Fatty Liver Disease
Diagnostic Challenges & Updates

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Current Issues 2014

Definitions

- **NAFLD** – Fat in the liver (imaging or histology) in a patient without secondary fat accumulation.
- **NASH** – NAFLD with histologic evidence of liver injury in the form of ballooned hepatocytes, inflammation and fibrosis
- **NAFL** – NAFLD without the above histologic findings associated with NASH
- **NASH cirrhosis** – Cirrhosis with current or previous evidence of NASH

Secondary Hepatic Fat

- **Macrovesicular**
  - Excess alcohol
  - HCV
  - Wilson Disease
  - Starvation/TPN
  - Abetalipoproteinemia
  - Medications (amiodarone, methotrexate, tamoxifen, corticosteroids)
- **Microvesicular**

Secondary Hepatic Fat

- **Macrovesicular**
- **Microvesicular**
  - Reye Syndrome
  - Acute Fatty Liver of pregnancy
  - Medications (e.g. antiretrovirals, valproate)
NAFLD: Extent of the problem

- 6.3-33% (median = 20%) in various populations
- 3-5% NAFLD represent NASH
- Prevalence of NASH cirrhosis in general population not known (in bariatric surgery population: 90% NAFLD and 5% unsuspected cirrhosis)
- Obesity (2009-2010, CDC)
  - US adults: 35.7%, 78 million; highest rate in women over age 60 (42.3%)
  - US children: 16.9%, 12.5 million; highest rate in boys age 6-11 (20.1%)
Histologic pattern and outcome

- Steatosis alone
- Steatosis + inflammation
  - Progression to cirrhosis <5%
- Steatosis + ballooning
- Steatosis + fibrosis
  - Progression to cirrhosis ~25%

Treatment Options

- Weight loss
- Exercise
- Vitamin E is a first line treatment for non-diabetic patients
- Pioglitazone, omega-3 FA, and bariatric surgery may also be effective
- Screening for esophageal varices and HCC is appropriate

Steatohepatitis: essential features

AASLD and NASH Clinical Research Network

- Steatosis (>5%)
- Inflammation (lobular)
- Hepatocellular injury
  - Ballooned hepatocytes
  - Pericellular fibrosis

Steatohepatitis: essential features

AASLD and NASH Clinical Research Network

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- Hepatocellular injury
  - Ballooned hepatocytes
  - Pericellular fibrosis
Mild Steatosis (Grade 1, scale 0-3)

Moderate Steatosis (Grade 2, scale 0-3)

Severe Steatosis (Grade 3, scale 0-3)

Steatohepatitis: essential features
AASLD and NASH Clinical Research Network

- Steatosis (>5%)
- Inflammation (lobular)
- Hepatocellular injury
  Ballooned hepatocytes
  Pericellular fibrosis
Lobular Inflammation in NASH

Portal Inflammation in NASH

Neutrophil Satellitosis

Steatohepatitis: essential features

AASLD and NASH Clinical Research Network

- Steatosis (>5%)
- Inflammation (lobular)
- Hepatocellular injury
  - Ballooned hepatocytes
  - Pericellular fibrosis
Ballooned Hepatocyte

Heptocellular Ballooning
- Large size
- Cytoplasmic clearing
- Eosinophilic globules

Multiple Ballooned Hepatocytes

BH Mimic – Small Droplet Fat
Steatohepatitis: essential features

AASLD and NASH Clinical Research Network

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- Inflammation (lobular)
- Hepatocellular injury
  - Ballooned hepatocytes
  - Pericellular fibrosis

Staging - Brunt/Kleiner Method

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>Stage 1A</td>
<td>Pericentral/sinusoidal Fibrosis – Delicate</td>
</tr>
<tr>
<td>Stage 1B</td>
<td>Pericentral/sinusoidal Fibrosis – Dense</td>
</tr>
<tr>
<td>Stage 1C</td>
<td>Periportal Fibrosis</td>
</tr>
<tr>
<td>Stage 2</td>
<td>Pericentral/sinusoidal and Periportal Fibrosis</td>
</tr>
<tr>
<td>Stage 3</td>
<td>Bridging Fibrosis</td>
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<tr>
<td>Stage 4</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>
Stage 3

Stage 4

Fibrosis Pitfall – Tangential

Fibrosis Pitfall - Subcapsular
Fibrosis Pitfall - Overstained

Fibrosis Pitfall – Overstained

Fibrosis Pitfall - Nodular
Perivenular Collagen

Fibrosis Pitfall – Branching Portal Tract
Fibrosis Pitfall – Histiocyte Aggregate

Fibrosis Pitfall – Histiocyte Aggregate

Steatohepatitis: non-essential features
- Mallory hyaline in Zone 3
- Mild iron deposits in hepatocytes or sinusoidal cells
- Megamitochondria
- Glycogenated nuclei
- Lipogranulomas
- Acidophil bodies (occasional)

Mallory Hyaline
NASH Activity Score (NAS)

- Steatosis (0-3)
  - none, mild, moderate, severe
- Lobular inflammation (0-3)
  - 0, <2, 2-4, >4 foci/20x
- Hepatocellular ballooning (0-2)
  - none, few, many
- Total = 0-8

Histologic Variation

**PATTERN 1: CLASSIC STEATOHEPATITIS**

Steatosis with mild inflammation, hepatocellular ballooning, and pericellular fibrosis
Histologic Variation

PATTERN 2: STEATOSIS WITHOUT HEPATOCELLULAR INJURY

Steatosis without hepatocyte ballooning or pericellular fibrosis is insufficient for a diagnosis of steatohepatitis and represents NAFL

Low rate of progression (~5%) to significant fibrosis

Histologic Variation

PATTERN 3: STEATOSIS WITH SWOLLEN HEPATOCYTES/NON-CLASSIC BALLOONED HEPATOCYTES

Borderline for steatohepatitis; if clinical risk factors are present, it is best to manage the patient as appropriate for steatohepatitis

Histologic Variation

PATTERN 4: BALLOONED HEPATOCYTES OR PERICELULAR FIBROSIS WITHOUT STEATOSIS

Uncommon in patients with metabolic risk factors

<table>
<thead>
<tr>
<th>Ballooned Hepatocytes Only</th>
<th>Pericellular Fibrosis Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent cessation of Alcohol</td>
<td>Chronic venous outflow obstruction</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>Remote CZ injury</td>
</tr>
</tbody>
</table>
Chronic Venous Outflow Obstruction

Histologic Variation

**PATTERN 5: STEATOSIS WITH PERICELLULAR FIBROSIS, BUT NO BALLOONED HEPATOCYTES**

Chronic steatohepatitis in the appropriate clinical context

Other considerations: chronic venous outflow obstruction, drug (e.g. oxaliplatin), remote parenchymal rejection (post-transplant)

Histologic Variation

**PATTERN 6: CIRRHOSIS WITH STEATOSIS AND/OR BALLOONED HEPATOCYTES**

Cirrhosis with histologic features of NAFLD is best considered NASH cirrhosis. Some cases may show residual pericellular fibrosis.

Diagnostic Challenges

1. Alcoholic steatohepatitis
2. Burnt out NASH cirrhosis
3. Centrizonal Arteries
4. Drug induced steatohepatitis
5. Hereditary hemochromatosis
6. Metabolic disorders
7. Microvesicular steatosis
8. More than mild portal inflammation
Alcoholic Steatohepatitis

- Alcoholic steatohepatitis can not be definitively distinguished from NASH by histology

<table>
<thead>
<tr>
<th></th>
<th>NASH</th>
<th>ASH</th>
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<tbody>
<tr>
<td>Steatosis</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Ballooned hepatocytes</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lobular inflammation</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Mallory hyaline</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Neutrophil infiltrate</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Obliterated CV</td>
<td>+/-</td>
<td>+</td>
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Burnt-out NASH Cirrhosis

- Typical steatohepatitis features regress with progression of fibrosis and may be lost with cirrhosis
- Many cases labeled as cryptogenic cirrhosis; since this population has a high incidence of type 2 DM, NASH is considered to be the most likely etiology
- Rule out other etiologies and correlate with NASH risk factors

Centrizonal Arteries

- Identification of arterioles is used to orient the pathologist to lobular architecture
- Centrizonal arterialization is common and is under-recognized
- Mis-identification of a central zone as a portal tract can lead to erroneous classification as a portal based disease
- Glutamine synthetase can be helpful in problem cases (stains pericentral hepatocytes)
Drug Induced Steatohepatitis

- Histologic changes identical to NASH have been identified in patients without NASH risk factors exposed to certain drugs

<table>
<thead>
<tr>
<th>Definite Association</th>
<th>Possible Association</th>
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<tbody>
<tr>
<td>Amiodarone</td>
<td>Tamoxifen</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Steroids</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Estrogen</td>
</tr>
<tr>
<td>Perhexiline Maleate</td>
<td>Diethyldihydroxyestradiol</td>
</tr>
<tr>
<td>Diethylaminoethoxyh</td>
<td>exesterol</td>
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**Hereditary Hemochromatosis**

- A mild to moderate hepatocyte siderosis (generally nonzonal) and/or Kupffer cell siderosis is seen in ~20% of NAFLD patients.
- Serum ferritin is an acute phase reactant that is commonly increased in NAFLD patients.
- Increased iron saturation would more strongly suggest hereditary hemochromatosis.
- C282Y HFE mutation in an established NASH patient may warrant biopsy to evaluate iron overload.

**Periportal Siderosis in HH**
Unrelated Steatosis in HH

Periportal Siderosis in NAFLD

Secondary Siderosis in NAFLD

Metabolic Disorders

- **Glycogenic hepatopathy**
  - Type 1 DM with poor glycemic control
  - Glycogenosis, minimal fat, and abundant megamitochondria
- **Diabetic hepatosclerosis**
  - Non-zonal perisinusoidal fibrosis and BM deposition in patients with long standing insulin dependent DM, minimal steatosis, no ballooning
- **Wilson disease**
  - Steatosis (non-zonal), glycogenated nuclei, Mallory hyaline, swollen hepatocytes, portal inflammation and fibrosis
Microvesicular Steatosis

- Pure microvesicular steatosis does not occur in NASH and indicates severe mitochondrial injury
- Reye syndrome, acute fatty liver of pregnancy, alcoholic foamy liver degeneration, drug (cocaine, tetracycline, valproic acid, zidovudine), and rare genetic disorders.
- Many NAFLD cases will have a minor component of microvesicular fat
Diffuse Microvesicular Steatosis

More than Mild Portal Inflammation

- NASH portal inflammation is typically mild
- Prominent portal inflammation raises consideration of other causes (HBV, HCV, AIH, PBC, Wilson disease)
- If other etiologies are excluded, this can be considered NASH with prominent portal inflammation
- May be associated with a higher degree of fibrosis

Pediatric NASH

- NASH cirrhosis seen as young as 8 years of age
- AST/ALT screening has been considered for obese children starting at age 10
- Type 1 pediatric NASH: Identical to adult type NASH
- Type 2 pediatric NASH: Severe panacinar steatosis, no ballooned hepatocytes, early portal based fibrosis (stage 1C)
- Children younger than age 2 with fatty liver should be evaluated for rare genetic disorders
Severe Pan-acinar Steatosis

Tumors Arising in NAFLD

- Hepatocellular Adenomas
  - Adenomas, especially the inflammatory variant, occur commonly in obese and diabetic patients
- Focal Nodular Hyperplasia
  - May be fatty
- Hepatocellular carcinoma
  - HCC in NASH explants had less aggressive features and longer recurrence free survival compared to HCC in HCV explants

Hepatocellular Adenoma

Serum Amyloid A (SAA) Immunostain
QUESTIONS?

What causes ballooning

- Oxidative damage to cytoskeleton
- Intermediate filaments K 8 and 18

Loss of K8/K18 in ballooned cells

Lackner, J Hepatol 2008

Hepatitis C with steatohepatitis

- Steatohepatitis increases the risk of disease progression in hepatitis C
- Reduces efficacy of antiviral therapy
- Hepatitis C increases insulin resistance and exacerbates steatohepatitis

Younossi, Liver Int 2009