**Fatty Liver Disease**  
Diagnostic Challenges and Updates  
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Department of Pathology  
University of California, San Francisco

**Obesity in Antiquity**  
Obesity Treatment  
Brisk walking  
Wrestling

**Definitions**

- **NAFLD** – Fat (>5%) 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 and inflammation +/- fibrosis.

- **NAFL** – NAFLD without the above histologic findings associated with NASH.
Secondary Hepatic Fat

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

Secondary Hepatic Fat

- Macrovesicular
- Microvesicular
  - Reye Syndrome
  - Acute Fatty Liver of pregnancy
  - Medications (e.g. antiretrovirals, valproate)

Natural History

- **NASH**: Can progress to cirrhosis and liver failure (and rarely hepatocellular carcinoma)
- **NAFL**: Risk of progression to cirrhosis and liver failure is considered minimal (with increased risk associated with NAFL with inflammation)
Genetic Factors

- **PNPLA3** — encodes adiponutrin. A SNP at position 148 is associated with hepatic steatosis, NASH, and increased fibrosis stage (as well as incidence of HCC)

- **TM6SF2** — a SNP at position 167 has similar associations as **PNPLA3 SNP**
Scoring Systems

- **NAS** - Unweighted composite of steatosis, lobular inflammation, and ballooning scores. Useful to measure changes in biopsies in clinical trials. Fibrosis is scored separately.

- **SAF score** – Semiquantitative score consisting of steatosis amount, activity (lobular inflammation and ballooning) and fibrosis.

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Conclusions

• With continued high rates of adult obesity and diabetes, in an aging population, NAFLD related liver disease and mortality will increase in the US
• Strategies to slow growth of NAFLD and therapeutic options are necessary to mitigate disease burden

Response to the Crisis

Number of Publications by Five Year Intervals, Keyword: "Non-alcoholic Steatohepatitis"

Outline

1. Essential histologic criteria for diagnosis of steatohepatitis
2. Centrizonal arteries
3. Aggressive NASH
4. Diagnostic pitfalls
5. Revisiting the NAS
Steatohepatitis: Essential Features

AASLD and NASH Clinical Research Network

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

 +/- Pericellular fibrosis

Estimation of Steatosis


Mild Steatosis (Grade 1, scale 0-3)

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.

Moderate Steatosis (Grade 2, scale 0-3)

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.
Severe Steatosis (Grade 3, scale 0-3)

Steatohepatitis: Essential Features

AASLD and NASH Clinical Research Network

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• Inflammation (lobular)
• Hepatocellular injury
  Ballooned hepatocytes

+/- Pericellular fibrosis
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Steatohepatitis: Essential Features

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  Ballooned hepatocytes

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Staging - Modified Brunt Method

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

Regression
Steatohepatitis: Non-essential Features

- Mallory hyaline in Zone 3
- Mild iron deposits in hepatocytes or sinusoidal cells
- Megamitochondria
- Glycogenated nuclei
- Lipogranulomas
- Acidophil bodies (occasional)
- Centrilobular arteries

Mallory Hyaline

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>
Histologic Variation

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

Borderline for steatohepatitis in the appropriate clinical context

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

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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.

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**ORIGINAL ARTICLE**

Centrilobular Arteries and Microvessels in Nonalcoholic Steatohepatitis

Ryan M. GR, M.D., PH.D.*, Patrick P. V., R.T. - Laura Wilson, M.S.M.; Nishan M. Kass, M.D., PH.D.† and Linda D. Perrillo, M.D.*


<table>
<thead>
<tr>
<th>Grade</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Total (%)</th>
</tr>
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<tbody>
<tr>
<td>Stage 1b/1c</td>
<td>17</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2/19 (11%)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>23</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>9/31 (29%)</td>
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<tr>
<td>Stage 3</td>
<td>13</td>
<td>8</td>
<td>10</td>
<td>3</td>
<td>21/36 (58%)</td>
</tr>
<tr>
<td>Stage 4</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>8/11 (73%)</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>18</td>
<td>13</td>
<td>9</td>
<td>58/100 (58%)</td>
</tr>
</tbody>
</table>

Artery grades: 0, no central zones with artery; 1, 1 to 2 central zones with artery; 2, >2 and <50% of central zones with artery; 3, >50% of central zones with artery. Definitions of stages: 1b, centrilobular fibrosis only; 1c, centrilobular fibrosis with pericentral fibrosis; 2, centrilobular fibrosis and pericentral fibrosis; 3, bridging fibrosis; 4, cirrhosis.

*P < 0.001 using univariate ordinal logistic regression.

Aggressive non-alcoholic steatohepatitis following rapid weight loss and/or malnutrition

[Unrelated text]
Aggressive NASH

- NASH presenting as ALF
- We described 6 patients who developed ALF following rapid loss or malnutrition
- 4 patients either died or required urgent liver transplant
- Pathologic findings similar to advanced alcoholic steatohepatitis

Pathologic Features

- Extensive/circumferential centrilobular pericellular fibrosis
- Central scar with perivenular sclerosis/veno-occlusion with superimposed hepatocellular dropout
- Abundant/prominent hepatocellular balloons, and numerous Mallory-Denk bodies
- Centrilobular arteries often prominent

Severe Centrizonal Scarring

![Image of histopathology slides showing severe centrilobular scarring.](Image)
Prominent BH and Centrizonal Arteries

Ductular Reaction, Cholestasis, and Central Vein Occlusion

Diagnostic Challenges

1. Alcoholic steatohepatitis
2. Burnt out NASH cirrhosis
3. Drug induced steatohepatitis
4. Hereditary hemochromatosis
5. Metabolic disorders
6. Microvesicular steatosis
7. 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>
</tr>
</thead>
<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>
</tr>
</tbody>
</table>

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

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>
</tr>
</thead>
<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>Perhexaline</td>
<td>Diethylstilbestrol</td>
</tr>
<tr>
<td>Methotrexate/maleate</td>
<td>Diethylstilbestrol</td>
</tr>
<tr>
<td>Maleate/Methotrexate</td>
<td>Diethylstilbestrol</td>
</tr>
</tbody>
</table>
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

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
Glycogenic Hepatopathy

Diabetic Hepatosclerosis
Steatosis and Portal Inflammation in Wilson Disease

Periportal Fibrosis in Wilson Disease

Wilson Disease with Swollen Hepatocytes
Wilson Disease with Pericellular 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 (cocaíne, 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

NASH CLINICAL RESEARCH NETWORK (CRN)

NIDDK Workshop on Fatty Liver Disease 1998

- No good estimates of disease prevalence or severity (but suspected that this was a big problem)
- Little information on the natural history
- No non-invasive diagnostic tests
- No standard methods for evaluating liver biopsy
- No approved therapies

Courtesy of Dr. David Kleiner, NIH
NASH Clinical Research Network

- Sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases
- 18 Participating Academic Clinical Centers (8 Adult, 10 Pediatric), 1 Data Coordination Center, and the NIDDK Project Scientists
- Established to focus on the etiology, contributing factors, natural history, complications, and therapy of nonalcoholic steatohepatitis

NASH CRN Studies

Foundation for trials:
- Pathology standardization → NAFLD Activity Score ("NAS")
- Utility of laboratory ALT reference ranges
- Impact of T2D on nonalcoholic liver disease

Primary Goal of the Pathology Committee
Create a scoring system for evaluating liver biopsies that could be used for clinical trials and natural history studies

Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease

- H&E and Trichrome only
- 9 pathologists, 2 independent reads
- Scoring system included features for grading/staging plus other findings
- Defined an “Activity Score” for use in clinical trials to objectively measure composite histologic change
- Score based on results of multivariable analysis
- Excluded fibrosis to avoid mixing "stage" with "grade"

NAFLD Activity Score (NAS)
= Steatosis (0-3) + Lob. Inf. (0-3) + Ballooning (0-2)

Hepatology 41: 1313; 2005

Courtesy of Dr. David Kleiner, NIH
Ballooning is Associated with Long Term Survival, Whereas Steatosis is Not

Angulo et al., Gastroenterology 149: 389; 2015

Steatosis

Ballooning

Problem
Steatosis accounts for more weight in the NAS than Ballooning

Possible Solutions
- Drop Steatosis from the score
- Extend the Ballooning Scale
NASH CRN Studies

Pathology committee discussions on better characterization of ballooning
Defined two new concepts for prospective evaluation:
- Classical vs Non-Classical Ballooning
- Severe vs Not Severe Ballooning
New definitions implemented with the first case in DB2

Classical vs Non-Classical

- **Classical ballooning**
  - Enlarged (>1.5x normal)
  - Cytoplasmic clearing
  - Cytoplasmic clumping
  - May have Mallory-Denk bodies

- **Non-Classical ballooning**
  - Typically in zone 3, perivenular
  - Smaller
  - Same cytoplasmic alterations
  - Lack Mallory-Denk bodies

Non-Classic Ballooned Hepatocyte

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.
Classical vs. Non-Classical HB

Substantial agreement (weight kappa 0.76 (95% CI=0.64, 0.88))

Severe Hepatocyte Balloons

- Several foci of classic hepatocyte balloons immediately apparent at low magnification (4x)

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Extending the Ballooning Score Beyond 2: A Proposal for a New Balloon Score

David E. Kleiner1, Elizabeth M. Brunt2, Patricia H. Balf2, Cynthia A. Bahling2, Ryan M. Gill3, Cynthia D. Guy4, Brian A. Neuschwan-der-Tett5, Anu J. Sanyal6, Mark L. Van Natta7. 1Laboratory of Pathology, National Cancer Institute, Bethesda, MD; 2Johns Hop-kins School of Public Health, Baltimore, MD; 3Washington Univer-sity, St. Louis, MO; 4Sharp Memorial Hospital, San Diego, CA; 5University of California, San Francisco, San Francisco, CA; 6Duke University Medical Center, Durham, NC; 7Saint Louis University, St. Louis, MO; 8Virginia Commonwealth University, Richmond, VA

- 1226 biopsies
- Demographic, anthropometric, laboratory data within 6 months of biopsy extracted
### Proposed Modified Hepatocyte Balloon Score

<table>
<thead>
<tr>
<th>Old Ballooning Score</th>
<th>Classical?</th>
<th>Severe?</th>
<th>New Ballooning Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - None</td>
<td>No</td>
<td>No</td>
<td>0</td>
<td>No ballooning</td>
</tr>
<tr>
<td>1 - Few or 2 - Many</td>
<td>No</td>
<td>No</td>
<td>1</td>
<td>Only Non-classical</td>
</tr>
<tr>
<td>1 - Few</td>
<td>Yes</td>
<td>No</td>
<td>2</td>
<td>Few Classical</td>
</tr>
<tr>
<td>2 - Many</td>
<td>Yes</td>
<td>No</td>
<td>3</td>
<td>Many Classical</td>
</tr>
<tr>
<td>3 - Many</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Severe, Many Classical</td>
</tr>
</tbody>
</table>

- Reduces effect of many “non-classical” hepatocyte balloons when no classical ballooning seen
- Gives more weight to ballooning
- Better correlation with diagnosis

### Highlights presented at AASLD

1. Diagnosis
2. Fibrosis
3. Age and gender associations
4. Diabetes and metabolic syndrome
5. Liver enzymes

### Summary and Conclusions

- We have proposed a new ballooning score based on careful morphological characterization of the range of ballooned hepatocytes
- The new balloon score doubles the dynamic range of the current balloon score
- The score shows excellent correlation with clinical disease features, as well as with patient demographics
Acknowledgments
*Writing Group Members

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