NON-ALCOHOLIC STEATOHEPATITIS – DIAGNOSTIC CHALLENGES

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OVERVIEW

*Non-alcoholic fatty liver disease* (NAFLD) indicates evidence of fat in the liver, either by imaging or histology, in a patient without a reason to have secondary fat accumulation (e.g. significant alcohol consumption, use of certain medications, or inherited storage defects, see Table 1). Significant alcohol consumption for the purpose of clinical consideration of fatty liver is defined as ongoing or recent consumption of >21 drinks on average per week, in men, and > 14 drinks on average per week in women [1].

**Table 1. Secondary hepatic macrovesicular fat deposition**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cause</th>
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<tbody>
<tr>
<td>Excess alcohol</td>
<td>Abetalipoproteinemia</td>
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<tr>
<td>Hepatitis C (particularly genotype 3)</td>
<td>Medications (e.g.)</td>
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<tr>
<td>Wilson disease</td>
<td>Amiodarone</td>
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<tr>
<td>Lipodystrophy</td>
<td>Methotrexate</td>
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<tr>
<td>Starvation</td>
<td>Tamoxifen</td>
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<tr>
<td>Parenteral nutrition</td>
<td>Corticosteroids</td>
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Histologic examination of liver tissue is required to sub-classify NAFLD as *non-alcoholic fatty liver* (NAFL) or *non-alcoholic steatohepatitis* (NASH) [1]. NAFL represents steatosis without histologic liver injury while NASH represents steatosis with histologic evidence of liver injury (i.e. ballooned hepatocytes, inflammation, and fibrosis).

**Non-alcoholic steatohepatitis (NASH) key pathologic features**

- Steatosis >5%
- Mixed acinar inflammation
- Hepatocellular ballooning and/or pericellular fibrosis

The risk of progression to advanced fibrosis in NAFL is minimal while in NASH, progression to cirrhosis and/or development of hepatocellular carcinoma (HCC) is well described [2]. *NASH cirrhosis* is defined as cirrhosis with current or previous evidence of NAFLD.

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1 Gill R. M. and Kakar S. Non-alcoholic steatohepatitis, an update on diagnostic challenges, Surgical Pathology Clinics, Volume 6, Issue 2, Pages 227-257, June 2013, adapted with permission from Elsevier.
The median worldwide prevalence of NAFLD is estimated at 20% and the prevalence of NASH reportedly ranges between 3-5% [1, 2]. The prevalence of NAFLD in the overweight/obese US adult population is probably significantly higher [1]. Risk factors for NASH include metabolic syndrome, dyslipidemia, diabetes mellitus type 2, and obesity [1] (Table 2).

<table>
<thead>
<tr>
<th>Metabolic diseases (acquired)</th>
<th>Drugs</th>
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<tbody>
<tr>
<td>Obesity</td>
<td><em>Definite association</em></td>
</tr>
<tr>
<td>Diabetes, type 2</td>
<td>Amiodarone</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>Chemotherapeutic agents like irinotecan</td>
</tr>
<tr>
<td>Rapid weight loss</td>
<td><em>Questionable etiologic association; may exacerbate or precipitate NASH</em></td>
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<tr>
<td>Malnutrition</td>
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<tr>
<td><strong>Metabolic diseases (genetic)</strong></td>
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<tr>
<td>Wilson disease</td>
<td>Tamoxifen</td>
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<tr>
<td>Tyrosinemia</td>
<td>Steroids</td>
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<tr>
<td>Abetalipoproteinemia</td>
<td>Estrogens</td>
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<tr>
<td><strong>Other</strong></td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Lipodystrophy</td>
<td>Calcium channel blockers (like nifedipine, Verapamil, and diltiazem)</td>
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<td>Jejunoileal bypass</td>
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Metabolic syndrome is defined as central obesity and insulin resistance, which characteristically manifests with at least three of the following: blood pressure >130/85 mmHg, increased waist circumference (>102 cm in men and >88 cm in women), fasting blood sugar >110 mg/dL, triglycerides >150 mg/dL, and low HDL (i.e. <40 mg/dL in men, <50 mg/dL in women).

There are no clinical or radiological tests that can reliably diagnose steatohepatitis and serum transaminases often correlate poorly with biopsy findings [1, 3]. Microscopic examination of a liver biopsy by a pathologist remains the gold standard for diagnosis of NAFLD.

Comprehensive practice guidelines have recently been published for the diagnosis and management of NAFLD [1]; some of these recommendations (Chalasani et al. make a total of 45 recommendations) are highlighted in this syllabus. For example, it is recommended that liver biopsy should be considered in all NAFLD patients who are at increased risk for steatohepatitis and advanced fibrosis [1]. They recommend that the presence of metabolic syndrome and the NAFLD fibrosis score be used to identify patients who are at increased risk for steatohepatitis and advanced fibrosis [1]. Liver biopsy should also be considered in NAFLD patients if there are competing etiologies for hepatic steatosis (as detected by imaging) and whenever coexisting chronic liver disease cannot be excluded without a liver biopsy [1]. Biopsy is currently not recommended in asymptomatic patients with incidental hepatic steatosis on imaging and no other evidence of liver disease [1].
MIROSCOPIC FEATURES
Three microscopic features are essential for the diagnosis of steatohepatitis [4, 5] (Table 3): steatosis, inflammation, and hepatocellular injury in the form of hepatocyte ballooning and/or pericentral fibrosis. Significant steatosis is defined as >5% macrovesicular steatosis (includes both large and small droplet fat), usually with pericentral accentuation. Inflammation is usually mild and more prominent in the lobular parenchyma, with or without a component of mild portal inflammation. Neutrophils are typically present, usually in small numbers, but can surround ballooned hepatocytes (i.e. neutrophil satellitosis), as is more commonly seen in alcoholic steatohepatitis.
Lymphocytes and histiocytes are also common with lipogranuloma formation often noted. Occasional eosinophils, pigmented macrophages, and microgranulomas may be present. Steatohepatitic hepatocellular injury manifests as ballooned hepatocytes or, in the chronic phase, as pericellular fibrosis around central veins. Hepatocellular ballooning is characterized by an increase in cell size, rarefaction of cytoplasm and condensation of cytoplasm into eosinophilic globular areas [4]. When conspicuous, the globular structures are referred to as Mallory hyaline. All three characteristics must be present for definite interpretation as hepatocellular ballooning. Hepatocytes with small droplet steatosis or with excessive glycogen can be enlarged or clear, but do not have all three characteristics and should not be interpreted as ballooned hepatocytes. Spotty hepatocyte necrosis is also common and does not necessarily support a viral etiology. Pericellular fibrosis involving sinusoids in zone 3 results in a “chicken-wire” or “spider-web” pattern of fibrosis.

| Table 3. Histologic features of nonalcoholic steatohepatitis. Adapted from AASLD conference summary on NASH, 2002 [4] |
|---|---|
| **Essential features** | **Often present, not essential for diagnosis** |
| 1. Steatosis, predominantly macrovesicular, concentrated in zone 3 | 1. Glycogenated nuclei in zone 1 |
| 2. Mild mixed acinar inflammation | 2. Lipogranulomas in the lobular parenchyma or portal tracts |
| 3. Hepatocellular injury in the form of | 3. Occasional acidophil bodies |
|   (a) Hepatocellular ballooning, often most prominent in zone 3, and/or | |
|   (b) Pericellular fibrosis | |
| **May be present, not essential for diagnosis** | **Unusual features** |
| 1. Mallory hyaline in zone 3, typically inconspicuous | 1. Predominantly microvesicular steatosis |
| 2. Mild iron deposits in hepatocytes or sinusoidal cells | 2. Prominent portal and/or acinar inflammation, numerous plasma cells |
| 3. Megamitochondria | 3. Prominent bile ductular reaction, cholestasis |
| 4. Glycogenated nuclei in zone 1 | 4. Perivenular fibrosis, hyaline sclerosis |
| 5. Lipogranulomas in the lobular parenchyma or portal tracts | 5. Marked lobular inflammation |

NASH Grading and Staging
The extent of fibrosis documented in a biopsy can influence clinical decisions and should always be included in the pathology report as the fibrosis “stage.” For example, presence of fibrosis may prompt the hepatologist to pursue underlying risk factors more aggressively and treatment consideration may include more aggressive management (e.g.
gastric bypass surgery for obesity or enrollment in clinical trials). The degree of fibrosis is important for monitoring disease progression in subsequent biopsies and documentation of advanced fibrosis (stage 3 or 4) will allow for adequate planning for transplantation. Unlike chronic viral hepatitis, which is a portal-based disease, fibrosis in steatohepatitis typically starts around the central vein. Therefore the staging systems used in chronic viral hepatitis such as the Batts-Ludwig methodology [6], are not appropriate for NASH. Two staging schemas are available for NASH, the Brunt methodology [7] and the NASH clinical research network (CRN)/Kleiner [5] modification to this methodology (Tables 4 and 5). In practice the Brunt methodology is sufficient for most clinical reports.

<table>
<thead>
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<th>Table 4. Staging of steatohepatitis: Brunt methodology [7]</th>
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<tr>
<td>Stage 0</td>
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<td>Stage 1</td>
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<td>Stage 2</td>
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<td>Stage 3</td>
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<td>Stage 4</td>
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<th>Table 5. Staging of steatohepatitis: NASH CRN modified Brunt methodology [5]</th>
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<tr>
<td>Stage 0</td>
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<tr>
<td>Stage 1</td>
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<tr>
<td>1A. Mild zone pericellular fibrosis, requires trichrome for identification</td>
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<tr>
<td>1B. Moderate zone 3 pericellular fibrosis, often apparent on H&amp;E stain</td>
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<tr>
<td>1C. Portal/periportal fibrosis only</td>
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<tr>
<td>Stage 2</td>
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<td>Stage 3</td>
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<td>Stage 4</td>
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In addition to the general pitfalls of staging chronic hepatitis (e.g. subcapsular biopsy, fragmentation, limited sample, tangentially cut portal tracts), some problems specific to steatohepatitis include trichrome staining quality (overstaining can lead to a trichrome stain with thin blue strands lining the sinusoids mimicking pericellular fibrosis), lipogranuloma related staining (i.e. focal areas of fibrosis in a nodular configuration, especially around central veins, may be due to lipogranulomas and should not be considered in staging; fibrosis should be considered to be present only if pericellular extension along the sinusoids is seen), and recognition of portal-based fibrosis in the absence of pericellular fibrosis around the central vein, which while uncommon in steatohepatitis, can occur in children and some adult patients with NASH [8].

Unlike staging, the clinical utility of grading in steatohepatitis is not clearly established. At present, clinical decisions are not dictated by the histologic grade. Therefore, grading of steatohepatitis is not considered a necessary component of the pathology report. Hepatologists are often interested in the grade of steatosis (as described in Table 6), but not in grading of other elements like inflammation and ballooning.
The NAFLD activity score (NAS) scheme [5] builds on the earlier Brunt grading scheme [7] and is used by the NASH CRN in case evaluation (Table 6). The NAS score is defined as the sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2). NAS can therefore range from 0-8. The NAS is not intended to replace a pathologist’s diagnostic determination of steatohepatitis, but can be reported if grading is desired.

<table>
<thead>
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<th>Table 6. NASH activity score (NAS) [5]</th>
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<tr>
<td><strong>Histological feature</strong></td>
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<tr>
<td>Steatosis</td>
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<tr>
<td>Lobular inflammation*</td>
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<tr>
<td>Ballooning**</td>
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*The number of foci was counted per 200x field for lobular inflammation
**Few ballooned cells indicate rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline

**DIFFERENTIAL DIAGNOSIS**

While many cases of NASH will have classic morphologic features of pericentral ballooned hepatocytes, significant steatosis, lobular mixed inflammation, and pericellular fibrosis, we have encountered a number of variations on these classic features that can result in diagnostic confusion. Overall there are six patterns of NAFLD to recognize:

**NAFLD patterns of injury***

1. Steatosis, mixed inflammation, ballooned hepatocytes, and pericellular fibrosis → NASH
2. Steatosis without hepatocellular injury → NAFL
3. Steatosis with swollen hepatocytes → NAFL vs. borderline NASH
4. Ballooned hepatocytes or pericellular fibrosis without steatosis → NASH in the appropriate clinical context
5. Pericellular fibrosis and steatosis, no ballooned hepatocytes → NASH
6. Cirrhosis with steatosis or history of NAFLD risk factors → NASH cirrhosis

*NASH = non-alcoholic steatohepatitis; NAFL = non-alcoholic fatty liver; NAFLD = non-alcoholic fatty liver disease. Classification as a form of NAFLD requires no history of significant alcohol consumption.

**Pattern 1:** Steatosis with mild inflammation, hepatocellular ballooning, and/or pericellular fibrosis
Classic steatohepatitis pattern of injury, which can be considered “chronic active steatohepatitis.”

**Pattern 2: Steatosis without hepatocellular injury**
Steatosis without hepatocellular injury in the form of ballooning or pericellular fibrosis is insufficient for a diagnosis of steatohepatitis and therefore represents NAFL, which has a low rate of progression (<5%) to significant fibrosis or cirrhosis [9].

**Pattern 3: Steatosis with swollen hepatocytes**
Hepatocellular ballooning should be interpreted only if all three features of ballooning are present (i.e. swelling, cytoplasmic clearing, and condensation of cytoplasm into eosinophilic globular areas). Well-formed Mallory hyaline is not an essential feature, but supports the diagnosis when present. If clear-cut hepatocellular ballooning is not present, but hepatocellular changes are suspicious for ballooning, then the findings should be regarded as borderline for steatohepatitis and it may be best to manage the patient as appropriate for steatohepatitis. It is thought that injured hepatocytes in steatohepatitis have defective protein turnover with ubiquitination and loss of keratin 8/18 (and thus loss of keratin 8/18 by immunohistochemical staining may be used for identification of ballooned hepatocytes in challenging cases)[10, 11].

**Pattern 4: Ballooned hepatocytes or pericellular fibrosis without steatosis**
Ballooned hepatocytes without steatosis can be seen with amiodarone use or in patients who have recently stopped taking alcohol. This pattern is uncommon in patients with metabolic risk factors. In addition to steatohepatitis, pericentral/sinusoidal fibrosis can be seen in chronic vascular outflow obstruction or parenchymal rejection (in the post-liver transplant setting). Sinusoidal fibrosis also occurs in diabetic hepatosclerosis, but tends to be patchy and non-zonal.

**Pattern 5: Steatosis with pericellular fibrosis, but no ballooned hepatocytes**
In the absence of ballooning, these changes are likely to represent chronic steatohepatitic injury. Pericellular fibrosis can also be due to chronic venous outflow obstruction (as in right heart failure or Budd-Chiari syndrome), chemotherapeutic agents like oxaliplatin (a drug used to treat colorectal cancer), or, in the post-transplant setting, with remote parenchymal rejection, as noted for Pattern 4, but together with significant steatosis, steatohepatitis is the likely etiology. Other possible etiologies can be mentioned in the pathology report, depending on the clinical context.

**Pattern 6: Cirrhosis with steatosis and/or ballooned hepatocytes**
Cirrhosis with histologic evidence of NAFLD is best considered NASH cirrhosis. Some cases may also show residual pericellular fibrosis, though this is somewhat less specific in the setting of established cirrhosis. Over time the features of NAFLD often disappear (i.e. burn out) in cirrhosis, as described below.

**DIAGNOSTIC CHALLENGES AND PITFALLS**
Beyond the common patterns of NAFLD, a number of clinical and diagnostic scenarios can present challenges to the pathologist. The following nine scenarios are described to assist in avoiding common pitfalls in NASH diagnosis:

NASH Pitfalls

- Alcoholic steatohepatitis
- Burnt-out NASH cirrhosis
- Centrizonal arterialization
- Drug induced liver injury and steatohepatitis
- Hereditary hemochromatosis
- Metabolic disorders (glycogenic hepatopathy, diabetic hepatosclerosis, Wilson disease)
- Microvesicular steatosis
- More than mild portal inflammation
- Pediatric NASH
- Steatohepatitis with an acute/subacute presentation

Alcoholic steatohepatitis
It is usually not possible to definitively distinguish alcoholic from nonalcoholic steatohepatitis based on liver histology. In general, NASH is characterized by more prominent steatosis and less severe signs of hepatocellular injury like ballooning and inflammation. There are also certain histologic features that can occur in alcoholic steatohepatitis, but are less common in NASH, such as abundant Mallory hyaline, prominent neutrophil infiltrate (with neutrophil satellitosis), cholestasis, and obliteration of central veins.

Burnt-out NASH cirrhosis
The typical features of steatohepatitis often regress with progression of fibrosis and may be lost with cirrhosis (i.e. “burnt-out” steatohepatitis). These cases end up being labeled as cryptogenic cirrhosis. Since the patient population with cryptogenic cirrhosis has a high prevalence of obesity and type 2 diabetes, it is believed that most cases of cryptogenic cirrhosis are related to nonalcoholic steatohepatitis [12]. In this setting, the correct diagnosis can only be established by ruling out other etiologies and through correlation with risk factors for steatohepatitis.

Centrizonal arterialization
Identification of arterioles is often helpful in orientation of a liver biopsy, since they normally constitute one component of the portal triad. However, arterioles can also be seen in central zones in NASH liver biopsies [13], most commonly with advanced fibrosis [13]. This scenario may result in misidentification of a central zone as a portal tract and thereby lead to an erroneous interpretation of a portal-based disease process, potentially resulting in a missed NASH diagnosis.

Drug induced liver injury and steatohepatitis
A vast majority of NASH cases can be attributed to obesity, type 2 diabetes, and hyperlipidemia and, among NASH patients, most have metabolic syndrome. NASH may
in fact represent a hepatic component of the metabolic syndrome. However, in patients without risk factors, histologic findings identical to NASH have been observed, so it may not be possible to separate drug induced steatohepatitis from NASH, in some clinical scenarios (Tables 1 and 2) [14]. Amiodarone, irinotecan, perhexiline and diethylaminoethoxyhexestrol are drugs with a definite steatohepatitis association:

Amiodarone
Amiodarone is a potent antiarrhythmic agent, which causes elevated liver enzymes in up to 30% of patients and steatohepatitis in ~1-2% of patients [15-17]. Amiodarone steatohepatitis is characterized by prominent Mallory hyaline (occasionally in zone 1) and neutrophil satellitosis, while steatosis is less conspicuous. The findings can be similar to alcoholic steatohepatitis [15]. Reversal of liver injury often occurs with discontinuation of the drug, but may be delayed by weeks or months. In addition, amiodarone is also associated with “phospholipidosis,” which is characterized by accumulation of drug in lysosomes [18, 19]. This leads to hepatocellular and Kupffer cell “foamy” change. The foamy areas show lamellar lysosomal inclusion bodies on ultrastructural evaluation [18]. Phospholipidosis is not always seen in amiodarone toxicity [18] and is independent of steatohepatitis [16].

Irinotecan
Irinotecan is a chemotherapeutic agent, used preoperatively in colorectal cancer patients with hepatic metastases, which can cause steatohepatitis-like injury to the liver. This has been referred to as chemotherapy-associated steatohepatitis (CASH) in the oncology literature [20].

Methotrexate
Methotrexate is a folate antagonist used for long-term treatment of rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Liver toxicity typically manifests as steatosis, anisonucleosis, portal inflammation and portal-based fibrosis. It may also exacerbate or precipitate steatohepatitis in patients with risk factors like obesity and diabetes. The risk of liver toxicity is also exacerbated with heavy alcohol use, pre-existing liver disease, daily dosing, and high cumulative dose [21]. Some patients with high cumulative dose can develop steatohepatitis without metabolic risk factors [22, 23].

Perhexiline maleate/diethylaminoethoxyhexestrol
Perhexiline maleate (Pexid), an anti-angina drug, and diethylaminoethoxyhexestrol (Coralgil), a vasodilator, were extensively used in Europe and Japan, respectively. Both drugs can cause NASH and phospholipidoses similar to amiodarone [24, 25].

Other drugs have been described in association with NAFLD, but the evidence linking them to NASH is less strong. It is unlikely that these drugs have a direct role in NASH etiology, but rather that they precipitate steatohepatitis in the presence of other risk factors:

Other drugs
Tamoxifen is an anti-estrogen drug used in breast cancer patients. Steatohepatitis risk may only be increased in overweight or obese women [26]. Steroids, estrogen and diethylstilbestrol often lead to hepatic steatosis, but steatohepatitis is rare [17, 27]. Nifedipine is a calcium channel blocker that has been implicated in NASH, but risk factors for NASH are often also present in patient’s treated with this medication, creating some uncertainty about a definite association between this drug and steatohepatitis [28].

**Hereditary hemochromatosis**
A mild to moderate hepatocyte siderosis (generally non-zonal), and/or Kupffer cell siderosis, has been described in up to 21% of NAFLD patients with known wild type HFE [29] (though only 6% of these patients had isolated hepatocellular iron [29]). Serum ferritin is an acute phase reactant, which can be elevated in NAFLD patients, which may result in clinical suspicion for hereditary hemochromatosis. High serum ferritin and increased iron saturation, however, may more strongly suggest co-existent hereditary hemochromatosis and, especially with C282Y HFE mutation, which is associated with greater iron deposition in liver tissue, may warrant biopsy even in a patient with well-established NASH, to rule out iron overload and associated fibrosis [1]. On iron stain, hereditary hemochromatosis is usually characterized by a more intense iron deposition that predominantly occurs in hepatocytes and is more pronounced in the perportal region (see **Table 7**), though there is some overlap; typical histological features of NASH are not observed in isolated hereditary hemochromatosis. The role of hepatic iron and HFE mutations in progression of NASH fibrosis is uncertain [30-32]. Interestingly, the H63D HFE mutation has recently been reported to be associated with higher steatosis grade and NAFLD activity scores in NASH patients [29].

**Table 7. Patterns of siderosis in NASH**

<table>
<thead>
<tr>
<th>Distribution of iron</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>Pattern 1: KC*</td>
<td>Secondary Siderosis</td>
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<tr>
<td>Pattern 2: KC and mild/mod HC (random)</td>
<td>Secondary Siderosis</td>
</tr>
<tr>
<td>Pattern 3: Mild/mod HC (random)</td>
<td>Secondary Siderosis</td>
</tr>
<tr>
<td>Pattern 4: Mild/mod HC (periportal)</td>
<td>HH or Secondary Siderosis</td>
</tr>
<tr>
<td>Pattern 5: Marked HC (any)</td>
<td>HH or Secondary Siderosis</td>
</tr>
</tbody>
</table>

* KC = Kupffer cell; HC = Hepatocellular; HH = Hereditary hemochromatosis (untreated)

**Metabolic disorders**
Several metabolic disorders can show overlapping features with NASH:

**Glycogenic hepatopathy**
Glycogenic hepatopathy develops in diabetic patients with poor glycemic control and is characterized by swollen hepatocytes and glycogenated nuclei similar to NASH. However, it occurs more commonly in type 1 diabetes, as opposed to type 2 diabetes, which is more common in NASH. Numerous megamitochondria can be present in glycogenic hepatopathy; fat, Mallory hyaline, significant inflammation and pericellular fibrosis are usually absent or inconspicuous [33].
**Diabetic hepatosclerosis**
Diabetic hepatosclerosis is a term used to describe dense non-zonal perisinusoidal fibrosis and basement membrane deposition that occur in patients with long standing insulin-dependent diabetes. It is associated with severe microvascular disease in other organs and may represent a form of hepatic microangiopathy [34]. In addition to sinusoidal fibrosis, perivenular fibrosis and hyaline thickening of small hepatic artery branches may be present. Features typical of NASH such as steatosis or hepatocellular ballooning are not seen. These patients often have elevated alkaline phosphatase, a finding unusual for NASH.

**Wilson disease**
Wilson disease is often associated with steatosis and glycogenated nuclei. In some cases, swollen hepatocytes and Mallory hyaline can be present and can mimic steatohepatitis. The non-zonal distribution of fat and Mallory hyaline in Wilson disease, “chronic hepatitis-like” pattern of inflammation, and portal-based fibrosis are helpful in the differential diagnosis as these findings would be unusual in NASH. Also, it would be very unusual for Wilson disease to present after age 55, so this consideration can be excluded in an older patient. In difficult cases, quantitative copper determination from urine and/or paraffin-embedded liver tissue can allow for definitive diagnosis. Note that ceruloplasmin levels may be falsely elevated into the normal range in patients with active disease, resulting in a false negative value. Histochemical copper stains are not reliable in ruling out Wilson disease on biopsy.

**Microvesicular steatosis**
Pure microvesicular steatosis is a rare phenomenon and does not occur in NASH. It signifies severe mitochondrial injury and is seen in alcoholic foamy liver degeneration, Reyes syndrome (in children), acute fatty liver of pregnancy, rare genetic diseases (e.g. carnitine deficiency), and as an adverse effect of drugs/toxins like cocaine, tetracycline, valproic acid and zidovudine. A substantial number of NAFLD cases have a component of microvesicular steatosis ([35]), which has been associated with higher grades of steatosis, ballooning cell injury, presence of Mallory hyaline, megamitochondria, higher NAS score, and more advanced fibrosis, though a definite role for microvesicular steatosis in the pathophysiology of NASH related liver injury remains uncertain.

**More than mild portal inflammation**
Portal inflammation in steatohepatitis is typically mild. If prominent portal inflammation is present, other etiologies such as hepatitis B (HBV) or C (HCV), autoimmune hepatitis, primary biliary cirrhosis, or Wilson disease have to be clinically excluded. The presence of lymphoid aggregates (as in hepatitis C), viral inclusions (as in hepatitis B), numerous plasma cells (as in autoimmune hepatitis), or bile duct injury (as in primary biliary cirrhosis) is unusual in NASH. After other etiologies have been clinically excluded, the case can be regarded as steatohepatitis with unusually prominent portal inflammation. These cases are likely to be associated with a higher degree of fibrosis [36].

The presence of autoantibodies does not necessarily signify autoimmune hepatitis, as various serum autoantibodies have been detected in up to 36% of steatohepatitis patients.
Antinuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) have been reported in 20% and 3-6% of patients, respectively [37-39]. Both autoantibodies may be present together in rare patients [37-39]. Patients with autoantibodies generally show typical histologic features of NASH [39] and probably do not benefit from steroid therapy [37]. AMA has been rarely detected in NASH patients (~1%) and was not associated with primary biliary cirrhosis [37]. In all of these studies, the autoantibody titers are generally low, so high titer autoantibodies should raise more concern for another or co-existent etiology. On biopsy, the presence of steatosis, ballooning, and pericellular fibrosis as well as the absence of high liver transaminases, prominent portal inflammation, numerous plasma cells and prominent hepatocellular damage reliably distinguish steatohepatitis from autoimmune hepatitis.

Pediatric NASH
NASH related cirrhosis has been described in children as young as 8 years of age (with NAFLD documented as early as 2 years of age) [1, 8, 40]. In one study of obese school children, 23% of 17-18 years olds had unexplained elevated ALT (>40 IU/L) [40]. In addition to obesity, older age, male gender, and Hispanic ethnicity are independent predictors of NAFLD prevalence in children [1]. Currently, AST/ALT screening is recommended (in one study) for obese children, starting at age 10, with BMIs in the 85th to 94th percentile and with other risk factors [41]. However, the more recent practice guidelines did not make a formal recommendation for pediatric screening [1], given the lack of sufficient evidence at this time. Current recommendations suggest a need for liver biopsy in children when NAFLD is suspected, but the diagnosis is unclear, or before starting hepatotoxic medicines or pharmacologic therapy for NASH [1].

The histologic features of NASH in children may differ from adult NASH. It has been proposed that pediatric steatohepatitis can be classified into two types based on histological features.

Type 1 NASH
Resembles adult NASH with zone 3 predominance of steatosis, hepatocellular ballooning, and fibrosis [8].

Type 2 NASH
Steatosis is often severe and may lack zone 3 predominance. Ballooned hepatocytes, Mallory hyaline, and acinar inflammation may be mild or absent, while portal-based chronic inflammation may be prominent. Portal-based fibrosis in the absence of pericentral/sinusoidal fibrosis can occur (i.e. stage 1C, see Table 5) [8]. Note that some adults may also show this pattern of NASH injury, but the finding is rare (only 3 out of 288 adult biopsies evaluated by the NASH CRN) and not related to gender or race/ethnicity differences [42]. The differential diagnosis may include other hepatitic etiologies, such as autoimmune hepatitis, but severe steatosis should raise suspicion for type 2 NASH in this scenario and result in clinical correlation.

The majority of pediatric patients with NASH in one series showed type 2 features [8], while mixed type 1 and type 2 features were seen in the majority of cases in another
study [43]. The pathology report in cases with type 2 features should reflect that classic criteria for steatohepatitis are not fulfilled (as occurs in many pediatric cases), but that it may be prudent to manage the patient as appropriate for steatohepatitis.

Genetic and metabolic liver disease
Children < 2 years of age with fatty liver should be evaluated for rare genetic disorders such as fatty acid oxidation defects, lysosomal storage disorders, and peroxisomal disorders [1]. Fatty acid oxidation defects would typically manifest with microvesicular steatosis and possibly ductular reaction, cholestasis, and fibrosis [44]. Abetalipoproteinemia and familial hypobetalipoproteinemia may also present with steatosis in young patients and some abetalipoproteinemia patients have reportedly developed progressive fibrosis [45]. Other disorders of lipoprotein and lipid metabolism (e.g. familial high-density lipoprotein deficiency (Tangier disease), familial hypercholesterolemia, Wolman disease, ceramidase deficiency (Farber disease), Glycosyl ceramide lipidosis (Gaucher disease), and sphingomyelin-cholesterol lipidosis (Niemann-Pick disease)) may variably demonstrate vacuolated/foamy change in the liver, predominantly in Kupffer cell (consisting of products related to lipoprotein and lipid metabolism) [46-53]. While lipogranulomas are common in NASH, diffuse Kupffer cell foamy change is not a feature of NAFLD and would suggest a storage disorder. Hereditary tyrosinemia is a genetic disease with features of steatohepatitis and would demonstrate steatosis with pericellular fibrosis, but also prominent hepatocanalicular cholestasis and possibly also hepatocellular dysplasia [54]. Cystic fibrosis changes may also include hepatic steatosis and periportal fibrosis, but with ductular reaction and dilatation (possibly with inspissated secretions), and possibly with progression to multilobular biliary cirrhosis [55].

Familial NASH refers to identification of NASH (or cryptogenic cirrhosis) in high frequency within several kindreds in which obesity and type 2 diabetes mellitus are prevalent [56], which suggests a common mechanism of disease and perhaps also some heritable risk. A steatohepatitis pattern of injury has also been described in partial Lipodystrophy [57], Bardet-Biedl syndrome [58], Alstrom syndrome [59], and Bloom syndrome [60]. Prominent steatosis, without fibrosis, has also been noted in Dorfman-Chanarin syndrome [61, 62].

Steatohepatitis with an acute/subacute presentation
The majority of patients with NASH are detected due to an asymptomatic elevation of liver transaminases, presence of steatosis on imaging, or incidental hepatomegaly on a routine physical examination. Some patients can also present with cirrhosis that can be labeled cryptogenic, if typical histologic features of NASH are not present.

In rare cases, a subacute to acute presentation with rapid progression to liver failure can occur. Most of the patients are obese women with onset of liver failure 4-16 weeks after initial presentation [63]. The liver in these patients shows cirrhosis and typical features of active steatohepatitis. It is thought that these patients develop silent cirrhosis related to NASH and that the subacute presentation is triggered by an additional liver injury. Rapid
weight loss may also possibly precipitate NASH [64] and an increase in fibrosis has been reported in one study, after gastric bypass surgery [65].

**PROGNOSIS AND TREATMENT**

NAFLD is associated with increased mortality, most commonly due to cardiovascular disease, and NASH has an increased liver-related mortality rate [1, 9, 66-73]. As noted below, NASH cirrhosis patients may develop HCC, but at a lower rate than HCV patients [74, 75], and overall, NASH cirrhosis reportedly has a lower rate of decompensation and mortality versus patients with hepatitis C cirrhosis [74]. Nevertheless, overall mortality is similar to HCV (i.e. 10 year survival at 81.5%) [75].

Management of NAFLD requires attention to risk factors with emphasis on weight loss, diet, exercise, and improved control of diabetes [1]. Weight loss usually reduces hepatic steatosis after a 3-5% reduction in weight [76]. More significant weight loss (>10%) appears necessary to reduce histologic evidence of liver injury [76, 77]. Exercise alone, without weight loss, may improve steatosis, but any effects on liver injury are unknown [78, 79]. Some murine research has implicated a possible role for particular dietary constituents in NASH, such as trans fats [80].

Clinical trials are ongoing and treatment choices are evolving. For example, metformin (a biguanine which may act as an insulin-sensitizing agent) is no longer recommended as a specific treatment for NASH [1, 81] while Vitamin E (800 IU/day) (an antioxidant) is considered a first line pharmacotherapy for non-diabetic adults with biopsy proven NASH [82]. It is interesting to note that children with NAFLD may have a diet low in vitamin E, which may contribute to NAFLD pathophysiology in this population [83], though vitamin E therapy has not proven to be efficacious in this group [84].

Pioglitazone (a thiazolidinedione which may act through PPAR-\(\gamma\) receptors) may be used to treat biopsy proven NASH, but long term safety and efficacy has not been established [1, 2, 82]. Ursodeoxycholic acid (a bile acid with a variety of potential actions) is also not recommended for treatment of NASH [1, 85]. The benefit of treatment with omega-3 fatty acids (FA) (polyunsaturated fatty acids) remains unknown, though omega-3 FA may be used to treat hypertriglyceridemia in patients with NAFLD and there is some preliminary support for therapeutic use in NAFLD [1, 86]. Bariatric surgery (foregut type) may be considered in NAFLD patients without cirrhosis [1], but safety and efficacy in the setting of cirrhosis (due to NAFLD) is not well established [1, 87, 88]. Statins (HMG-CoA reductase inhibitors that lower total serum cholesterol and LDL concentrations) may be used to treat dyslipidemia in patients with NAFLD, but should not be used to specifically treat NASH at this time [1]. Patients with NASH cirrhosis should be screened for esophageal varices and possibly for HCC [1, 89, 90]. Patients transplanted for NASH with HCC have had less aggressive tumor features (e.g. less frequent vascular invasion and poorly differentiated foci), and long recurrence free survival when compared to HCC in HCV cirrhosis[91]. Recent data has also indicated that hepatocellular adenomas, especially the inflammatory variant, occur commonly in obese and diabetic patients [92, 93].
REFERENCES


