Do We Always Need Donor-Specific Antibodies to Diagnose ABMR?

Mark Haas
Cedars-Sinai Medical Center
Los Angeles, California, USA
Brief review of current Banff diagnostic criteria for acute/active and chronic, active ABMR

Can we use one or more surrogate markers to diagnose ABMR in the absence of detectable DSA?

- C4d
- Molecular markers
  - DSA – specific transcripts (DSASTs)
  - Molecular ABMR classifier
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.
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\textsuperscript{b} and/or ptc > 0)
   - Intimal or transmural arteritis (v > 0)\textsuperscript{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 (\([g + ptc] > 2\))\textsuperscript{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)

\textsuperscript{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.

\textsuperscript{b} Recurrent/de novo glomerulonephritis should be excluded

\textsuperscript{c} These lesions may be indicated of ABMR, TCMR, or mixed ABMR/TCMR

\textsuperscript{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.
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

<table>
<thead>
<tr>
<th>% with CAABMR</th>
<th>Banff 2007</th>
<th>18%</th>
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<td></td>
<td>Banff 2013</td>
<td>36%</td>
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HR of CAABMR for combined endpoint

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<th></th>
<th>Banff 2007</th>
<th>1.6 [0.7-3.8]</th>
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<tr>
<td></td>
<td>Banff 2013</td>
<td>2.5 [1.2-5.2]</td>
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</table>
1. What to do with a biopsy showing (g + ptc) >1, C4d+, + TG, and NO DSA?

2. What to do with a biopsy showing (g + ptc) >2, C4d-, + TG, and NO DSA?
Microvascular Inflammation (MVI) is NOT Specific for Active ABMR

Examine expression of pathogenesis-based transcript sets (PBTs) previously found to be associated with ABMR in 356 clinically indicated renal allograft biopsies.

209 with MVI = 0 (25% DSA+, 8% C4d+)
67 with MVI = 1 (36% DSA+, 15% C4d+)
80 with MVI ≥ 2 (54% DSA+, 50% C4d+)

P values for all PBTs, DSA+ vs. DSA-, within MVI = 1 and MVI ≥1 were not significant except for DSASTs
and Neither is Transplant Glomerulopathy (TG) Specific for Chronic ABMR - TG Has Multiple Etiologies

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 (?)

What about C4d?
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

- **Maveyyedi 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)*
Should DSA be required for ABMR diagnosis in C4d+ biopsies?
Gaston et al (DeKAF Study), Transplantation 90: 68-74, 2010
Influence of DSA and C4d on Outcomes in Chronic, Active ABMR with Transplant Glomerulopathy
Lesage et al (Quebec City), Transplantation 99: 69-76, 2015

61 patients with late indication biopsy (median 79 mo), TG and MVI
  45 C4d- and DSA- (‘isolated TG’)
  14 C4d+ and DSA- (6) or C4d- and DSA+ (8)
  12 C4d+ and DSA+
Influence of DSA and C4d on Outcomes in Chronic, Active ABMR with Transplant Glomerulopathy
Lesage et al (Quebec City), Transplantation 99: 69-76, 2015

\[ P = 0.01 \]
FOR YOUR CONSIDERATION:

Given the high specificity of C4d for DSA and these outcomes data, can DSA requirement for ABMR diagnosis be waived in biopsies of ABO-compatible kidneys with MVI and C4d?
What to do with a biopsy showing (g + ptc) ≥2, C4d-, + TG, and NO DSA?

Test for non-HLA DSA
- Not all labs do such testing for all relevant non-HLA Abs
- In most labs, routine DSA testing does not include HLA-C and HLA-DP

Consider molecular testing
- DSAST transcript set highly correlated with anti-HLA DSA in two independent labs (U. Alberta, Albert Einstein)
- Not known if expression increased with non-HLA DSA
- Doesn’t distinguish between IgG subclasses, C1q-binding vs. non-binding, 1 vs. >1 DSA, high vs. low MFI
Defining a Transcript Set Associated with DSA (DSASTs)  
Hidalgo et al (Edmonton), Am J Transplant 8: 1812-22, 2010

A.  
- DSA+ n=54  
- DSA neg n=91  
- 290 transcripts (FDR <0.05)  
- 132 transcripts (FDR <0.005)

B.  
- DSA II+ (DSA II+, DSA I/II+) n=41  
- DSA II neg (DSA I+, DSA neg) n=104  
- DSA I+ (DSA II+, DSA I/II+) n=23  
- DSA I neg (DSA II+, DSA neg) n=122  
- 272 transcripts (FDR <0.05)  
- 0 transcripts (FDR <0.05)

C.  
- DSA+ > DSA neg (n=290)  
- 92  
- 198  
- 74  
- DSA II+ > DSA II neg (n=272)
Defining a Transcript Set Associated with DSA (DSASTs)
Hidalgo et al (Edmonton), Am J Transplant 8: 1812-22, 2010

B - Transcripts preferentially expressed in NK cells
C - Transcripts preferentially expressed in endothelial cells
D - Transcripts expressed in endothelial and other cell types
Defining a Transcript Set Associated with DSA (DSASTs)
Hidalgo et al (Edmonton), Am J Transplant 8: 1812-22, 2010
Based on 30 non-redundant probes, selected from comparisons between biopsies + or - histologic ABMR (DSA+, C4d+ or C4d-)
Cell types of highest expression, based on literature and/or expression in cell cultures:
Endothelial cells – 17
NK cells – 5
Tubular epithelial cells – 4
T cells – 3
Macrophages – 2
IFN Gamma-induced - 2
Unknown cell type - 5
Association of molecular ABMR score with histologic diagnosis (mixed rejections excluded)

<table>
<thead>
<tr>
<th>ABMR Score</th>
<th>ABMR</th>
<th>No ABMR</th>
<th>Total</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
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<tr>
<td>&gt;0.2</td>
<td>64</td>
<td>66</td>
<td>130</td>
<td>0.49</td>
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<tr>
<td>≤0.2</td>
<td>46</td>
<td>499</td>
<td>545</td>
<td></td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>110;</td>
<td>565;</td>
<td>675;</td>
<td></td>
<td></td>
<td>0.83</td>
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sensitivity=0.58; specificity=0.87

P. Halloran et al, JASN 26: 1711-1720, 2015
A Probabilistic Approach to Histologic Diagnosis of Antibody-Mediated Rejection in Kidney Transplant Biopsies

P Halloran et al, AJT 17: 129-139, 2017

Relative variable importance: predicting ABMR score > 0.2 (N=703)
Additive value of the ABMR Molecular Score for reclassification of risk of allograft failure (continuous net reclassification improvement)

Alexandre Loupy et al. JASN 2014;25:2267-2277
One and Three Year Post-Biopsy Graft Survival As a Function of Microarray and Histologic Diagnosis of ABMR/Mixed Rejection


M+ = ABMR score >0.2; C+ = diagnostic or suspected ABMR C4d+ or C4d-
Schematic of an analysis of a new biopsy sample in relation to a reference set of samples from indication biopsies

What to do with a biopsy showing (g + ptc) >2, C4d-, ± TG, and NO anti-HLA DSA or non-HLA antibodies against the graft?

These are cases where molecular diagnostics have great potential for clinical usefulness, and should now be a specific focus for investigation.
Thank you for your attention. Any questions?