Detection and Monitoring of Antibodies Pre-Heart Transplant

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I have financial relationship(s) with:
List Commercial Interest(s) and Type of Financial Relationship
Grant/Research- Immucor, CSL Behring, NIAID
Consultant- Genentech

AND

My presentation does not include discussion of off-label or investigational use.

Learning Objectives:
• Outline the methods and approaches to detection of HLA antibodies for heart transplant candidates
• Explain strategies and risk factors for monitoring HLA antibodies prior to heart transplant
• Describe strategies for Virtual Crossmatching for optimizing donor selection for sensitized heart transplant candidates
Goals of Pre-Transplant HLA Antibody Assessment in Heart Transplant:

- Establish if patient is has immunologic memory “allosensitized” and the likelihood of finding a compatible donor: calculated PRA (cPRA)
- Virtual XM to optimize selection of histocompatible/incompatible donor, increase donor pool
- Sensitization with Mechanical Device
- Risk assessment for rejection
- Risk assessment for long-term outcome
- Optimization of immunosuppressive regimens
- Monitoring response to desensitization therapy

Histocompatibility Techniques

- Sensitivity & Specificity
- CDC
- AHG-CDC
- Flow XM
- ELISA
- Luminex
- Flow PRA
- Solid-phase assays

- Amount of Antibody

Interfering Factors in Lymphocyte XM

- mAb therapy and Xeno-Ab
  - Rituxin, anti-CD20 - remove CD20 on B cell
  - Thymoglobulin/ ATG - causes lysis in CDC assay, positive Flow XM/Luminex when 2nd Ab binds IgG

- Immune complexes in serum binding Fc receptors on lymphocytes

- Pronase - proteolytic enzyme produced by Streptomyces Griseus with Aminopeptidase activity against Fc receptors
  - Reduces Fc receptor-mediated binding
  - Increases sensitivity of B cell crossmatch
  - Reduces plasma clotting due to autoreactive antibodies
  - Limitation: can mediate cleavage of HLA antigens so enzymatic activity must be monitored
MFI is a Measurement of the Antibody Bound to Beads

Single Antigen Beads
Class I and II
DR15
DQ5
A3
A2
A24

Denatured Human Leukocyte Antigen Ab in Sensitized Kidney Recipients: Prevalence, Relevance, & Impact on Organ Allocation


Flow XM

SAB MFI

Denatured
Non-Denatured

IgM can cause false negatives in single antigen

Zachary et al. Human Immunology. 2009
Complement Components Can Interfere with Single Antigen Tests

Steric hindrance by C1 complex is refuted as a mechanism of interference; C4 and C3 product deposition seems to be important.

Caveats of Antibody Strength Measurements in Bead Mixes vs. Cells

Broadly reactive clone antibody binds many beads. Antigen with more restricted repertoire binds fewer beads. On cell only one allele is expressed, but has the public epitope recognized by that antibody (that was present on multiple beads). So now antibodies are not diluted across multiple specificities and may reflect TRUE Ab strength/titer.

What is Prozone? The Hook Effect

Noted in 1948 by Karl Landsteiner in "The specificity of serological reactions". In agglutination assays, "Some sera show an inhibition zone ("prozone"), and frequently agglutination is slowed down at high agglutinin concentrations. This phenomenon was found to be well-marked with some heated immune sera and was attributed to modified agglutins—agglutinoids—that are capable of combining but do not cause clumping, or to coating of the cells with non-specific protein or other substances..."

Prozone is typically defined as antibody-excess, whereas antigen-excess is post-zone. Demonstrated by testing a sample at neat and at dilution, where the signal in the diluted sample is higher than in the neat—essentially a false negative.
Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information

CPRA: Key Features
- Based on unacceptable antigens
  - Defined by HLA Ab specificity and strength
  - Defined by transplant center practice
- CPRA calculated from HLA frequencies
  - Using frequencies derived from 12,000 recent US donors
  - Includes both Class I and Class II HLA antigen frequencies and linkages
- Provides sensitive and consistent antibody identification across US
- Estimates probability of transplant
- Eliminates avoidable, positive XMs.
  - Streamline organ allocation
  - Increases donor pool for sensitized transplant candidates
  - Increases organ utilization

Defining Unacceptable Antigens to Facilitate Transplantation and Risk Assessment
- Defined by antibody specificity
- Defined by strength of antibody
- Defined by transplant center practice
- Considers immunological history
  - Previous mismatched transplants
  - Previous pregnancies, Blood transfusions
  - Mechanical Device
  - Infections
- An unacceptable antigen is one that causes a high risk of graft loss
### Correlation of Virtual XM, SAB, FCXM & CDC XM

<table>
<thead>
<tr>
<th>Single Antigen MFI</th>
<th>FCXM</th>
<th>CDC XM</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3000</td>
<td>Neg</td>
<td>Neg</td>
<td>Low</td>
</tr>
<tr>
<td>3000-5000</td>
<td>Expect some Pos</td>
<td>Neg</td>
<td>Low-Intermediate</td>
</tr>
<tr>
<td>5000</td>
<td>Mostly Pos</td>
<td>Neg</td>
<td>Intermediate</td>
</tr>
<tr>
<td>8000-10,000</td>
<td>&gt; 200 MCS</td>
<td>Neg</td>
<td>High</td>
</tr>
<tr>
<td>15,000+</td>
<td>250-400 MCS</td>
<td>Pos</td>
<td>Contraindication</td>
</tr>
</tbody>
</table>

Q. Zhang, M Hickey, N Valenzuela, M Cecka, E Reed, UCLA Immunogenetics

### Virtual Crossmatch

**Donor 1**

- HLA Typing: A1, A29, B8, B44, Bw4, Bw6, C7, C16, DR17, DR7, DR52, DR53, DQ5, DQ6, DP2, DP4 (GPA2)

**Donor 2**

- HLA Typing: A2, A33, B44, Bw4, C5, C10, DR14, DR17, DR52, DR53, DQ5, DQ2, DP5 (GPA2)

**Donor 3**

- HLA Typing: A1, A30, B8, B42, Bw4, C7, C17, DR17, DR18, DR52, DQ2, DQ4, DQ7

### De Novo HLA Ab Following BIVAD vs. VAD Placement

<table>
<thead>
<tr>
<th>Device</th>
<th>Baseline</th>
<th>Maximum</th>
<th>Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BiVAD</td>
<td>16.7%+30</td>
<td>50.8%+33</td>
<td>34%+33</td>
<td>0.03</td>
</tr>
<tr>
<td>HMII</td>
<td>48%+15</td>
<td>52%+20</td>
<td>4%+7.9</td>
<td>0.045</td>
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Keen and Reed EF. The Journal of Thoracic and Cardiovascular Surgery
Humoral immune response to donor HLA after implantation of human heart valve allografts


UCLA ADULT HEART PRE-TRANSPLANT CARDIAC KIQLATION and ANTIBODY ASSESSMENT

First Encounter – Initial Antibody Evaluation
(Includes: Cytotoxic Antibody PRA Class I and II, Flow PRA & ID Class I and Class II, and Single Antigen Antibody Class I and Class II

Status 2
Low Risk: Stable VAD outpatient, patient with history of pregnancy, transfusion and/or previous surgery, prior transplant.

Status 1
Low Risk: No clinical risk, repeated negative HLA PRA.

**High Risk: History of pregnancy, blood transfusion and/or previous surgery, HLA PRA positive; prior transplant.

Complement Activating HLA DSA Mediate Graft Injury

- AMR remains a major contributor of kidney allograft loss
- Activation of the classical complement pathway is a dominant mechanism of injury in AMR
- In vitro assays can be used to assess the complement-activating potential of DSAs
IgG vs C1q Correlation with AMR in Pediatric Heart Transplant Biopsies (n=18)

<table>
<thead>
<tr>
<th>Pt</th>
<th>CPRA</th>
<th>IgG/C1q</th>
<th>Flow XM</th>
<th>T/B MCS</th>
<th>VXM</th>
<th>IgG/C1q</th>
<th>Post-HTx</th>
<th>DSA</th>
<th>IgG/C1q</th>
<th>AMR</th>
<th>DSA</th>
<th>IgG/C1q</th>
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<tbody>
<tr>
<td>A</td>
<td>16/36</td>
<td>0/0</td>
<td>-</td>
<td>0/0</td>
<td>0/0</td>
<td>None</td>
<td>None</td>
<td>4.5</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>B</td>
<td>100/88</td>
<td>0/0</td>
<td>276/50</td>
<td>100/100</td>
<td>0/0</td>
<td>Pre</td>
<td>1/1</td>
<td>11.7</td>
<td>Pre</td>
<td>1/1</td>
<td>1/1</td>
<td>11.7</td>
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<tr>
<td>C</td>
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<td>0/0</td>
<td>93/0</td>
<td>93/0</td>
<td>0/0</td>
<td>Pre</td>
<td>1/1</td>
<td>3.4</td>
<td>Pre</td>
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<td>3.4</td>
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<tr>
<td>D</td>
<td>50/30</td>
<td>0/0</td>
<td>478/456</td>
<td>1/1</td>
<td>1/1</td>
<td>Pre</td>
<td>1/1</td>
<td>12.7</td>
<td>Pre</td>
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<td>449/430</td>
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<td>1/1</td>
<td>Pre</td>
<td>1/1</td>
<td>10.4</td>
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<tr>
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<td>0/0</td>
<td>44/38</td>
<td>44/38</td>
<td>0/0</td>
<td>Pre</td>
<td>1/1</td>
<td>6.6</td>
<td>Pre</td>
<td>1/1</td>
<td>1/1</td>
<td>6.6</td>
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<tr>
<td>G</td>
<td>50/30</td>
<td>0/0</td>
<td>367/408</td>
<td>1/1</td>
<td>1/1</td>
<td>Pre</td>
<td>1/1</td>
<td>8.3</td>
<td>Pre</td>
<td>1/1</td>
<td>1/1</td>
<td>8.3</td>
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<tr>
<td>H</td>
<td>44/40</td>
<td>0/0</td>
<td>320/264</td>
<td>1/1</td>
<td>1/1</td>
<td>Pre</td>
<td>1/1</td>
<td>6.5</td>
<td>Pre</td>
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<td>6.5</td>
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<tr>
<td>I</td>
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<td>0/0</td>
<td>2/24</td>
<td>2/24</td>
<td>0/0</td>
<td>Pre</td>
<td>1/1</td>
<td>3.0</td>
<td>Pre</td>
<td>1/1</td>
<td>1/1</td>
<td>3.0</td>
</tr>
</tbody>
</table>

One-Year Survival in Heart Recipients with DSA+/C4d+DSA was Dramatically Lower than DSA+/C4d- Patients

John Smith and Marlene Rose, JHLT 2014

**ONE TRANSPLANT FOR LIFE**
**MANY PATHWAYS TO SUCCESS**

HLA-Ab induction of C3d is inhibited by TNT003 in Luminex-based assay

Thomas KA, Valenzuela NM, Reed EF AJT, 2014
Anti-Endothelial Cell Antibodies

Tissue specific Auto-Ag or Polymorphic Ag

- Artery
- Venule
- Capillary
- Skin
- Heart
- Lung

Endothelial Progenitor cell

Vimentin: Rose ML
MICA: Reed EF, Rose ML
Collagen V: Wilken DS, Mohanakumar T
Epithelium K-α1 tubulin: Mohanakumar T

Agrin: Paul LC
Angiotensin II type-1 Receptor: Dragun D
MICA: Stastny P, Terasaki PI, Abramowicz D

Anti-Endothelial Cell Antibodies
Tissue specific Auto-Ag or Polymorphic Ag

Effects of DSA on Graft Pathogenesis

Determinants
- Intrinsic
- Extrinsic
- Rheologic

Effectors Functions
- Antibody
- Complement
- Monocytes

Mechanisms of Injury
- Leukocyte
- Acute Allograft
- Synovial Arthritis

Histological Manifestations of Injury
- Acute Rejection
- Chronic Rejection
- Endothelial Changes
- Vascular

Impact of Pre-transplant sensitization on Heart Allograft Survival

- No Pre-transplant Sensitization
- Pre-transplant Sensitization

Impact of DSA on Graft Pathogenesis

KA Thomas, NM Valenzuela, EF Reed, Trends in Molecular Medicine 2015
Th17 Responses to Collagen Type V, α1-Tubulin, and Vimentin Are Present Early in Human Development and Persist Throughout Life

Sullivan JA and Burlingham WJ. AJT 2016

Increased Negative Impact of HLA-DSA Together With Non-HLA-Specific Antibodies on Heart Allograft Outcome