Diagnosis of Liver AMR: Perspectives of a Kidney Pathologist

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Disclosures

Consultant Alexion (Clinical Trial Central Pathologist)

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I am a renal pathologist

Multiple Effects of Antibody on Kidney Allografts

Hyperacute AMR → Accommodation

Acute AMR

Chronic AMR → “Smoldering” AMR
How was antibody mediated rejection defined in the kidney?

Required evidence of:

1. graft injury
2. antibody interaction with graft
3. donor specific antibody

AMR Diagnostic Milestones: Kidney Transplantation

1991, 1993 C4d stain of peritubular capillaries → PRA, graft loss (Feucht)
(minimal pathology, no DSA)

1992, 1996 Distinct pathology (Capillaritis/fibrinoid necrosis) in acute rejection with class I DSA (Halloran)

1999 Acute humoral rejection triad (Collins)
DSA, C4d, capillaritis/fibrinoid necrosis

2001 Chronic humoral rejection triad (Mauiyyedi)
DSA, C4d, glomerulopathy/arteriopathy

2002 Capillaritis and glomerulitis in chronic AMR (Regele)

AMR Diagnostic Milestones: Kidney Transplantation

1999-2003 Solid phase DSA assays (Pei)

Banff consensus criteria
2003 Acute AMR
2007 Chronic AMR

2009 Endothelial gene expression in AMR (Sis)

2009, 2011 Recognition of C4d negative AMR with capillaritis (Sis, Loupy, Haas)

2012 Natural history of chronic AMR (Wiebe)
Advantages of C4d as a diagnostic test

Deposition in peritubular capillaries in the kidney is the most specific marker for DSA (>95% DSA+)
Rarely, if ever, seen in other conditions
Indicates DSA is interacting with the endothelium
Supports a role of complement activation
Transient (days) indicates current activity

Limitations of C4d

Variable, fluctuating presence in serial biopsies
(Nicke1ei 2003, Loupy 2011, Nickerson 2012)
Techniques differ in sensitivity and reproducibility
IHC paraffin (worst)
IF, two step and three step (best)
Chronic AMR typically less C4d than acute AMR, and may be entirely negative
(Sis 2009, 2012, Loupy 2011)
It is possible, even likely, that AMR is mediated by complement independent as well as complement dependent mechanisms
(Hirohashi 2012)

C4d Negative AMR

Comparison of Studies

<table>
<thead>
<tr>
<th>Patients</th>
<th>Time of Bx</th>
<th>Be type</th>
<th>DSA+</th>
<th>C4d-MTR</th>
<th>C4d-AMR</th>
<th>Definition of C4d-</th>
<th>Tech</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presens</td>
<td>3 m</td>
<td>P</td>
<td>14</td>
<td>22</td>
<td>61%</td>
<td>&lt;10%</td>
<td>HC=IF</td>
<td>Loupy 2009</td>
</tr>
<tr>
<td>Presens</td>
<td>&lt;3 m</td>
<td>I</td>
<td>12</td>
<td>11</td>
<td>44%</td>
<td>&lt;10%</td>
<td>HC=IF</td>
<td>Loupy 2011</td>
</tr>
<tr>
<td>Mostly presens</td>
<td>6-27m</td>
<td>I</td>
<td>19</td>
<td>19</td>
<td>50%</td>
<td>&lt;10%</td>
<td>IHC</td>
<td>de Kort 2013</td>
</tr>
<tr>
<td>Non presens Mostly &gt;1y</td>
<td>I</td>
<td>30</td>
<td>29</td>
<td>49%</td>
<td>&lt;10%</td>
<td>IF</td>
<td>Sis 2012</td>
<td></td>
</tr>
<tr>
<td>Non presens &gt;12 m</td>
<td>I</td>
<td>67</td>
<td>16</td>
<td>19%</td>
<td>&lt;10%</td>
<td>IF3x</td>
<td>Farkash unpub</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneous Studies
Presensitization
Type of biopsy (protocol vs indication)
Timing of biopsy (early, late)
Tech of C4d (IF, IHC)
Definition of C4d neg 0% or <10%
Definition of MI (>0, 1 or 2)
C1q fixation by DSA in serum correlates with C4d deposition in tissue

NonHLA? IgM?

Clinical Categories

Antibody Mediated Rejection (or Reaction)

<table>
<thead>
<tr>
<th>Category</th>
<th>Tissue Injury</th>
<th>DSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accommodation</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>Hyperacute</td>
<td>Necrosis, thrombosis, polys</td>
<td>+</td>
</tr>
<tr>
<td>Acute</td>
<td>Necrosis, thrombosis, polys</td>
<td>+</td>
</tr>
<tr>
<td>&quot;Smoldering&quot;</td>
<td>Capillaritis, mononuc</td>
<td>+</td>
</tr>
<tr>
<td>Chronic</td>
<td>↑Matrix, BM, proliferation</td>
<td>+</td>
</tr>
<tr>
<td>Plus</td>
<td>One indicator that antibody is interacting with tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C4d, capillaritis/glomerulitis, endothelial transcripts, endothelial activation</td>
<td></td>
</tr>
</tbody>
</table>

Renal pathologists agree on:

Pattern of C4d linked to DSA
peritubular capillaries

Specificity of C4d
>95% have HLA DSA

Technique: IF vs IHC
IF ↑sensitivity, ↑agreement, less artifact

Improvement needed
markers for C4d- AMR
Pathogenesis

Three Pathways to Antibody Mediated Rejection

Antibody Alone
Complement Mediated
Cell Mediated (FcR)

Capillaritis

Endo Gene Expression

Farkash and Colvin, Nat Rev Nephrol 8:255, 2012

Adaptive transfer of DSA to donor class I causes chronic allograft arteriopathy

B10.BR heart → B6/RAG1-/-
anti-H-2K^d Control

Uehara et al (MGH) AJT 7:57, 2007
NK Cells in Ab mediated Tx Arteriopathy

Ly49g

Chronic Antibody Mediated Transplant Arteriopathy
Dependent on NK Cells


DSA
+Complement, FcR/Cells

Endothelium

Glomeruli
Loss/activation
Thrombosis
Loss fenestrations
Duplication of BM
Filtration
Proteinuria

Peritubular Cap
Loss/activation
Thrombosis
Loss of capillaries
Duplication of BM
Ischemia

Arteries
Loss/activation
Thrombosis
Fibrinoid necrosis
Intimal fibroplasia
Ischemia

Portal vessels
?

Sinusoids
?

Kidney
Kidney
Kidney
Heart
Liver
Liver Diagnostic Criteria:
Acute Antibody Mediated Rejection

If antibody mediated rejection does occur in the liver, how do we recognize it?

Is C4d deposited and in what pattern?

C4d+ Portal capillaries and stroma

- Portal capillaries
- Portal stroma

Both considered positive
Stroma staining also in + cross match
No sinusoidal C4d

Sakashita (Kyoto) Mod Pathol 20:676, 2007

C4d+ Portal capillaries (also vein, artery)
More common in acute rejection (68%) than HCV (12%)

Kruckemeyer (Berlin) Transplantation 78:65, 2004
Schmedling (Berlin) AJT 6:523, 2006
Three patterns of C4d in liver allografts

- Hepatocyte cytoplasmic
- Sinusoidal
- Portal venule/capillary garland

Bellamy (Edinburgh) Histopathol 50:739, 2007

Significance of C4d pattern in liver allografts

- Hepatocyte cytoplasmic
- Sinusoidal
- Portal venule/capillary garland

Bellamy (Edinburgh) Histopathol 50:739, 2007

C4d+ Sinusoids and central veins (the real thing!)

- HLA presensitized patient (HLA-B7): day zero biopsy
- Developed chronic damage (bridging fibrosis, ductular proliferation)
- Ischemia reperfusion control = neg

Watson Kozlowski Nickelet... (Chapel Hill NC) AJT 6:3022, 2006
What C4d pattern in frozen sections correlates with pretx DSA?

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Diffuse Sinusoidal*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Portal Vein</td>
</tr>
<tr>
<td>Post perfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA+</td>
<td>13</td>
<td>0%</td>
</tr>
<tr>
<td>DSA-</td>
<td>42</td>
<td>54%</td>
</tr>
<tr>
<td>Indication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA+</td>
<td>15</td>
<td>87%</td>
</tr>
<tr>
<td>DSA-</td>
<td>17</td>
<td>0%</td>
</tr>
<tr>
<td>Preperfusion</td>
<td>47</td>
<td>0%</td>
</tr>
<tr>
<td>Native</td>
<td>7</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Linear or granular

Kosilowski et al (UNC), Liver Transpl 18:641, 2012

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Sinusoidal C4d Patterns

Diffuse Linear

Diffuse Granular

Kosilowski et al 2012

---

IF more sensitive than IHC

same case

IF Frozen

IHC Paraffin

Kosilowski et al 2012
IF (frozen) is better than IHC (paraffin)

15 paraffin embedded tissues from biopsies with diffuse sinusoidal C4d in frozen tissue were stained by IHC

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>IF</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusoidal C4d</td>
<td>15</td>
<td>100%</td>
<td>13%</td>
</tr>
</tbody>
</table>

Kozlowski et al 2012

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IHC C4d Patterns Associated with +Crossmatch (XM)

<table>
<thead>
<tr>
<th>Early Biopsies (&lt;21 days)</th>
<th>n</th>
<th>%</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antery</td>
<td>17</td>
<td>87</td>
<td>0.001</td>
</tr>
<tr>
<td>C.D</td>
<td>25</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>103</td>
<td>95</td>
<td>0.0001</td>
</tr>
<tr>
<td>C.D</td>
<td>27</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Central vein</td>
<td>198</td>
<td>100</td>
<td>0.001</td>
</tr>
<tr>
<td>C.D</td>
<td>17</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Portal vein</td>
<td>196</td>
<td>100</td>
<td>0.001</td>
</tr>
<tr>
<td>C.D</td>
<td>12</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Total Lymphocytes</td>
<td>195</td>
<td>95</td>
<td>0.004</td>
</tr>
<tr>
<td>Sinusoidal</td>
<td>38</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>C.D</td>
<td>4</td>
<td>100</td>
<td>0.005</td>
</tr>
<tr>
<td>Total mean</td>
<td>2.0 (1.1)</td>
<td>64 (44)</td>
<td>0.010</td>
</tr>
<tr>
<td>Median</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0-12</td>
<td>4-14</td>
<td></td>
</tr>
</tbody>
</table>

*Values obtained using regression models adjusted for gender, XMT, crossmatch negative, XMx = crossmatch positive, N = none, M in minimal, F = focal, D = diffuse.

Lunz, Demetris, AJT 12:171, 2012

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MGH Case


de novo DSA

Class II high MFI, minimal class I
Which pattern of C4d indicates acute AMR in liver transplants?

1. Portal stroma
2. Hepatic lobules/hepatocytes
3. Portal arterial endothelium
4. Central vein endothelium
5. Portal vein endothelium \( ? \)IHC
6. Portal capillary endothelium \( ? \)IHC
7. Sinusoids (IF>IHC)

In the kidney chronic AMR accounts for about 60% of late graft failures.

Does chronic antibody mediated rejection of the liver exist and how is it manifested?

<table>
<thead>
<tr>
<th>Table 1. C4d Staining in Liver Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>Native liver, biopsy, or explant</td>
</tr>
<tr>
<td>No evidence of rejection, biopsy</td>
</tr>
<tr>
<td>Acute (portal) rejection, biopsy</td>
</tr>
<tr>
<td>Acute central rejection, biopsy</td>
</tr>
<tr>
<td>Ductopenic rejection, biopsy, or explant</td>
</tr>
<tr>
<td>Chronic vascular rejection, explant</td>
</tr>
<tr>
<td>Chronic rejection</td>
</tr>
</tbody>
</table>

All Portal capillary pattern
Lessons from combined liver-kidney tx

Use kidney AMR to identify pattern of liver cases with AMR (rosetta stone)

Compare susceptibility of liver and kidney to AMR

Evaluate kidney AMR protection by liver

Bellamy (Edinburgh) Histopathol 50:739, 2007
Liver and Kidney Acute AMR

Kidney d14
Liver d11

DSA HLA A26, DR4

Both recovered

Hadaya Transpl Int 22:242, 2009

Lessons from Combined Liver-Kidney Transplant

The kidney can get AMR without notable involvement of the liver

Barth Clin Transpl 24:685, 2010
(+portal venous C4d)

MGH Case
Liver is less susceptible to AMR than Kidney

Simultaneous liver-kidney tx-- DSA+

C4d - Kidney
C4d - Liver
MGH Case
Combined Liver-Kidney Tx

62 yo woman with NASH and MPGN I
Combined kidney and liver transplant 2-4-12
9 days later Cr rose to 3.8 (from 1.4 post tx) anuric
Liver enzymes coming down, no spike (no bx)

de novo DSA to HLA-A2, C3 and DR13

RxC: PEX, IVIG, Thymoglobulin, rituximab
Followup
3 wks later Cr & LFT nl
4 mo DSA neg
1 yr nl Cr, LFT

Endarteritis
C4d

Recommendations from the kidney world

Practical
Use frozen sections
Take serum for DSA regularly and with every biopsy

Organizational
Develop a consensus among liver pathologists and clinicians on criteria for C4d positivity

Investigation
Test newer approaches (gene expression, endothelial activation markers…)
Seek evidence of C4d neg AMR
Focus on chronic lesions
ANTIBODY MEDIATED REJECTION IN LIVER TRANSPLANTATION
MARCH 22-23, 2013 • ADOLPHUS HOTEL • DALLAS, TX 75202

[Image of a page with text and a diagram]

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C4d

I HAVE NOTHING FURTHER TO SAY

But I will return...