Do Histological Features Inform DILI mechanisms?

Drug-Induced Liver Injury (DILI) Conference XVII
June, 2017

David Kleiner, M.D., Ph.D.
NCI/Laboratory of Pathology
Disclosures

• No financial or other conflicts of interest relevant to this presentation
Role of Liver Biopsy in DILI

- Characterize the morphologic changes
  - Morphologic changes may confirm drug injury by matching known/reported patterns
  - **Morphologic changes may suggest mechanism of injury**
- Assess the degree of injury
- Rule out other causes of hepatic injury
- May help to make diagnosis of DILI in complex cases by careful clinical-pathological correlation
- Sometimes biopsy can exclude DILI, permitting continued use of a necessary drug
Morphologic Considerations

- Cell types affected
  - Hepatocytes
  - Ducts and Ductules
  - Endothelial cells and vessels
  - Inflammatory cells
    - Type
    - Location
    - Targets
- Evidence of injury
  - Necrosis
  - Apoptosis
  - Cholestasis
  - Steatosis/Vacuoles
  - Inclusions
  - Cell swelling
  - Fibrosis
  - Pigments
Necrosis
### Necrosis in iDILI

#### Degree of Necrosis

<table>
<thead>
<tr>
<th>Degree of Necrosis</th>
<th>All Cases</th>
<th>Hepatocellular</th>
<th>Mixed</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>186 (75.3)</td>
<td>83 (65.4)</td>
<td>41 (87.2)</td>
<td>62 (84.9)</td>
</tr>
<tr>
<td>&lt;5%</td>
<td>23 (9.3)</td>
<td>14 (11.0)</td>
<td>1 (2.1)</td>
<td>8 (11.0)</td>
</tr>
<tr>
<td>5-33%</td>
<td>20 (8.1)</td>
<td>16 (12.6)</td>
<td>1 (2.1)</td>
<td>3 (4.1)</td>
</tr>
<tr>
<td>33-67%</td>
<td>13 (5.3)</td>
<td>10 (7.9)</td>
<td>3 (6.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>&gt;67%</td>
<td>5 (2.0)</td>
<td>4 (3.2)</td>
<td>1 (2.1)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

#### Location of Necrosis

<table>
<thead>
<tr>
<th>Location of Necrosis</th>
<th>All Cases</th>
<th>Hepatocellular</th>
<th>Mixed</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone 1</td>
<td>1 (1.6)</td>
<td>1 (2.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Zone 3</td>
<td>36 (59.0)</td>
<td>29 (65.9)</td>
<td>2 (33.3)</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>Panacinar</td>
<td>6 (9.8)</td>
<td>5 (11.4)</td>
<td>1 (16.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Azonal</td>
<td>18 (29.5)</td>
<td>9 (20.5)</td>
<td>3 (50.0)</td>
<td>6 (54.5)</td>
</tr>
</tbody>
</table>

*Hepatology 2014;59:661-670*
Zonal Necrosis

- Coagulative type necrosis with cell ghosts
- Susceptibility related to zonal variation in enzymes, oxygen, relative exposure to toxin

Non-zonal Necrosis

- Non-coagulative drop-out of hepatocytes
- Injury related to inflammation
Mechanism of Acetaminophen-Induced Coagulative Necrosis

Dig Dis 2015;33:464-471
INH-associated injury

Risk Factors
• Slow acetylation
• Polymorphisms in oxidation pathways
  – Glut S-transferase
  – CYP2E1
  – Mn SOD
  – UDP glucuronosyltranferase
  • J Hepatol 47:128-34

Likely Immune-mediated injury
• Adduct formation
• Auto-antibodies
  – Hepatology 59: 1084-93
• Positive lymphocyte transformation test
  – Clin Allergy 12:217-22
• Th17 response
Cholestasis
## Cholestasis in idILI

<table>
<thead>
<tr>
<th>Cholestasis Grade</th>
<th>All Cases</th>
<th>Hepatocellular</th>
<th>Mixed</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>123 (49.6)</td>
<td>77 (60.6)</td>
<td>24 (50.0)</td>
<td>22 (30.1)</td>
</tr>
<tr>
<td>1</td>
<td>38 (15.3)</td>
<td>22 (17.3)</td>
<td>6 (12.5)</td>
<td>10 (13.7)</td>
</tr>
<tr>
<td>2</td>
<td>49 (19.8)</td>
<td>20 (15.7)</td>
<td>8 (16.7)</td>
<td>21 (28.8)</td>
</tr>
<tr>
<td>3</td>
<td>38 (15.3)</td>
<td>8 (6.3)</td>
<td>10 (20.8)</td>
<td>20 (27.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>All Cases</th>
<th>Hepatocellular</th>
<th>Mixed</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocellular</td>
<td>112 (45.2)</td>
<td>41 (32.3)</td>
<td>23 (47.9)</td>
<td>48 (65.8)</td>
</tr>
<tr>
<td>Canaliclar</td>
<td>120 (48.4)</td>
<td>50 (39.3)</td>
<td>23 (47.9)</td>
<td>47 (64.4)</td>
</tr>
<tr>
<td>Cholangiolar</td>
<td>9 (3.7)</td>
<td>3 (3.2)</td>
<td>2 (4.3)</td>
<td>3 (4.2)</td>
</tr>
<tr>
<td>Ductal</td>
<td>0 (0)</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Hepatology 2014;59:661-670
Implicated in drug-induced cholestasis
Transporter Specific Mechanisms

- Cyclosporin, rifampin, bosentan, troglitazone, glibenclamide, sulindac competitively inhibit BSEP
- Luminal inhibition of BSEP by estrogen, progesterone
  - Gastro 118: 422-30
- Inhibition of MRP2
  - Biochem Pharmacol 64:151-8
- BSEP, MDR3 polymorphisms associated with drug induced cholestasis
  - Pharmacogenet Genomics 17:47-60
Inflammation and cell injury mediated cholestasis

- Pro-inflammatory cytokines reduce expression of transporter genes, increase NO production in cholangiocytes leading to ductular cholestasis (sepsis mechanisms)
  - Gastro 124:737-53; Hepatology 28:1637-44
- Alterations in actin microfilaments (chlorpromazine)
  - Am J Pathol 98:603-16
- Disruption of microtubules by drugs
- Alteration of membrane lipid composition and fluidity
Steatosis
# Steatosis in iDILI

<table>
<thead>
<tr>
<th></th>
<th>All Cases</th>
<th>Hepatocellular</th>
<th>Mixed</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steatosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Grade of Steatosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>86 (34.8)</td>
<td>41 (32.5)</td>
<td>20 (41.7)</td>
<td>25 (34.2)</td>
</tr>
<tr>
<td>&lt;5%</td>
<td>96 (38.9)</td>
<td>57 (45.2)</td>
<td>12 (25.0)</td>
<td>27 (37.0)</td>
</tr>
<tr>
<td>5-33%</td>
<td>43 (17.4)</td>
<td>14 (11.1)</td>
<td>12 (25.0)</td>
<td>17 (23.3)</td>
</tr>
<tr>
<td>33-67%</td>
<td>14 (5.7)</td>
<td>8 (6.3)</td>
<td>4 (8.3)</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td>&gt;67%</td>
<td>8 (3.2)</td>
<td>6 (4.8)</td>
<td>0 (0)</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td><strong>Type of Steatosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrovesicular</td>
<td>118 (73.3)</td>
<td>59 (69.4)</td>
<td>20 (71.4)</td>
<td>39 (81.3)</td>
</tr>
<tr>
<td>Mixed</td>
<td>22 (13.7)</td>
<td>12 (14.1)</td>
<td>3 (10.7)</td>
<td>7 (14.6)</td>
</tr>
<tr>
<td>Microvesicular</td>
<td>21 (13.0)</td>
<td>14 (16.5)</td>
<td>5 (17.9)</td>
<td>2 (4.2)</td>
</tr>
<tr>
<td><strong>Location of Steatosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone 3</td>
<td>45 (28.0)</td>
<td>20 (23.5)</td>
<td>10 (35.7)</td>
<td>15 (31.3)</td>
</tr>
<tr>
<td>Zone 1</td>
<td>2 (1.2)</td>
<td>0 (0)</td>
<td>1 (3.6)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>Panacinar</td>
<td>29 (18.0)</td>
<td>19 (22.4)</td>
<td>2 (7.1)</td>
<td>8 (16.7)</td>
</tr>
<tr>
<td>Azonal</td>
<td>85 (52.8)</td>
<td>46 (54.1)</td>
<td>15 (53.6)</td>
<td>24 (50.0)</td>
</tr>
</tbody>
</table>

Hepatology 2014;59:661-670
Distinguishing Micro- from Macrovesicular Steatosis

- **Microvesicular**
  - Innumerable tiny vacuoles filling cell imparting a foamy appearance to cytoplasm

- **Macrovesicular**
  - Generally “countable” numbers of vacuole, variable in size, may not fill cytoplasm
Microvesicular steatosis is frequently associated with mitochondrial injury

<table>
<thead>
<tr>
<th>Drug</th>
<th>MPTP$^a$ opening</th>
<th>Direct inhibition of mitochondrial FAO$^b$</th>
<th>OXPHOS uncoupling</th>
<th>Direct inhibition of the MRC$^c$</th>
<th>mtDNA depletion or damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen (APAP)$^d$</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alpidem</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aminoptine *</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amiodarone *</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Didanosine (ddl) *</td>
<td>*</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Disulfiram</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fialuridine (FIAU) *</td>
<td>*</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nilutamide</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Nimesulide</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panadiplon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perhexiine</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pirprofen *</td>
<td>*</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Salicylic acid *</td>
<td>*</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Stavudine (d4T) *</td>
<td>*</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tacrine</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline (and its derivatives) *</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Troglitazone</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Valproic acid (VPA) *</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Zidovudine (AZT) *</td>
<td>*</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^*$ = microvesicular steatosis
Abnormal Chain Termination with incorporation of FIAU, FMAU and FAU

Chronic exposure gradually depletes mtDNA in HepG2 cells

Proc Natl Acad Sci U S A 1996;93:3592-3597
Mechanisms of Drug-Induced Macrovesicular Steatosis

1. Moderate impairment of FAO
   - Amiodarone
   - Perhexiline
   - Tamoxifen
   - NRTI's
   - Glucocorticoids

2. Decreased VLDL secretion
   - Amiodarone
   - Perhexiline
   - Tetracycline

3. Direct activation of transcription factors (SREBP1c, PPARγ, PXR)
   - Interferon-α
   - Troglitazone
   - Tamoxifen
   - Nifedipine
   - Glucocorticoids
   - Risperidone (obesity)
   - Olanzapine (obesity)
   - Glucocorticoids (obesity)
   - Valproate (obesity)
   - NRTIs (lipoatrophy)
   - Protease Inhibitors (lipoatrophy)

4. Obesity, or lipoatrophy

Increased de novo lipogenesis
- Interferon-α
- Troglitazone
- Tamoxifen
- Nifedipine
- Glucocorticoids

Insulin resistance (with hyperinsulinemia)

Mitochondrion

Alteration of the MRC

ROS

Oxidative stress and lipid peroxidation

Macrovacular Steatosis

If chronic exposure

Steatohepatitis

Hepatocyte

Peroxisomal FAO
Microsomal CYPs
Association of Steatosis with Necrosis

Acetaminophen

Isoniazid

TABLE 5.6. Toxic agents that produce hepatic steatosis, necrosis, or both

<table>
<thead>
<tr>
<th>Agent administered</th>
<th>Steatosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCl₄</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CCl₄ and promethazine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CCl₄ and antioxidant</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>CCl₄ and SKF525A</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Thioacetamide</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ethionine</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Orotic acid</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

From Zimmerman, 2001
Inflammatory Infiltrates

• The presence of inflammation implies some role for the immune system, although does not necessarily suggest primary vs secondary

• The type and pattern of inflammation can be suggestive of particular immune responses, but the association is loose
  – Plasma cell rich infiltrates with interface hepatitis => autoimmune hepatitis-like
  – Numerous eosinophils and/or epithelioid granulomas => immunoallergic-like
Inflammatory Cell Detection

- B-cells (CD20, CD19, PAX5)
- Plasma cells (H&E, CD38, CD138, MUM-1)
- T-cells (CD3, CD4, CD8)
- T regulatory cells (CD25, FoxP3)
- NK-cells (CD56, CD57)
- Cytotoxic cells (perforin, granzyme, TIA-1)
- Macrophages/Kupffer cells (CD68, CD168, CD11b)
- Eosinophils (H&E, Major Basic Protein)
- Neutrophils (H&E, CD15)
- Mast cells (tryptase, CD117)
Comparative analysis of portal hepatic infiltrating leucocytes in acute drug-induced liver injury, idiopathic autoimmune and viral hepatitis

Dual staining allowed conservation of tissue
Portal hepatic infiltrating B cells [CD20+] were primarily found in non-DILI related injury; presence of NK cells [CD56+] allowed further discrimination between VH vs. AIH patients. (A) B cells [CD20+] and (B) NK cells [lymphoid CD56+] and normalized to portal triad size. Mean + SD are displayed for each subject.
SHORT COMMUNICATION

T Cells Infiltrate the Liver and Kill Hepatocytes in HLA-B*57:01-Associated Floxacillin-Induced Liver Injury

Natascha Wuillemin,*† Luigi Terracciano,‡ Helmut Beltraminelli,§ Christoph Schlapbach,¶ Stefano Fontana,** Stephan Krähenbühl,‖ Werner J. Pichler,* and Daniel Yerly*

Figure 1  IHC staining of a liver biopsy. H&E, CD3, CD8, and TIA-1 staining revealed periportal infiltration with CD3+ CD8+, and TIA-1+ lymphocytes. CD3 and CD8 staining were performed on serial sections. Original magnification: ×200.

Am J Pathol 2014;184:1677-1682
Other Tools
Spectral Imaging

- Method of image microscopy in which each pixel is represented by a spectrum rather than a single color
- Conventional or Fluorescent
- Software can de-convolute spectra given example of “pure” colors
Mass-Spectral Imaging

Lipid Zonation and Phospholipid Remodeling in Nonalcoholic Fatty Liver Disease

Zoe Hall,1,2 Nicholas J. Bond,2 Tom Ashmore,1 Francis Sanders,2 Zsuzsanna Ament,2 Xinzhu Wang,1 Andrew J. Murray,3 Elena Bellafante,4 Sam Virtue,5 Antonio Vidal-Puig,5 Michael Allison,6 Susan E. Davies,7 Albert Koulman,2 Michele Vacca,1,2,5 and Julian L. Griffin1,2

• Matrix-assisted laser desorption ionization mass spectral imaging (MALDI MSI) of frozen sections, coupled with lipidomic analysis

• Allowed microscopic mapping of lipid distributions across the hepatic acinus in human biopsies and animal models of NAFLD

Hepatology 2017;65:1165-1180
Model in panel C – ob/ob high fat diet
Dual-photon microscopy can identify individual collagen fibrils in unstained paraffin embedded tissue sections and can quantify many aspects of their architectural organization:

- Number of fibrils
- Length
- Diameter
- Cross-linking
- Orientation
• Four parameters out of 70 were independently associated with stage:
  – No. of strands
  – Strand eccentricity
  – Strand solidity
  – Strand length (near vessels)
Summary

• There is not a 1:1 correspondence between histology and particular mechanisms of injury
• However, histology can lead the way to investigations of mechanism and can distinguish between varied presentations of similar histologies
• A variety of tools currently exist for probing tissue while maintaining information about architectural relationships