The Bugaboos of Fatty Liver Disease:
Ballooning and Fibrosis

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Challenges in the Diagnosis of Steatohepatitis

• Ballooning – Importance and Characterization
• Fibrosis – The Complexity of Fibrosis Progression in NASH

Why is Ballooning So Important?

Steatohepatitis is a Specific Pattern of Liver Injury

• Steatosis and Inflammation
  – Necessary but not sufficient
• (Zone 3 centered) hepatocellular injury
  – Required for specific diagnosis
  – Ballooning degeneration, Mallory-Denk bodies
• Fibrosis
  – Characteristic pattern: Pericentral/sinusoidal “chicken-wire” fibrosis then periportal fibrosis and central-central and central-portal bridging
  – But an outcome, not cause and may persist when other features resolve/diminish

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NASH CRN SCORING SYSTEM

• Steatosis – 0-3 scale
  – <5%, 5-33%, 34-67%, >67%
• Lobular inflammation – 0-3 scale
  – 0, <2 foci/20x hpf, 2-4 foci, >4 foci
• Ballooning – 0-2 scale
  – None, Few, Many
• Portal inflammation – 0-2
  – None, Mild, More than mild
• Fibrosis – 0-4 (later slide)
Chronic Hepatitis C

Ballooning is Associated with Long Term Survival, Whereas Steatosis is Not

Angulo et al., Gastroenterology 149: 389; 2015

Definition of Ballooning

- Cell is enlarged beyond background, non-steatotic hepatocytes
- Cytoplasm is altered by clearing and clumping, not dominated by fat vacuoles
- Mallory-Denk bodies may be present

Steatosis

Steatosis

Ballooning

Ballooning
### Agreement

<table>
<thead>
<tr>
<th>Feature</th>
<th>Inter-rater Kappa</th>
<th>Intra-rater Kappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis Grade</td>
<td>0.79</td>
<td>0.85</td>
</tr>
<tr>
<td>Lobular Inflammation</td>
<td>0.45</td>
<td>0.91</td>
</tr>
<tr>
<td>Portal Inflammation</td>
<td>0.45</td>
<td>0.7</td>
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<tr>
<td>Ballooning</td>
<td>0.56</td>
<td>0.7</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0.84</td>
<td>0.91</td>
</tr>
<tr>
<td>Diagnostic Classification</td>
<td>0.61</td>
<td>0.59 to 0.88</td>
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</table>

*Hepatology 2005; 41:1313-21*

So, what do you do with cells that don’t seem to fit all the criteria?
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 and often in groups
  - Same cytoplasmic alterations
  - Lack Mallory-Denk bodies

There are Significant Clinical and Histological Differences Between Categories of Ballooning

The Degree of Ballooning is Strongly Associated with Fibrosis

Clear Relationship of Ballooning Degree to Diagnosis

Ballooning Summary

- Ballooning is important to recognize because
  - It makes steatohepatitis a specific pattern of injury even in the absence of fibrosis
  - It is prognostically important
- Ballooning has distinctive features that allow us to pick out specific balloon cells
- There may be relevance to cells that meet some, but not all, of the criteria
The Problem of Fibrosis Staging in NASH

Staging Fibrosis

NASH CRN classification

0 No fibrosis
1 Perisinusoidal or periportal (but not both)
   1A Zone 3 perisinusoidal fibrosis (mild, Trichrome only)
   1B Zone 3 perisinusoidal fibrosis (moderate, seen on H&E)
   1C portal fibrotic expansion/periportal fibrosis
2 Both perisinusoidal and periportal
3 Bridging fibrosis (any type)
4 Cirrhosis

Questions

• Why is it so complicated?
• Why don’t the different stages have progressively more fibrosis?
• Why is stage 1 split between perisinusoidal and pericentral fibrosis?

Pediatric Biopsies are More Likely to Show Periportal rather than Central Perisinusoidal Fibrosis

Fibrosis Progression In Viral Hepatitis

Fibrosis Progression Pathways in NASH
Diagnosis of Periportal Fibrosis in NAFLD/NASH

- Extension of pericellular septations
- Trapping of periportal hepatocytes

Stage 2 Fibrosis

The distinction between Stage 2 and Stages 1 and 3 is important

Progression and Regression of Fibrosis

<table>
<thead>
<tr>
<th>Stage at Last Follow-up Biopsy</th>
<th>0</th>
<th>1A</th>
<th>1B</th>
<th>1C</th>
<th>2</th>
<th>3</th>
<th>4</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>47</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>17</td>
<td>3</td>
<td>0</td>
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<tr>
<td>1A</td>
<td>17</td>
<td>10</td>
<td>7</td>
<td>1</td>
<td>12</td>
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<tr>
<td>1B</td>
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<td>3</td>
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<td>0</td>
<td>6</td>
<td>10</td>
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375 patients
Long-term Prognostic Relevance of Liver Histology in Nonalcoholic Fatty Liver Disease

- International collaborative longitudinal study of patients with NAFLD
- 619 patients followed for a median of 12.6 years (range 0.3-35.1)
- Inclusion based on liver biopsy with at least 5% steatosis (minimum diagnostic criterion for NAFLD) read by single pathologist

<table>
<thead>
<tr>
<th>Liver biopsy features</th>
<th>Mortality/liver transplantation (n = 619)</th>
<th>Liver-related events (n = 619)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted cumulative (%)</td>
<td>P-value</td>
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<tr>
<td>Portal inflammation, grade 0</td>
<td>25.5</td>
<td>1.9</td>
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<tr>
<td>1</td>
<td>28.3</td>
<td>3.5</td>
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<tr>
<td>2</td>
<td>32.7</td>
<td>4.6</td>
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<td>3</td>
<td>42.2</td>
<td>5.3</td>
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<td>4</td>
<td>58.0</td>
<td>7.1</td>
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Mortality/liver transplantation (n = 619)
Liver-related events (n = 619)

Inflammation is Redistributed between Stage 1B and Stage 2 in NASH

Findings
- Portal inflammation was strongly correlated with fibrosis stage and ductular reaction (DR)
- Portal inflammation was enriched for macrophages and CD8+ T-cells
- Infiltration of portal areas by macrophages was the earliest change and preceded the increased expression of proinflammatory cytokines

Conclusion: Detailed characterization of portal inflammation, especially in the DR/progenitor cell niche, may be important to understand progression of disease in NASH
Portal Inflammation Becomes More Severe as Periportal Fibrosis Develops and Progresses
Brunt et al., Hepatology 2009;49:809-820

Portal Inflammation is Also Related to Long-Term Outcome
Gastroenterology 2015;149:389-397

Portal Inflammation becomes more severe as periportal fibrosis develops and progresses. Fibrosis summary includes:
- Fibrosis progression in NAFLD/NASH is much more complex than progression in viral hepatitis.
- The development of periportal fibrosis appears to be part of the progression pathway in most cases.
- The degree of periportal vs central perisinusoidal fibrosis appears to vary significantly.
- Nonetheless, in cross-sectional analysis, patients with stage 2 fibrosis have a worse prognosis than those with stage 1.
- The appearance of periportal fibrosis is linked with the development of increased portal inflammation.

Fibrosis Summary

References

Staging and Grading Systems in Use as Clinical Research Tools