence of oxidants by increasing synthesis of protective antioxidant pathways, such as those based on reduced glutathione (GSH). If GSH levels are depleted (as with fasting, toxins such as alcohol, or consumption by pro-oxidants), the products of lipid peroxidation create and amplify oxidative stress. In turn, oxidative stress can cause liver injury (e.g. by triggering apoptosis and inciting inflammation). The mechanisms that may trigger and perpetuate inflammatory recruitment in NASH, and the importance of cytokines such as tumour necrosis factor-α (TNF-α) are discussed in Chapter 10.

Evidence has been deduced from human studies as well as in experimental models that cytochrome P450 2E1 (CYP2E1) is overexpressed in steatohepatitis [66–68], most likely because of impaired insulin receptor signalling. CYP2E1 is a potential source of reduced (reactive) oxygen species (ROS). In the absence of CYP2E1, CYP4A takes on the role as an alternative microsomal lipid oxidase, and it too may generate ROS [67]. CYP2E1 and CYP4A catalyze the ω and ω-1 hydroxylation of long-chain fatty acids. The products are dicarboxylic fatty acids, which cannot be subjected to mitochondrial β-oxidation and are so targeted to the peroxisome for further oxidation. In turn, this generates hydrogen peroxide (coupled to catalase) as an essential by-product [69].

The relative importance of metabolic sites of ROS generation in hepatocytes (mitochondria, endoplasmic reticulum, peroxisomes), and products of the inflammatory response in contributing to oxidative stress in steatohepatitis remains unclear; interactive processes are likely to operate [64]. However, mitochondria could be a critical source of ROS in fatty liver disorders (see Chapter 11) [38,70].

Hepatic inflammation and cellular injury to hepatocytes can induce and activate transforming growth factor-β (TGF-β), which has a key role in activating stellate cells to elaborate extracellular matrix as part of the wound healing process. It is now apparent that leptin has a key role in hepatic fibrogenesis, and leptin also appears to be necessary for appropriate liver regeneration as part of the ‘wound healing’ response to chronic steatohepatitis and other forms
of liver injury (see Chapter 12). Thus, leptin, originally characterized as an anti-obesity hormone acting on the central nervous system to regulate appetite, could have multiple roles in the pathogenesis of NASH by modulating fat deposition in hepatocytes (anti-lipotoxicity), and regulating the hepatic fibrotic and regenerative response to steatohepatitis. A more detailed account of the cell biology of NASH is presented in Chapter 12.

**Approaches to management of NASH**

**Lifestyle adjustments**

Attempts to correct steatosis and liver injury in NASH can begin before the diagnostic process is complete (see Chapter 15). The aim is to correct insulin resistance and central obesity. Rapid and profound weight loss is potentially dangerous for the person with fatty liver disease [3]. It is prudent and more realistic to recommend slow reductions in body weight that are achievable and sustainable by permanent changes in lifestyle. It has been shown that such reductions improve liver tests [71], and there is mounting evidence that this is associated with removal of fat from the liver, decreased necroinflammatory change and even resolution of fibrosis [72,73].

In accordance with the results of recent type 2 diabetes intervention studies [74,75], physical activity should include at least 20 min of exercise each day (140 min/week), equivalent to rapid walking. The essentials of dietary modification are the same as for diabetes: reduce total fat to less than 30% of energy intake, decrease saturated fats, replace with complex carbohydrates containing at least 15 g fibre, and rich in fruit and vegetables. Consideration of low versus high glycaemic foods (e.g. brown or basmati rice versus conventional long or short-grain white rice); reduction of simple sugars and alcohol intake is also likely to be beneficial.

Some authors have advocated referral to a dietitian or ‘personal case manager’ to provide education and closer supervision of dietary regimens and lifestyle interventions [73–75]. Approaches to lifestyle modification and weight reduction are discussed in more detail in Chapter 15. The effectiveness and cost-efficacy of such approaches are important aspects that warrant further study.

**Measures to control hyperlipidaemia and hyperglycaemia**

Increased physical activity and low-fat diet improve insulin sensitivity and can, in some cases, reverse insulin resistance. The value of exercise in improving glycaemic control in diabetes is now generally accepted. In other respects, treatment of diabetes in patients with NASH should conform to conventional approaches, although this may change in future if drugs that help reverse insulin resistance live up to initial promise against NAFLD/NASH without causing unacceptable weight gain. These agents include metformin and the thiolazinediones (see Chapter 16). Drugs that correct lipid disorders, anti-oxidants (vitamin E, betaine) and other hepatoprotective agents (ursodeoxycholic acid) are also under study in NASH (see Chapter 16).

**Concluding remarks: can NAFLD/NASH be prevented or reversed?**

Because liver failure does not occur in NAFLD/NASH unless cirrhosis has developed, reducing or reversing fibrotic progression must be the ultimate objective of treatment. While several agents improve liver tests over the short term in patients with NAFLD/NASH (see Chapter 16), none have yet (June 2003) been shown to have long-term efficacy and to impact on fibrotic progression (but see Chapter 24). In the absence of evidence of such efficacy, patients should currently only receive drug therapy directed at NASH within the context of a clinical trial, particularly as some of the compounds presently under study carry toxic potential or other unwanted effects (see Chapters 16 and 24).

There is now compelling evidence that type 2 diabetes can be prevented (or at least delayed in onset) by lifestyle interventions [74,75]. Both the Finnish and US Diabetes Intervention Projects showed a 58% reduction in incidence of type 2 diabetes among those at high risk could be achieved with only modest reductions in body weight [74,75]. NASH, another consequence of insulin resistance (see Chapter 5), should also be preventable by changes in diet and physical activity. There is now evidence that weight reduction and lifestyle changes nearly always improve liver tests in NAFLD, and also have potential to improve liver
histology in obese patients with hepatitis C or fatty liver disorders [71–73] (Chapter 24). Whether this approach would be a cost-effective way to reduce the number of patients progressing to cirrhosis and liver failure is clearly worthy of study.

References


41 www.cdc.gov/nccdphp/dnpa/obesity/trends/maps/index.htm


Abstract

This chapter provides general background information on the pathology of NAFLD/NASH for non-pathologists, as well as practical help for anatomical pathologists who report liver biopsies. The main emphasis is on the definition and illustration of the various patterns of liver injury that form the broad spectrum of injury encompassed by the terms non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH). Difficult concepts, such as the essential requirements and minimal requirements for a diagnosis of NASH, are addressed. Currently, the broader term NAFLD is probably preferable because it embraces simple steatosis and non-specific steatohepatitis than does the more narrow term NASH, in which the pathology is virtually identical to that seen in alcoholic hepatitis and which is usually complicated by fibrosis. An approach is suggested for the diagnosis of cirrhosis associated with NASH and ‘cryptogenic’ cirrhosis seen in people with clinical risk factors for NASH. Finally, the relatively new concept that hepatocellular carcinoma (HCC) forms part of the spectrum of NASH complicated by cirrhosis is discussed briefly.

Introduction

In a landmark study in 1980, Ludwig et al. [1] described a series of patients who lacked a history of ‘significant’ alcohol intake but in whom the liver histology resembled that of alcoholic liver disease. They coined the term NASH to describe the principal features of
this condition; namely, hepatic steatosis and inflammation and an aetiology that was ‘non-alcoholic’. During the next two decades it became apparent that the histopathological definition of NASH was subject to a wide range of interpretations. In many studies, the presence of mild focal macrovesicular steatosis and lobular inflammation, mainly or exclusively composed of mononuclear cells, was regarded as sufficient for the histological diagnosis of NASH, while some insisted on the presence of ballooning degeneration, and still others required neutrophils and/or fibrosis. There is still no international consensus regarding the histopathological criteria for the diagnosis of NASH. Some have proposed that in addition to steatosis and lobular inflammation, either ballooning degeneration or perivenular or pericellular fibrosis should be present [2,3] (see also Chapter 24).

In their first paper on grading and staging NASH, Brunt et al. [4] stated that in grade 1 injury one ‘may see occasional ballooned zone 3 hepatocytes’, but in a subsequent review article Brunt et al. [5] required hepatocellular ballooning to be present for the diagnosis of NASH. Burt et al. [6] used the term steatohepatitis when steatosis, ballooning of hepatocytes and any degree of centrilobular fibrosis was present, while Diehl et al. [7] regarded centrilobular fat accumulation, and Mallory bodies or zone 3 perivenular and pericellular fibrosis as cardinal features of NASH. Some pathologists occasionally make a diagnosis of ‘NASH’ even in the absence of steatosis (B. Brunt, personal communication, see also Chapter 24). Presumably, this is when the clinical setting is appropriate for NASH and the biopsy shows all the features required for a diagnosis of NASH apart from steatosis. However, it seems counter-intuitive to use a diagnostic term that includes steatosis in cases where there is no steatosis.

Clinicopathological studies have been vexed by these inconsistencies, leading to considerable confusion amongst pathologists, clinicians and patients. In an attempt to ‘tighten the screws’, Lee [2] suggested that the diagnosis of NASH should be reserved for liver biopsies in which the pathology closely resembles that of alcoholic steatohepatitis. Sheth et al. [3], Brunt et al. [4], Brunt [5] and Burt et al. [6], amongst others, have supported this suggestion. The features in liver biopsies diagnosed as NASH should fulfil the criteria for alcoholic hepatitis laid down by the International Hepatopathology Study Group: hepatocyte necrosis and the presence of neutrophils amongst the inflammatory cells, with or without Mallory bodies [8]. Although liver injury diagnosed as NASH should be indistinguishable from alcoholic hepatitis, the liver injury is generally less severe, with fewer or no Mallory bodies [6,8–11]. In addition, some of the patterns of injury (e.g. sclerosing hyaline necrosis) seen in alcoholic hepatitis are not usually evident in NASH [9].

According to such rigid criteria, milder forms of steatohepatitis, which bear little resemblance to alcoholic hepatitis, are effectively excluded from being designated as NASH, leading to the apparent paradox that ‘steatosis + inflammation + insignificant alcohol intake’ do not necessarily equal ‘non-alcoholic steatohepatitis’. In addition, many hepatopathologists, who work with animal models for alcohol-induced liver injury, point out that alcohol-related liver injury in humans is also frequently non-specific-without Mallory bodies and with few or no polymorphs, rather than ‘classic’ steatohepatitis with ballooning, neutrophil polymorphs and Mallory bodies, to support the validity of their models [12]. Again, it is paradoxical that the same non-specific pattern of steatohepatitis in human non-drinkers, which is identical to that seen experimentally in association with alcohol, should not be designated by the words ‘non-alcoholic steatohepatitis’.

To overcome some of the problems outlined above, Matteoni et al. [13] suggested the term ‘non-alcoholic fatty liver diseases’ (NAFLD), which they divided into four categories:

- **Type 1**: steatosis alone
- **Type 2**: steatosis plus lobular inflammation
- **Type 3**: steatosis, lobular inflammation and ballooning degeneration of hepatocytes
- **Type 4**: steatosis, ballooning degeneration and Mallory bodies and/or fibrosis

NAFLD is a useful ‘umbrella’ term that covers a broad spectrum of liver injury and encompasses steatosis (type 1), a pattern of non-specific steatohepatitis that does not resemble alcoholic hepatitis (type 2) and NASH (types 3 and 4). The finding that NAFLD types 3 and 4 are associated with the worst clinical outcomes provides support for such a classification [13].

Ludwig et al. [1], in the initial paper on NASH, used the term ‘insignificant amounts of alcohol’ and reported that ‘most patients had less than one drink a week’. However, there is a lack of consensus as to what constitutes ‘insignificant’ or ‘negligible’ alcohol intake. A recent review on NASH reports on studies that have allowed from 40 to 210 g/week ethanol [14]. It is

**CHAPTER 2**
possible that the alcohol, particularly the higher doses, is contributing to liver injury, in at least some if not all of these patients.

**Liver pathology**

**Light microscopy**

**Steatosis**

Steatosis (fatty liver) is characterized by the accumulation of fat droplets in hepatocytes. In NAFLD the fat is seen mainly as large single macrovesicular droplets that displace the nucleus to the periphery of the cell (Plate 1, facing p. 22); a lesser amount of microvesicular fat may be seen as large numbers of smaller droplets surrounding a central nucleus (Plate 2). In early or mild NAFLD, the fat is seen in zone 3 hepatocytes. Simple steatosis is reversible in a matter of days to weeks.

Biochemically, steatosis is defined as an accumulation of lipid in the liver exceeding 5% of the liver weight [15]. We, and others, consider the presence of fat droplets in up to 5% of hepatocytes as within normal limits [16], while others regard the presence of any steatosis as abnormal and allocate a score of 1 for even the mildest forms (Table 2.1) [4,5]. When the steatosis is entirely microvesicular in type, other aetiologies including alcohol and drugs and, where appropriate, acute fatty liver of pregnancy should be considered. Steatotic livers may also contain ‘fat cysts,’ and lipogranulomas that are mainly located in zone 3, and are composed of aggregates of lipid-laden macrophages that stain positively with an antibody to CD 68.

There is uncertainty about the minimum criteria for the diagnosis of any type of hepatitis in fatty livers. The presence of one or two focal collections of mononuclear cells in the parenchyma (Plate 1) or occasional mononuclear cells in the portal tracts is not sufficient to warrant a diagnosis of NAFLD/NASH types 2–4. Nor does the existence of one or more clinical risk factors for NASH justify the designation of simple fatty liver as NASH in the absence of hepatocyte injury and a mixed inflammatory infiltrate. However, a diagnosis of NAFLD type 1 would be appropriate in such livers.

**Alcoholic hepatitis**

The essential features are steatosis, hepatocyte necrosis and a neutrophil polymorph infiltrate. Ballooned hepatocytes and Mallory bodies are frequently seen but are not obligatory for the diagnosis of alcoholic hepatitis [7,17].

**Steatohepatitis**

This is a term that implies the presence of both fatty change and hepatocyte injury accompanied by inflammation. Ludwig et al. [1] made the selection criteria for inclusion in their study ‘moderate to severe macrovesicular fatty change and lobular inflammation’. They further described the features in liver biopsies as focal necrosis and a mixed inflammatory infiltrate. Most of their cases contained Mallory bodies and showed varying degrees of fibrosis. Thus, the originally described features of NASH (Plates 3–6) clearly resemble those of alcoholic hepatitis.

Hepatocyte injury can be in the form of ballooning degeneration that is reversible, or hepatocyte necrosis or apoptosis that is irreversible. Some [4,5,16], but not all authors [7,18], consider the presence of ballooning degeneration as an absolute requirement for a diagnosis of NASH. Ballooned hepatocytes are enlarged and have pale cytoplasm as a result of fluid retention (Plates 3, 4). The problem is that small fat droplets can give the cytoplasm a ‘cobweb-like’ appearance that closely resembles that of mildly hydropic cells. Further, in end-stage cirrhosis, bile stasis, particularly in hepatocytes at the periphery of the regeneration nodules, results in hydropic change that gives the cells a ballooned appearance.

Fat stains (oil red O on frozen tissue, or post-fixation in osmium tetroxide), which are not routinely performed, are required to reliably distinguish between fluid and fat. Apoptotic hepatocytes, seen as shrunken eosinophilic cells with pyknotic nuclei, can be seen in NASH but are never as prominent as in viral hepatitis. Necrotic hepatocytes are not usually prominent, but a mixed inflammatory infiltrate comprising neutrophils, lymphocytes and ceroid-laden Kupffer cells can be seen at the sites where necrotic hepatocytes have disappeared. Again, some authors [4,5], but not others [7,18], require neutrophils for a diagnosis of NASH.

**Table 2.1** Grading of steatosis. (After Brunt [5], with permission of the author.)

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Fat droplets in &lt; 33% hepatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2</td>
<td>Fat droplets in 33–66% hepatocytes</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Fat droplets in &gt; 66% hepatocytes</td>
</tr>
</tbody>
</table>
Mallory bodies are seen in hepatocytes, particularly in zone 3 and especially in those cells showing balloon-
degeneration. They appear as irregularly shaped, deep-
ey eosinophilic masses in the cytoplasm (Plate 3). Mallory bodies are composed of cytokeratin polypeptides, which stain with an antibody to ubiquitin [19]. They are often seen in NASH but, as the case in alco-
holic hepatitis [8], the presence of Mallory bodies is not obligatory for a diagnosis of NASH.

Another unresolved problem is how much necroin-
flammation is required for a diagnosis of NASH; to some extent this can be overcome by using a grading system either as words (mild, moderate, marked) or a numerical grade (1–3) (Table 2.2).

**Fibrosis and cirrhosis**

In both alcoholic hepatitis and NASH, fibrosis is first seen in zone 3 (centrilobular region). The fibrosis is characteristically pericellular in distribution (Plate 4), but perivenular fibrosis may also be present. Some authors advocate the presence of early fibrosis as an essential feature for the diagnosis of NASH [6,18]. In children with NASH (see Chapter 19 for a more detailed discussion) the fibrosis tends to be in the portal tracts rather than in zone 3 (Plate 5A, B).

In clinical series, approximately 20% of patients with NASH progress to cirrhosis [2,7,13]. In the staging of NASH, the fibrosis can progress, albeit slowly, to cirrhosis (Plate 6). In established cirrhosis, there is complete loss of the normal lobular architecture and replacement by regenerative nodules of hepatocytes that are completely surrounded by bands of fibrous tissue [7]. Marked fibrosis can be seen in haematoxylin and eosin (H&E) stained sections, but special stains such as Sirius red (Plate 7), van Gieson or Masson trichrome are needed for a more accurate estimate of the severity of fibrosis. The Sirius red stain is preferred for morphometric analysis because, unlike the Masson trichrome stain, it reacts specifically with collagen and does not stain other matrix proteins [20].

Brunt [5] has developed and refined a staging system for fibrosis in NASH (Table 2.3). Cirrhotic livers usually show active steatohepatitis (personal observation of the editors). However, in end-stage liver disease coming to transplantation, the hepatic steatosis and necroinflammation may no longer be apparent. In this situation, the cirrhosis is described as ‘inactive’ (cryptogenic cirrhosis) and NASH is sometimes designated as ‘burnt-out’ [21].

**Glycogenated nuclei**

The presence of pseudo-inclusions of glycogen in hep-
atocyte nuclei is non-specific, but they are frequently seen in diabetes mellitus [22] and are therefore a frequent finding in NASH.

**Electronmicroscopy**

_Megamitochondria (giant mitochondria)_

Enlarged mitochondria, often containing paracrystal-
ineclusions, are a frequent ultrastructural finding in
NASH (Fig. 2.1) (Plate 5(b)) [23]. Megamitochondria are well recognized in alcoholic liver disease, even in the early stages, and occasionally they can even be recognized by light microscopy as eosinophilic intracytoplasmic globules in H&E-stained sections of liver. However, because giant mitochondria are now becoming increasingly recognized in NASH in both humans [23] and animal models [24], their presence in liver biopsies showing steatohepatitis does not necessarily point to an alcoholic aetiology. The causes and effects of mitochondrial injury in NASH are discussed in Chapter 11.

Clinicopathological correlation

Histopathologists cannot make a diagnosis of NASH purely on morphological grounds; clinicopathological correlation is essential. Problems for the histopathologist include the following:

• Whether or not to use the term NASH when the steatosis is mild, hepatocyte injury minimal, without ballooning, and the inflammatory infiltrate is composed purely of mononuclear cells. This is a particular problem when the patient does not drink alcohol but has one or more risk factors for NAFLD/ NASH. Currently, many pathologists prefer to term this pattern of injury NAFLD type 2 because the features do not meet the strict criteria for a diagnosis of NASH.

• What terminology to use when the alcohol history is not known or has not been stated on the request form. It is unwise to use the term ‘non-alcoholic’ in the diagnosis under these circumstances; rather, a diagnosis of ‘steatohepatitis of uncertain aetiology’ is suggested, along with a recommendation for clinicopathological correlation.

During the last decade, clinicians and pathologists have moved from an era of incorrectly diagnosing alcoholic hepatitis in people who were non-drinkers to overdiagnosing NASH, sometimes in excessive alcohol drinkers. At this stage, the significance of this milder form of non-specific hepatitis, especially in patients with risk factors for NASH, is uncertain. A small study of serial liver biopsies from patients with psoriasis receiving low-dose methotrexate showed a subset with risk factors for NASH. These patients all had non-specific steatohepatitis, not ‘classic’ NASH, yet showed progressive liver fibrosis while on methotrexate unlike those who had no risk factors for NASH [18].

Pathologists are frequently asked for advice about the need for liver biopsy in patients in whom NASH is suspected on clinical grounds. The results of a study by Angulo et al. [25] enable the pathologist to provide some guidance to clinicians. The only correlate for steatosis was the body mass index (BMI), while for steatohepatitis a high correlation was found between the severity of fibrosis and the following:

• BMI
• Older age
• Type 2 diabetes mellitus (insulin resistance)
• Aspartate aminotransferase : alanine aminotransferase (AST : ALT) ratio > 1
• Female gender [26].

Studies are currently in progress to determine whether female gender is indeed a risk factor for progressive liver injury (J. George, personal communication). This type of information has enabled better selection of patients in whom a liver biopsy is likely to yield significant pathology (NASH or NAFLD types 3 and 4) (see also Chapter 24).
Differential diagnosis

From the pathologist’s perspective, the main differential diagnosis is between alcoholic hepatitis and NASH [10,11]. It is not possible to differentiate between the two conditions purely on morphological grounds; however, the more severe the hepatitis in terms of the amount of necroinflammation, the greater the number of Mallory bodies and the higher the stage of fibrosis, the more likely the injury is caused by alcohol rather than metabolic factors [7].

Increasingly, drugs are being reported as a cause of liver injury that is identical to alcoholic hepatitis and often of equal or greater severity. As discussed in Chapter 1, the editors recommend that this be called drug-induced steatohepatitis rather than NASH, particularly because different pathogenic mechanisms may be involved.

Coexistent liver disease

NAFLD/NASH may coexist with a number of other liver diseases including hepatitis C, hepatic iron overload (both primary in association with HFE mutations, and secondary overload resulting from a range of causes), primary biliary cirrhosis and α_{1}-antitrypsin deficiency (for a detailed discussion see Chapter 23) [27].

Chronic hepatitis C

Steatosis has long been recognized as a frequent morphological finding in liver biopsies from patients with hepatitis C [28]. The steatosis in hepatitis C may be related to the direct ability of the virus, in particular genotype 3, to induce steatosis [29,30], and/or to alcohol or to the presence of risk factors for NASH (see also Chapter 24).

Steatosis in hepatitis C, when associated with risk factors for NASH, is an independent predictor of fibrosis [29] and has been shown to accelerate the progression of liver damage [31].

From a histological perspective, steatosis associated with hepatitis C per se differs from that of NAFLD in that it is usually focal, without a zonal distribution, and is not associated with ballooning degeneration, Mallory bodies or pericellular or perivenular fibrosis [28]. The presence of these features in a biopsy from a patient with hepatitis C is strongly suggestive of coexistent NASH.

Hepatic siderosis

Increased stainable iron, usually mild (grade 1–2), has been documented in liver biopsies from patients with NASH; the frequency varies from 18 to 65% (Plate 8) [4,31].

Several studies have reported an increased prevalence of HFE gene mutations in patients with NASH [32–34]. One study suggested a correlation between increased hepatic iron and fibrosis [32], but this has not been confirmed by later studies [26,35]. Hepatic iron overload has been documented in liver biopsies from patients with the insulin resistance syndrome [36].

It has been suggested that iron may serve as a ‘second hit’ in steatotic livers, leading to the development of NASH [37]. This suggestion is supported by a study using an animal model of NASH in which excess dietary iron increased the amount of necroinflammation in steatotic livers (see Chapters 7 and 8) [38].

A Perls’ Prussian blue stain for iron should be performed on liver biopsies showing NASH; the report should include a comment about the absence or presence of excess iron, and the grade of iron 1–4 (the iron grading relates to the percentage of hepatocytes that contain stainable iron: grade 1, up to 25%; grade 2, 25 to 50%; grade 3, 50 to 75%; and grade 4, iron in 75 to 100% of hepatocytes).

NAFLD/NASH in children

NAFLD is increasingly recognized in the paediatric population, particularly in obese children in the peri-pubertal years [39–42]. Although the full spectrum of morphological changes seen in adult NAFLD can be seen in children [40,41], it is noteworthy that in paediatric NAFLD the tendency is for inflammation and fibrosis to be predominantly in the portal tracts (Plate 5A–D) [39,40,42]. In particular, Baldridge et al. [39] drew attention to the invariable presence of a mixed inflammatory infiltrate in portal tracts, and portal fibrosis with only rare foci of lobular inflammation in some cases, and only one case with Mallory bodies. Hepatic fibrosis is usual and tends to occur early, but cirrhosis is not regarded as a frequent component of paediatric NASH [39,43]. Nevertheless, there have been recent case reports of children developing cirrhosis within 1–3 years of presentation with NASH [41]. Other causes of steatohepatitis, such as metabolic