HLA ANTIBODY TESTING
Problems and Solutions

Howard M. Gebel, Ph.D., D(ABHI)
Professor of Pathology
Co-Director, Histocompatibility and Molecular Immunogenetics Laboratory
Emory University Hospital
Atlanta, GA
hgebel@emory.edu
Friday March 22 at 10:10-10:35 a.m.
Session: Current State-of the

Conflicts

None

<table>
<thead>
<tr>
<th></th>
<th>Rejection</th>
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<tbody>
<tr>
<td>Positive Crossmatch</td>
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<td>6</td>
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<tr>
<td>Negative Crossmatch</td>
<td>8</td>
<td>187</td>
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Patel and Terasaki, NEJM 280:735,1969
HLA antibodies and Rejection

- Lungs Lobo, LJ et al. DSA are associated with AMR, ACR, BOS and cystic fibrosis after lung transplantation J Heart Lung Transplant. 32: 70-7, 2013
- Islets Campbell et al. Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. AJT 7:1242-8, 2007

3.6.13 Histocompatibility Testing for Liver Transplantation
The transplant program and its histocompatibility laboratory must have a joint written policy on HLA typing, antibody screening, and crossmatching. Guidelines for policy development, including assigning risk and timing of crossmatch testing, are set out in Appendix D of Policy 3
METHODS FOR ANTIBODY EVALUATION

Cytotoxicity
- NIH
- Variations
  Washes
  Extended Incubation
  Antiglobulin
  DTT/DTE

Flow Cytometry
- T cell / B cell

Complement Dependent Cytotoxicity Assay (CDC)

**Transplantability Index**

The probability that a sensitized patient will have a negative crossmatch with a given donor.

\[ \text{Transplantability Index} = 100 - \% \text{PRA} \]

### Panel/Percent Reactive Antibody

<table>
<thead>
<tr>
<th>Tray Pos.</th>
<th>Cell ID</th>
<th>Panel Typing</th>
<th>A</th>
<th>B</th>
<th>Cw</th>
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<td>3</td>
<td>63</td>
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<td>38</td>
<td>73</td>
<td>12</td>
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</table>

### Panel/Percent Reactive Antibody

**T-Cell ONLY**

<table>
<thead>
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<td>11</td>
<td>37</td>
<td>52</td>
<td>6</td>
</tr>
</tbody>
</table>

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**Panel Typing**

- **Cell I.D.**
- **Tray Pos.**
- **Panel Typing**
- **A**
- **B**
- **Cw**
Problems

Sensitivity not optimal

Panel composition

Viability

Typically restricted to class I antibodies

Evolution of HLA Antibody Detection


3-Color Flow Cytometric Crossmatch
Virtual Crossmatching

Predicting the crossmatch based on:

1) Knowledge of the HLA antibody specificities present in the recipient.
2) The HLA phenotype of the potential donor.

A, B, C
DRB1, DQB1, DRB3/4/5
DQA, DPA, DPB

“We conclude that the approach of detailed antibody analysis can result in an improvement for successful transplantation of more dialysis patients who are highly sensitized to HLA alloantigens.”

Delmonico, et. al. Transplantation 36:692 1983

IgG FCXM: Renal Allograft Study
Frequency of rejection in a single center

Kerman et al Transplantation 68:1855-1858, 1999
METHODS FOR ANTIBODY EVALUATION

Cytotoxicity
- NIH
- Variations
  - Washes
  - Extended Incubation
  - Antiglobulin
  - DTT/DTE

Flow Cytometry
- T cell / B cell

Antigen Non-Specific

Solid Phase HLA Antigen Specific Assays

Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006

EBV Transformed Cell Line
HLA Transfected Cell Line

Purified HLA Antigens
phenotypes/single alleles
Class I/Class II

conjugation

Class I/Class II
coated microparticles

Potential donor:
DRB1*04, DRB1*07; DR53, +;
DQB1*02, DQB*0302 (8)

Potential donor:
DRB1*15, DRB1*16; DR51, -;
DQB1*05, DQB1*06

DQ 7,8,9; DR53  cPRA=69%
**5 Year Deceased-Donor Renal Allograft Survival in Unsensitized PRA Patients with Negative FCXM**


**Calculated PRA: Initial Results Show Benefits for Sensitized Patients and a Reduction in Positive Crossmatches**

J. M. Cook*, A. Y. Rachireddy*, R. L. Retiroment* and M. S. LeMell*

Table 1: Number of positive crossmatches reported as a reason for organ refusal

<table>
<thead>
<tr>
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<tbody>
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<td>100</td>
<td>120</td>
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<td>134</td>
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<td>60-194</td>
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<td>1069</td>
<td>2477</td>
<td>866</td>
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<tr>
<td>All</td>
<td>16,589</td>
<td>12,585</td>
<td>9,543</td>
<td>737</td>
<td></td>
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</tbody>
</table>

Leukocyte donor kidney transplants: 570/21


Catter Sikal, Jing Owens, Kinah Mahwood, Bernd Dieter, Sabine Scherer, Andrea Reinhardt, Thuy Vo Tram, Andreas Heinrich, and Gerhard Opitz

Transplantation 91:883-887, 2011
Solid phase HLA antibody detection assays:

a) benefit highly sensitized patients and promote more appropriate allocation of deceased donor organs

b) disadvantage highly sensitized patients and promote inappropriate allocation of deceased donor organs
Seven Stages of Technological Infatuation

- Initial skepticism
- Cautious experimentation
- Widespread enthusiasm
- Further investigation
- Widespread condemnation
- Reexamination of data
- Appropriate implementation

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Preformed Complement-Activating Low-Level Donor-Specific Antibody Predicts Early Antibody-Mediated Rejection in Renal Allografts

Christopher Lawrence, Michelle Williamson, Paul A. Brookes, Eva Serrano-Navarre, Jerrold Bajaj, Terry Cook, Candida Rosados, David Tansie, and Anthony N. Warren.

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Transplantation epublished ahead of print, 2013:
Are all antibodies detected on beads clinically relevant?

1) Vendors
2) Antigen source
   1) Native
   2) Recombinant
3) Antigen expression
   1) Conformationally correct
   2) Denatured
   3) Amount
4) Reagents
5) Platforms
   1) Flow cytometry
   2) Luminex

XM NEGATIVE WITH DR53+ TARGET;
XM+ WITH DR15+ TARGET
Donor-specific antibody against denatured HLA-A1: Clinically nonsignificant?

Additional Considerations
Assay variability
1) Inter/Intra lab
2) Bead to bead variation
3) Methodological variability
Additional Considerations
Assay variability

1) Inter/Intra lab
2) Bead to bead variation
3) Methodological variability
4) Patient variability
Consequences if vXM is incorrect

Negative vs Positive
- Inappropriate allocation
- Rejection
- Increased ischemic times

Positive vs Negative
- Missed opportunities

Consensus Guidelines on the Testing and Clinical Management Issues Associated With HLA and Non-HLA Antibodies in Transplantation

16) Kidney
a. Unacceptable HLA antigens should be a part of kidney allocation algorithms. [2]
b. Accurate XM prediction depends on complete HLA typing. To minimize the incidence of unexpected positive XM in paired exchange registries, the donor should be typed at HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA, HLA-DQB, HLA-DPA, and HLA-DPB loci. [2]
c. A renal transplantation can be performed without a prospective pretransplantation CDC or flow XM if SAB testing indicates the consistent absence of DSA against HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA, HLA-DQB, HLA-DPA, and HLA-DPB locus antigens. Each center needs to develop its policy in agreement with regulatory bodies and clinical programs. [3]