Clinical Implications of HLA Antibodies in Solid Organ Transplantation

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In the beginning...


Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells.

• 21 transplants, 2 with hyperacute rejection, both had transfusions and pregnancies
• both had extremely strong IgG leucocyte agglutinins with titers above 1/512

“...humoral antibodies active against antigens in the graft may have a decisive effect on the fate of the graft.”


Significance of the positive crossmatch test in kidney transplantation.

• 225 transplants
• eight of 195 with negative crossmatch failed to function immediately, in contrast to 24 of 30 with positive crossmatch (p<0.001)
• Immediate failure occurred in significantly higher numbers among patients with a higher risk of having antibodies, such as multiparous females and patients receiving secondary transplants.

“The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.”
Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and regraft outcome.

- 123 renal transplants performed in the presence of a positive cytotoxic crossmatch
  - either peak +/current + or peak +/current –
  - IgG vs IgM by DTT treatment
- IgG HLA class I Ab
  - poor survival
- IgM HLA class I Ab: peak +/current –
  - acceptable survival
- positive B cell XM due to IgM or IgG HLA antibodies
  - good primary survival but poor regraft survival
- IgM non-HLA Ab: peak +/current +
  - good primary and regraft survival
flow crossmatch
solid phase antibody testing

• much more sensitive
• detecting antibodies that previously went undetected by CDC
  – low titer
  – didn’t activate complement
• are these antibodies clinically relevant?
### Table 1. The clinical relevance of DSA varies amongst different studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>$N$</th>
<th>DSA+ ($n$)</th>
<th>Type</th>
<th>Donor</th>
<th>Selection</th>
<th>AMR (C4d+)</th>
<th>Graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patel et al. [12]</td>
<td>60</td>
<td>20</td>
<td>FCXM</td>
<td>LD</td>
<td>No</td>
<td>↑</td>
<td>←</td>
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<tr>
<td>Gupta et al. [13]</td>
<td>121</td>
<td>16</td>
<td>CDCXM</td>
<td>DD</td>
<td>No</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Berg Loonen et al. [14]</td>
<td>34</td>
<td>13</td>
<td>CDCXM</td>
<td>DD</td>
<td>AM</td>
<td>←</td>
<td></td>
</tr>
<tr>
<td>Billen et al. [15]</td>
<td>165</td>
<td>32</td>
<td>CDCXM</td>
<td>DD/LD</td>
<td>No</td>
<td>↓</td>
<td>← ↔ II</td>
</tr>
<tr>
<td>Eng et al. [16]</td>
<td>471</td>
<td>27</td>
<td>CDCXM</td>
<td>DD</td>
<td>BXM+</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Vlad et al. [17]</td>
<td>325</td>
<td>27</td>
<td>CDCXM</td>
<td>DD</td>
<td>No</td>
<td>↑</td>
<td>←</td>
</tr>
<tr>
<td>Amico et al. [18]</td>
<td>334</td>
<td>67</td>
<td>CDCXM</td>
<td>DD/LD</td>
<td>No</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Wahrmann et al. [19]</td>
<td>338</td>
<td>39</td>
<td>CDCXM</td>
<td>DD/LD</td>
<td>No</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Aubert et al. [20]</td>
<td>114</td>
<td>11</td>
<td>CDCXM</td>
<td>??</td>
<td>No</td>
<td>← ↔ II</td>
<td></td>
</tr>
<tr>
<td>Phelan et al. [21]</td>
<td>64</td>
<td>12</td>
<td>CDCXM (AHG)</td>
<td>LD</td>
<td>No</td>
<td>← ↔ II</td>
<td></td>
</tr>
<tr>
<td>Morris et al. [22]</td>
<td>149</td>
<td>15</td>
<td>CDC/FCXM</td>
<td>LD</td>
<td>No</td>
<td>← ↔ II</td>
<td></td>
</tr>
<tr>
<td>Lefaucheur et al. [23]</td>
<td>402</td>
<td>76</td>
<td>CDCXM</td>
<td>DD</td>
<td>No</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Riethmüller et al. [24]</td>
<td>37</td>
<td>20</td>
<td>CDCXM</td>
<td>LD</td>
<td>Sens</td>
<td>↑ ← II</td>
<td>↓</td>
</tr>
<tr>
<td>Bartel et al. [25]</td>
<td>68</td>
<td>51</td>
<td>CDCXM</td>
<td>DD</td>
<td>IA</td>
<td>← ↔ II</td>
<td></td>
</tr>
<tr>
<td>Couzi et al. [26]</td>
<td>45</td>
<td>34</td>
<td>CDC/FCXM</td>
<td>DD</td>
<td>FCXM+</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Couzi et al. [26]</td>
<td>45</td>
<td>11</td>
<td>CDC/FCXM</td>
<td>DD</td>
<td>FCXM−</td>
<td>← ↔ II</td>
<td></td>
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<tr>
<td>Willicombe et al. [27]</td>
<td>480</td>
<td>45</td>
<td>CDC/FCXM</td>
<td>DD/LD</td>
<td>AL</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Caro-Oleas et al. [28]</td>
<td>892</td>
<td>50</td>
<td>CDC</td>
<td>DD</td>
<td>No</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

AL, induction with alemtuzumab; AHG-XM, complement dependent cytotoxicity crossmatch in the presence of anti-human-immunoglobulin; AMR antibody-mediated rejection; BXM, B cell crossmatch; CDC, complement-dependent cytotoxicity; CDCXM, crossmatch in complement dependent cytotoxicity; DD, deceased donor; DSA, donor specific HLA antibodies; FCXM, flow cytometric crossmatch; IA, immunoadsorption; LD, living donor. ↔ No difference. ↑ or ↓ Increased or decreased.
PRE-TRANSPLANT DSA
Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads

Retrospective study of 334 kidney transplants 1999-2004
Current CDC XM- (2 had remote B CDC XM+) no prospective FXM test day of txp serum for DSA by Luminex (pos>500 MFI): 67 DSA+

Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation

observational study of 402 deceased donor kidney recipients 1998-2006
historic: 118 I/II Ab positive, 46 DSA by ELISA, 83 DSA by SAB, 24 remote CDC XM+
current: 76 DSA by SAB, CDC XM− (DSA pos >300 MFI)

historic (peak) DSA, class I or II, is associated with decreased graft survival
even in absence of AMR

Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation

Risk of AMR is higher in patients with preexisting DSA >465 MFI (by ROC analysis)

Table 2. RR for acute AMR according to the MFI of highest pregraft ranked DSA detected by Luminex (logistic regression)

<table>
<thead>
<tr>
<th>DSA MFI(_{\text{max}}) class</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤465</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>465 to 1500</td>
<td>24.8 (4.6 to 134.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1500 to 3000</td>
<td>23.9 (3.5 to 160.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>3000 to 6000</td>
<td>61.3 (11.5 to 327)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;6000</td>
<td>113.0 (30.8 to 414)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Graft survival is lower in patients with preexisting DSA >3000 MFI

A all patients

B no AMR

Pre-transplant donor specific anti-HLA antibody is associated with antibody-mediated rejection, progressive graft dysfunction and patient death.

Retrospective study of 258 renal transplants 2003-2007, median 5.6 yrs follow-up, all CDC T XM–, 4 BX+ DSA+, 30 BX+ DSA–, 1 DSA+ no BX

day of txp serum screened by luminex mixed then SAB if pos (DSA pos>500 MFI)

**DSA >8000 MFI, especially class II or class I +II leads to increased rates of acute AMR**

The Significance of Pretransplant Donor-Specific Antibodies Reactive with Intact or Denatured HLA in Kidney Transplantation

pretransplant sera with DSA from 156 kidney transplant recipients tested on class I regular SAB, iBeads and SAB with denatured antigen

reactivity to exclusively denatured HLA was not associated with decreased graft survival

Otten et al, Clin Exp Imm. 2013
Effect of Pretransplant Human Leukocyte Antigen Antibodies Detected by Solid-Phase Assay on Heart Transplant Outcomes

retrospective study of 85 heart transplants T CDC XM-tested pre-txp sera with flow XM(retrospective) and luminex SAB (DSA pos >1500 MFI)

patients with DSA >1500 and positive flow XM have higher rates of AMR and CMR

**Correlation of circulating donor-specific anti-HLA antibodies and presence of C4d in endomyocardial biopsy with heart allograft outcomes: a single-center, retrospective study**

112 cardiac allografts, 109 patients, 170 biopsies with concurrent C4d IF and DSA by luminex mixed, phenotype and SAB (pos >1,000 MFI) pre- and post-transplant DSA measurement

<table>
<thead>
<tr>
<th>DSA (n = 170 biopsies)</th>
<th>No C4d</th>
<th>Weak C4d</th>
<th>C4d &gt; 50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA Class I (n = 30)</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>HLA Class II (n = 61)</td>
<td>28 (46%)</td>
<td>24 (39%)</td>
<td>9 (15%)</td>
</tr>
<tr>
<td>HLA Class I and II (n = 39)</td>
<td>15 (39%)</td>
<td>9 (23%)</td>
<td>15 (38%)</td>
</tr>
<tr>
<td>Any DSA (n = 110)</td>
<td>48 (44%)</td>
<td>36 (32%)</td>
<td>26 (24%)</td>
</tr>
<tr>
<td>No DSA (n = 60)</td>
<td>43 (72%)</td>
<td>14 (23%)</td>
<td>3 (5%)</td>
</tr>
</tbody>
</table>

(B) Biostatistically Significant p-Values for Comparison of C4d IF Staining in Patients With DSA to Class I and/or Class II vs Without DSA

<table>
<thead>
<tr>
<th>DSA</th>
<th>Class I and II</th>
<th>No DSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>( p = 0.01 )</td>
<td>( p = 0.0004 )</td>
</tr>
<tr>
<td>Class II</td>
<td>( p = 0.004 )</td>
<td>( p = 0.003 )</td>
</tr>
</tbody>
</table>

* C4d IF staining score in EMBs during episodes of graft dysfunction in patients with positive DSA.

* Weak C4d: \( <1 \) Interstitial capillary staining in any percentage of myocardial parenchyma or any intensity of staining in \(<50\%\) of interstitial capillaries.


DSA to class I or class I+II correlates with C4d deposition and graft failure
Class II Alloantibody and Mortality in Simultaneous Liver-Kidney Transplantation

86 SLKT patients with pretransplant serum sample (76% had posttransplant serum sample) luminex SAB pos >2000 MFI: 30 with preformed DSA, 9 with de novo DSA

preformed class II DSA increased risk of renal AMR, liver AMR and ACR, and graft loss

Class II Alloantibody and Mortality in Simultaneous Liver-Kidney Transplantation

Donor-Directed MHC Class I Antibody Is Preferentially Cleared from Sensitized Recipients of Combined Liver/Kidney Transplants

liver allografts may not fully protect renal allografts in the presence of class II DSA

POST-TRANSPLANT *DE NOVO* DSA
Evolution and Clinical Pathologic Correlations of De Novo Donor-Specific HLA Antibody Post Kidney Transplant

315 consecutive renal transplants without pre-txp DSA (Flow PRA beads and Flow XM neg) 1999-2008 (mean follow-up 6.2±2.9 years)
serum collected at 0, 1, 2, 3, 6, 12, 18, 24 months and at time of biopsy for graft dysfunction,
biopsy when dnDSA was newly detected
protocol biopsy at 6 and 24 months for patients with no dnDSA or graft dysfunction
47 developed dnDSA

dnDSA vs without

 dnDSA vs dnHLA or pretxp HLA

 dnDSA vs other dysfunction

de novo DSA can lead to antibody mediated injury and progress to graft loss

De Novo DQ Donor-Specific Antibodies Are Associated With a Significant Risk of Antibody-Mediated Rejection and Transplant Glomerulopathy

retrospective study of 505 renal txp patients 2005-2010 without pre-txp DSA CDC B and T XM neg, Flow T XM neg, luminex SAB DSA neg (pos >300 MFI) 92(18.2%) developed DSA (MFI>300)with 50 (54.3% of the 92) having DQ DSA mixed bead screen first - then positive had SAB

de novo DQ DSA are associated with poor graft outcome

Willicombe, et al. Transplantation 2012;94:172
De Novo Donor HLA-Specific Antibodies after Heart Transplantation Are an Independent Predictor of Poor Patient Survival

retrospective analysis of 243 cardiac txp recipients with no preexisting DSA (CDC XM neg) measure HLA Ab every year after txp up to 13 years luminex SAB pos >1000 MFI considering patterns of reactivity and CREGs

<table>
<thead>
<tr>
<th>Table 3: Impact of persistent de novo DSA on patient survival in a multivariable Cox proportional hazards model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated hazard ratio</td>
</tr>
<tr>
<td>De novo and persistent DSA</td>
</tr>
<tr>
<td>HLA-DR mismatch</td>
</tr>
<tr>
<td>Donor age</td>
</tr>
<tr>
<td>Hemodynamic compromise</td>
</tr>
<tr>
<td>Treated rejection in the 1st year</td>
</tr>
</tbody>
</table>

de novo DSA are strongly associated with poor survival

De Novo Donor-Specific HLA Antibodies Decrease Patient and Graft Survival in Liver Transplant Recipients

retrospective study of 749 adult liver txp recipients without pre-txp DSA (pos >5000 MFI) test serum at 1 year posttransplant

deo novo DSA, mostly against DQ, lead to poor survival

Things to consider when reading papers

• crossmatching before transplant
  – CDC and/or flow?
  – CDC and flow XM both negative?
  – CDC negative / flow positive?
  – CDC positive / flow negative?
  – what is cutoff for positive flow XM?
  – was pronase used?
  – B cells vs T cells?
Things to consider when reading papers

- **antibody analysis**
  - what was MFI cutoff for positive?
    - 300-5,000
  - how was MFI cutoff chosen?
    - random, ROC analysis, based on XM positivity? CDC XM or Flow XM?
  - were all loci considered, including Cw and DP?
  - which bead platform – flow or luminex?
  - which vendor’s SABs were used?
  - are all donor alleles included in SABs?
  - what resolution of beads?
    - mixed screen, phenotype, single antigen
  - antibody spread across an epitope
    - might look negative
  - IgG vs IgM
  - complement fixing?
    - IgG subclass, C1q, C4d
    - C1q original method or with wash and/or AHG
Things to consider when reading papers

• patient characteristics
  – size of cohort
  – selection criteria
  – prospective or retrospective
  – more immunosuppression or desensitization for patients with DSA?
  – time of serum samples and biopsies
    • protocol or only when graft dysfunction?
  – number of years post-transplant follow-up
Thank You