Diagnosis of Acute and Chronic Antibody-Mediated Rejection: Banff Classification and Pathologic Correlates of Graft Survival

Mark Haas
Cedars-Sinai Medical Center
Los Angeles, California, USA
1. Pathology of acute ABMR in kidney: histology, C4d, DSA, and the 2013 Banff classification

2. Pathology of chronic, active ABMR in kidney: transplant glomerulopathy, DSA, and the 2013 Banff classification

3. Pathologic factors influencing graft survival following treatment of active ABMR
Statement of Disclosure

Mark Haas serves as a paid consultant on pathology adjudication committees for two industry-sponsored clinical trials:

Shire ViroPharma – Treatment of Acute ABMR
AstraZeneca – Treatment of Proliferative Lupus Nephritis

Neither represents a conflict of interest relevant to any of the material presented in this talk.
<table>
<thead>
<tr>
<th>Finding</th>
<th>Ab+ Rejection</th>
<th>Ab- Rejection</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe arteritis</td>
<td>10/24</td>
<td>0/20</td>
<td>0.001</td>
</tr>
<tr>
<td>Infarction</td>
<td>9/24</td>
<td>0/20</td>
<td>0.002</td>
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<tr>
<td>PMNs in PTC</td>
<td>11/24</td>
<td>1/20</td>
<td>0.003</td>
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<tr>
<td>Glomerulitis</td>
<td>11/24</td>
<td>2/20</td>
<td>0.01</td>
</tr>
<tr>
<td>Fibrin thrombi (glom and vasc)</td>
<td>11/24</td>
<td>3/20</td>
<td>0.05</td>
</tr>
<tr>
<td>Dilation of PTC</td>
<td>8/24</td>
<td>2/20</td>
<td>0.08</td>
</tr>
<tr>
<td>PMNs in glomeruli</td>
<td>7/24</td>
<td>3/20</td>
<td>NS</td>
</tr>
<tr>
<td>Moderate or severe tubulitis</td>
<td>12/24</td>
<td>19/20</td>
<td>0.002</td>
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CD68
C4d
C4d and Early Graft Loss
(H.E. Feucht et al, Kidney Int 43: 1333-8, 1993)

- 93 renal allografts biopsied for early dysfunction (mean 11 days post-transplant)
  - 43 biopsies – diffuse PTC C4d
    18 graft losses in 1st year (58% graft survival)
  - 8 biopsies – focal PTC C4d
    3 graft losses in 1st year (63% graft survival)
  - 42 biopsies – C4d negative in PTC
    4 graft losses in 1st year (90% graft survival)
- 3/4 cases of graft loss in C4d- group were C4d+ on a later biopsy
- C4d+ associated with re-transplant, elevated PRA
C4d Staining in Renal Allografts: correlation with donor-specific Ab

- **Collins et al, JASN 10: 2208-14, 1999**
  100% of AR with +DSA were C4d+
  No C4d in DSA- AR, CSA toxicity

- **Maueyyedi et al, JASN 13: 779-787, 2002**
  30% of early AR C4d+ - 90% had anti-donor antibody
  2 morphologic subtypes of AMR - capillary, arterial
  Arterial (fibrinoid necrosis) had worse outcome

- **Bohmig et al, JASN 13: 1091-9, 2002**
  21/24 C4d+ cases had DSA by flow cytometric XM
  50% of C4d- biopsies had DSA
  93% specificity, 31% sensitivity (IHC on paraffin sections)
Diagnostic Criteria for Acute AMR in Renal Allograft Biopsies

1. Morphologic evidence
   a. Neutrophils and/or monocytes/macrophages in PTC and/or glomeruli (peritubular capillaritis; glomerulitis)
   b. Arterial fibrinoid necrosis
   c. Thrombi in glomerular capillaries, arterioles, and/or small arteries
   d. Acute tubular injury, without other apparent causes

2. Immunohistologic evidence
   a. Diffuse C4d in PTC
   b. Immunoglobulin and/or complement in arterial fibrinoid necrosis

3. Serologic evidence
   a. Circulating antibodies to donor HLA or other specific anti-donor antibodies at the time of biopsy
Compared clinical, pathologic parameters in DSA-positive renal transplant recipients at 1 year post-transplant based on findings on a 3-month protocol biopsy:

Subclinical ABMR (14 patients)
C4d+ with glomerulitis and/or PTC WBC margination

Suspicious but not diagnostic (22 patients)
C4d- with glomerulitis and/or PTC WBC margination

No ABMR (9 patients)
C4d-, no glomerulitis or PTC WBC margination
Findings 1 year post-transplantation:

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean GFR</th>
<th>TA/IF CI + CT &gt;0</th>
<th>Transplant Glomerulopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC ABMR (14): (C4d+, g/ptc+)</td>
<td>39 +/- 14</td>
<td>100%</td>
<td>43%</td>
</tr>
<tr>
<td>“Suspicious” (22): (C4d-, g/ptc+)</td>
<td>46 +/- 18</td>
<td>77%</td>
<td>18%</td>
</tr>
<tr>
<td>No ABMR (9): (C4d-, g/ptc-)</td>
<td>62 +/- 19</td>
<td>33%</td>
<td>0%</td>
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</table>
Acute/Active ABMR; all 3 features must be present for diagnosis

1. Histologic evidence of acute tissue injury, including one or more of the following:
   - Microvascular inflammation (g > 0\(^b\) and/or ptc > 0)
   - Intimal or transmural arteritis (v > 0)\(^c\)
   - Acute thrombotic microangiopathy, in the absence of any other cause
   - Acute tubular injury, in the absence of any other apparent cause

2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ([g + ptc] >2)\(^d\)
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated

3. Serologic evidence of donor-specific antibodies (HLA or other antigens)

\(^a\) These lesions may be clinically acute, smoldering, or subclinical. Biopsies showing two of the 3 features may be designated as “suspicious” for acute/active ABMR.

\(^b\) Recurrent/de novo glomerulonephritis should be excluded

\(^c\) These lesions may be indicative of ABMR, TCMR, or mixed ABMR/TCMR

\(^d\) In the presence acute T cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc >2 alone is not sufficient to define moderate microvascular inflammation and g must be ≥1.
Different Etiologies of Transplant Glomerulopathy

1. Chronic/Persistent Antibody-Mediated Rejection
   (73% of for-cause biopsies with TG at mean of 5.5 yrs post-transplant were C4d+, had concurrent DSA, or both;
   Sis et al, AJT 7: 1743-1752, 2007)

2. Hepatitis C
   - Need to differentiate from recurrent or de novo MPGN, using IF and/or EM
   - Possibly related to TMA associated with anti-cardiolipin antibodies

3. Other forms of TMA

4. Cell-Mediated Rejection?

anti-Class II DSA
anti-Class II non-DSA
No anti-Class II

Proportion of patients with TG

Month of TG diagnosis

Gloor et al, AJT 7: 2124-32, 2007
Biopsies ≤3 mo post-transplant with MVI, DSA, glom. endothelial EM changes

Haas and Mirocha, AJT 11: 2123-31, 2011
Chronic, Active ABMR; all three features must be present for diagnosis

1. Morphologic evidence of chronic tissue injury, including 1 or more of the following:
   - Transplant glomerulopathy (cg >0)\(^g\), if no evidence of chronic TMA
   - Severe peritubular capillary basement membrane multilayering (requires EM)\(^h\)
   - Arterial intimal fibrosis of new onset, excluding other causes

2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ([g + ptc] >2)\(^i\)
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated

3. Serologic evidence of donor-specific antibodies (HLA or other antigens)

\(^f\) In the absence of evidence of current/recent antibody interaction with the endothelium (those features in section 2), the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time post-transplantation.

\(^g\) Includes GBM duplication by electron microscopy only (cg1a) or GBM double contours by light microscopy

\(^h\) >7 layers in 1 cortical peritubular capillary and >5 in 2 additional capillaries, avoiding portions cut tangentially

\(^i\) In the presence acute T cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc >2 alone is not sufficient to define moderate microvascular inflammation and g must be >1.
Comparison of Predictive Value of Banff 2013 vs. Banff 2007 Criteria for Chronic, Active ABMR

123 patients, single center, indication bx Jan 2006 – Oct 2014
45 reached combined endpoint of graft loss or doubling of SCr

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<tr>
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<th>Banff 2007</th>
<th>Banff 2013</th>
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<td>% with CAABMR</td>
<td>18%</td>
<td>36%</td>
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<tr>
<td>HR of CAABMR for</td>
<td>1.6 [0.7-3.8]</td>
<td>2.5 [1.2-5.2]</td>
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<tr>
<td>combined endpoint</td>
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Graft Survival After Treatment
“Pure” ABMR vs Mixed ABMR/TCMR
80 cases: 37 Type 1, 43 Type 2

P = 0.037

P = 0.073 by multivariable analysis

M Haas et al, Kidney Int, 2017
P = 0.0007

P = 0.013 by multivariable analysis

M Haas et al, Kidney Int, 2017
Thank you for your attention. Any questions?