Defining Antibody Mediated Rejection:
A UCLA Cross-Disciplinary Workshop to improve diagnosis and treatment of Acute and Chronic AMR

Friday, Oct. 25, 2013 / Tamkin Auditorium, B-130
Ronald Reagan UCLA Medical Center
Defining Antibody Mediated Rejection:
A UCLA Cross-Disciplinary Workshop to improve diagnosis and treatment of Acute and Chronic AMR

11:45 a.m. - 12:15 p.m. Lunch outside Tamkin Auditorium (RRMC B-130)

12:15 - 12:30 p.m. 1. Defining Donor Specific Antibodies- Immunogenetics
   a. HLA antibodies- Jennifer Zhang
   b. Non-HLA antibodies- Elaine Reed

12:30 - 1:15 p.m. 2. Renal transplant-
   a. Clinical definition of AMR Adult- Mike Bumgarnders & Gerry Lipshutz
   b. Clinical definition of AMR Pediatric- Eileen Tsai
   c. Graft Pathology- Fernando Palma Diaz

1:15 - 2 p.m. 3. Heart transplant-
   a. Clinical definition of AMR Adult- Mario Deng
   b. Clinical definition of AMR Pediatrics- Greg Perens
   c. Graft pathology- Dean Wallace

2 - 2:15 p.m. Break

2:15 - 2:45 p.m. 4. Lung transplant-
   a. Clinical definition of AMR- David Ross
   b. Graft Pathology- Dean Wallace

2:45 - 3:30 p.m. 5. Liver transplant-
   a. Clinical definition of AMR Adult- Doug Farmer
   b. Clinical definition of AMR Pediatrics- Laura Wozniak
   c. Graft Pathology- Bita Naini

3:30 - 4 p.m. 6. Small Bowel transplant-
   a. Clinical definition of AMR- Doug Farmer
   b. Graft Pathology- Bita Naini

4 - 5 p.m. Group Discussion-
   a. Developing optimal practices for diagnosis of AMR
   b. Identify knowledge Gaps/Recommend future basic and clinical research strategies
Defining Donor Specific Antibodies

Jennifer Q. Zhang

UCLA Immunogenetics Center
Department of Pathology
UCLA School of Medicine
Immunologic Barriers to Transplantation

**HLA antigens**
- MHC class I (HLA-A, B, C)
- MHC class II (HLA-DR, DP, DQ)

**ABO blood group antigens**

**Non-HLA antigens**
- Collagen Type IV, and VI
- Vimentin, Myosin
- MHC Class I related chain A (MICA)
- Angiotensin II type I receptor (AT1R)
Humoral Alloreactivity to HLA

- Pregnancy
- Blood Transfusion (Artificial devices)
- Transplantation (Use of tissue allografts for vascular reconstruction in congenital heart surgery)
- Bacterial / Viral Infection
Consequences of Preformed anti-donor HLA Antibodies

- Hyperacute rejection, Accelerated acute rejection
- Delayed graft function
- Chronic rejection
- Prolonged waiting times
- No transplantation

Sensitization rates:
- 30% of patients awaiting for renal/liver and small bowel transplant
- 15-20% of patients awaiting for heart/lung transplant
Goals of the Antibody Detection

- **Sensitivity**
  Is there an HLA antibody present?  
  What is the antibody titer?

- **Specificity**
  Is the antibody clinical relevant?  
  Precise detection of ALL HLA specificities  
  What are the isotypes – IgG or IgM  
  Are they able to fix complement (IgG_{1,3} vs IgG_{2,4})?  
  Are they donor-specific HLA antibodies?
Antibody Detection Methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>HLA Antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC Crossmatch</td>
<td>Native</td>
</tr>
<tr>
<td>Flow Crossmatch</td>
<td>Native</td>
</tr>
<tr>
<td>PRA</td>
<td>Phenotype Affinity Purified</td>
</tr>
<tr>
<td>SAB</td>
<td>Recombinant</td>
</tr>
<tr>
<td>C1q-SAB</td>
<td>Recombinant</td>
</tr>
</tbody>
</table>

Hyperacute Rejection (CDC+)

Accelerated Rejection (FLOW+/CDC-)

Chronic Rejection Accommodation?

T/B Flow Crossmatch

Solid-phase assays

Sensitivity & Specificity ↑
Luminex Single Antigen vs C1q Test

Anti-HLA antibodies
Non-complement fixing

Anti-HLA antibodies
Complement fixing

Detect complement fixing
IgG1/IgG3 and IgM antibodies

Detect all IgG antibodies

SAB

C1Q
Management of Unacceptable Antigens

• Cytotoxic antibodies
• Single Antigen Test
  – Renal/Pancreas: Luminex SAB > 8,000 MFI
    (Antibodies with predicted flow \(xm\) > 200)
  – Pediatric Renal: 1,000 MFI
  – Heart/Lung/Small Bowel: Luminex SAB >5,000 MFI
  – C1q+ for Heart/Lung
  – Liver?
• Luminex PRA > 2,000 MFI
• All specificities for regraft candidates

Update UNET when tests are reviewed
Challenges for Identifying DSA and Risk Stratification

- Out-of-date tests
- Multiple weak donor-specific antibodies
- Donor typing not complete
  - HLA-DP
  - HLA DQ alpha
# The Development of DSA Post-Transplant

<table>
<thead>
<tr>
<th>Organ</th>
<th>Author</th>
<th>N</th>
<th>% De Novo DSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Mustat</td>
<td>43</td>
<td>62.7% (39.5% Cd4+)</td>
</tr>
<tr>
<td></td>
<td>O’Leary</td>
<td>78</td>
<td>44% chronic rejector in 1-year, 62% in 20-year</td>
</tr>
<tr>
<td></td>
<td>Kaneku</td>
<td>749</td>
<td>8.1% in 1-year</td>
</tr>
<tr>
<td></td>
<td>Miyagawa</td>
<td>79</td>
<td>48% in 1-year (living donor)</td>
</tr>
<tr>
<td>Small Bowel</td>
<td>Tsai</td>
<td>13</td>
<td>31%</td>
</tr>
<tr>
<td>Heart</td>
<td>Smith</td>
<td>243</td>
<td>25.4% in one year</td>
</tr>
<tr>
<td></td>
<td>Zhang</td>
<td>168</td>
<td>22% in one year</td>
</tr>
<tr>
<td></td>
<td>Peng</td>
<td>183</td>
<td>33% in one year (pediatric)</td>
</tr>
<tr>
<td>Lung</td>
<td>Hachem</td>
<td>116</td>
<td>44.8% in 90 days, 56% in 18 months</td>
</tr>
<tr>
<td></td>
<td>Lobo</td>
<td>44</td>
<td>23% in 1-year, 30% in 3-year</td>
</tr>
<tr>
<td></td>
<td>Snyder</td>
<td>441</td>
<td>12% in 1-year</td>
</tr>
<tr>
<td>Kidney</td>
<td>Chaudhuri</td>
<td>124</td>
<td>6% in 2 years (pediatric)</td>
</tr>
<tr>
<td></td>
<td>Cooper</td>
<td>244</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>Wiebe</td>
<td>315</td>
<td>15% in 5-year</td>
</tr>
<tr>
<td></td>
<td>Everly</td>
<td>189</td>
<td>11% in 1-year, 20% in 5-year, 25% in 10-year</td>
</tr>
<tr>
<td></td>
<td>Loupy</td>
<td>1016</td>
<td>31% in 5-year</td>
</tr>
<tr>
<td></td>
<td>Freitas</td>
<td>284</td>
<td>19% in 1-year</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Cantarovich</td>
<td>167</td>
<td>15.5% in 1-year</td>
</tr>
</tbody>
</table>

*Note: SAB cutoff: 5000 MFI*
## Post-Transplant DSA Assessment

### Pediatric Renal

<table>
<thead>
<tr>
<th>Type</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>4w</th>
<th>2m</th>
<th>3m</th>
<th>4m</th>
<th>5m</th>
<th>6m</th>
<th>9m</th>
<th>12m</th>
<th>Bi-annual</th>
<th>Annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Sensitized</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitized</td>
<td>1m</td>
<td>3m</td>
<td>6m</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>6m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desensitization</td>
<td>1w</td>
<td>2w</td>
<td>3w</td>
<td>4w</td>
<td>2m</td>
<td>3m</td>
<td>4m</td>
<td>5m</td>
<td>6m</td>
<td>9m</td>
<td>12m</td>
<td>Quarterly</td>
<td>Bi-annual</td>
</tr>
</tbody>
</table>

### Adult and Pediatric Heart

<table>
<thead>
<tr>
<th>Type</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>4w</th>
<th>6w</th>
<th>2m</th>
<th>3m</th>
<th>4m</th>
<th>5m</th>
<th>6m</th>
<th>8m</th>
<th>10m</th>
<th>12m</th>
<th>Quarterly</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12m</td>
<td></td>
</tr>
</tbody>
</table>

### Adult Lung

<table>
<thead>
<tr>
<th>Type</th>
<th>1m</th>
<th>3m</th>
<th>6m</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>12m</th>
<th>Bi-annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12m</td>
</tr>
</tbody>
</table>

### Liver & Small Bowel combined

<table>
<thead>
<tr>
<th>Type</th>
<th>1m</th>
<th>3m</th>
<th>6m</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>12m</th>
<th>Bi-annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSA-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12m</td>
</tr>
<tr>
<td>DSA+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12m</td>
</tr>
</tbody>
</table>

### Isolated Small Bowel

<table>
<thead>
<tr>
<th>Type</th>
<th>1m</th>
<th>3m</th>
<th>6m</th>
<th>9m</th>
<th>12m</th>
<th>Bi-annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-Sensitized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regraft/DSA-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA+</td>
<td>1w</td>
<td>2w</td>
<td>4w</td>
<td>8w</td>
<td>3m</td>
<td>6m</td>
</tr>
</tbody>
</table>

### Adult Renal

<table>
<thead>
<tr>
<th>Type</th>
<th>6w</th>
<th></th>
<th></th>
<th>12m</th>
<th>Annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSA-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA+</td>
<td></td>
<td></td>
<td></td>
<td>6m</td>
<td></td>
</tr>
<tr>
<td>Desensitization</td>
<td>4-5d</td>
<td>2w</td>
<td>4w</td>
<td>8w</td>
<td>6m</td>
</tr>
</tbody>
</table>

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**All patients with suspected AMR**
### Remaining Questions

- The risk of DQ DSA
- The risk of Cw, DP
- The risk of C1q+ DSA vs non-C1q DSA

#### The treatment patient based on DSA?

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplant</td>
<td>Renal</td>
<td>Renal</td>
<td>Liver</td>
<td>Heart</td>
<td>Lung</td>
</tr>
<tr>
<td>De Novo DSA</td>
<td>18.2% (92/502)</td>
<td>18% (62/347)</td>
<td>63% (27/43)</td>
<td>33% (57/173)</td>
<td>10% (9/90)</td>
</tr>
<tr>
<td>DQ DSA</td>
<td>54.3% (50/92)</td>
<td>53% (33/62)</td>
<td>81% (22/27)</td>
<td>72% (41/57)</td>
<td>56% (5/9)</td>
</tr>
</tbody>
</table>
Thank You

"OK, the old one’s in my right hand, the donor’s in my left. Right?"
Anti-Endothelial Cell Antibodies in Organ Transplantation

- Endothelial Progenitor cell
  - Artery
    - Vimentin: *Rose ML*
    - MICA: *Reed EF, Rose ML*
  - Vein
    - collagen V: *Wilkes DS, Mohanakumar T*
    - K-alpha-1 tubulin: *Mohanakumar T*
  - Heart
  - Capillary
    - Agrin: *Paul LC*
    - Angiotensin II type-1 Receptor: *Dragun D*
  - Lung
  - Kidney
    - MICA: *Stastny P, Terasaki PI, Abramowicz D*
Anti-EC Antibodies Can Mediate Graft Injury Via Complement Dependent and Independent Pathways

Complement Dependant Pathways

- Natural IgM antibody
- Anti-MICA antibody
- Anti-Type V Collagen antibody
- Anti-Vimentin antibody
- Weible Palade Body excytosis
- Leukocyte Recruitment
- Anti-α tubulin antibody
- Anti-AT1R antibody
- PKC
- TCF5, cMyc
- NF-κB
- AP-1
- Fibrogenic growth factors
- Fibroproliferation
- Proliferation
- Cytokine production
- Tissue Factor

Complement Independent Pathways

- Anti-α tubulin antibody
- Anti-AT1R antibody
- NF-κB
- AP-1
- Fibrogenic growth factors
- Fibroproliferation
- Proliferation
- Cytokine production
- Tissue Factor
Diagnosis of Antibody Mediated Rejection

- **No Antibody**: 8
- **Class I +**: 33 (74%)
- **Class II +**: 
- **Class I&II +**: 

OR$_{90}=20$

P<0.001 (vs. Controls)

TCAD at 5yr

Ab+ =86%

Ab- =22%

Michaels et al. Journal of Heart and Lung Transplantation, 2003
**AMR is Accompanied by Development of DSA to HLA and MICA in Heart Transplants**

<table>
<thead>
<tr>
<th>DSA</th>
<th>Groups</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA</td>
<td>AMR</td>
<td>22 (60%)</td>
<td>15</td>
<td>37</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Non-AMR</td>
<td>6 (4.6%)</td>
<td>125</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>MICA</td>
<td>AMR</td>
<td>5 (26%)</td>
<td>14</td>
<td>19</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Non-AMR</td>
<td>1 (2%)</td>
<td>52</td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>

Q Zhang, Reed EF Transplantation 2011
**Combined Results Of HLA, MICA And Endothelial Cell Crossmatch**

<table>
<thead>
<tr>
<th>Categories</th>
<th>HLA DSA</th>
<th>MICA DSA</th>
<th>Both HLA/MICA</th>
<th>EC XM Pos</th>
<th>Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMR+</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>AMR-</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>41</td>
<td>53</td>
</tr>
</tbody>
</table>

*This analysis was restricted to patients with donor/recipient pairs of MICA genotyping*

Q Zhang, Reed EF Transplantation 2011
Proto Array Reactivity of Heart Transplant Rejection Sera

66 Candidate Proteins

- Auto Ag: 18%
- Anti-EC: 25%
- Cytoskeleton: 8%
- Nuclear: 8%
- Extracellular: 5%
- Other: 36%

P. Rao, Reed EF, 2012
Anti-HLA Antibodies Mediate Endothelial Cell Injury Via Several Mechanisms

- Complement Activation
- C4d Deposition
- Neutrophil, NK cell and Macrophage Infiltration
- Proliferation, Migration
- Cytoskeletal Regulation

**Fc-Dependent Functions**

**Fc-Independent Functions**
Phospho-S6K and S6RP Associate with AMR

- Increasing levels of phosphorylation of S6K and S6RP exhibited stronger association with AMR
- A level of 2+ or greater significantly augments the risk of AMR

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>pS6K, grade 1+</td>
<td>18</td>
<td>0.001</td>
<td>3 – 100</td>
</tr>
<tr>
<td>pS6K, grade 2+</td>
<td>52</td>
<td>&lt;0.001</td>
<td>6 – 425</td>
</tr>
<tr>
<td>pS6K, grade 3+</td>
<td>49</td>
<td>0.001</td>
<td>5 – 521</td>
</tr>
<tr>
<td>pS6RP, grade 1+</td>
<td>4</td>
<td>0.06</td>
<td>1 – 13</td>
</tr>
<tr>
<td>pS6RP, grades 2+/3+</td>
<td>10</td>
<td>0.008</td>
<td>2 – 52</td>
</tr>
</tbody>
</table>

C. Lai, J. Wei, F. Li, J Kobashigawa, MC Fishbein, DW Gjertson, EF Reed, 2012
Antibody-Mediated Rejection Workshop: Pediatric Renal Transplantation

Eileen Tsai, MD
October 25, 2013
Mattel Children’s Hospital UCLA
Kidney Transplantation is treatment of choice for ESRD with superior outcomes than long-term dialysis.

More than 90,000 patients with end-stage renal disease in the United States are on the renal transplant list as of August 2011, with wait times in excess of 6 years:
- 35% patients are sensitized on the wait list
- 16% patients highly sensitized with cPRA >80%

OPTN/SRTR 2008

Antibody-Mediated Rejection is one of the major causes of allograft failure and contributes significantly to the current organ shortage and increased wait times.
Prevalence of AMR

- Hyperacute
  - Very rare due to pre-transplant cross-matching

- Acute
  - Occurs in 5-7% of all kidney transplant recipients
  - Occurs in 20-48% patients undergoing desensitization

- Chronic
  - Over 50% of renal allografts are lost to chronic AMR after ten years post-transplantation
Proposed Natural History of DSA

Preceding cellular rejection or infection

Early Inflammatory Event(s)

De Novo DSA

Peritubular Capillaritis

+/- C4d

Glomerulitis

IFTA +/- TG

Rising Cr / Proteinuria

+/- Cellular Rejection

Weibe et al AJT 2012
Diagnosis of Antibody-mediated Rejection
updated Banff 2011 criteria

- Acute AMR: Diagnosis requires documentation of circulating anti-donor antibody MFI > 1000, C4d deposition or morphological evidence of tissue injury including (2 of 3):
  - Type I - An acute tubular necrosis-like histology, with minimal inflammation
  - Type II - glomerulitis, peritubular capillaritis, and microthrombosis
  - Type III – Arteritis
  - Renal dysfunction is not required

- Chronic active antibody-mediated rejection:
  - glomerular double counters, multilayering of the peritubular capillary basement membrane and/or IFTA or fibrous and intimal thickening in arteries
  - C4d deposition without morphologic evidence of acute rejection
Pre-transplant Antibody Monitoring

- Pre-transplant
  - Non-sensitized patients cPRA < 30%
    - Monthly Flow PRA
    - Quarterly Single Antigen Class I & II by Luminex
  - Sensitized patients cPRA ≥ 30%
    - Living donor/paired exchange
      - MICA antibody, endothelial crossmatch, Single Antigen Testing
    - Deceased donor
      - Monthly Single Antigen Testing when undergoing desensitization
      - if cPRA is >30% and/or patient has been on wait list for more than 6 months not receiving offers because of sensitization
      - Single Antigen MFIs < 3000 are removed from unacceptable antigen list
Prevalence of post-transplant De Novo DSA in Pediatric Renal Transplantation

Figure 1: Cumulative risk of developing DSA and NDSA in pediatric recipients of kidney transplantation. N = total number of patients; E = events (number of patients developing DSA or NDSA). Cumulative incidence at 10 years and 95% CI are reported.

Gineveri et al AJT Dec 2012
UCLA Pediatric Renal Transplant
De NOVO DSA Experience

Retrospective, single center study
Jan, 2005 – Dec 2011 (n=201)

Excluded (n=85)
- Insufficient data (n=70)
- Non-HLA AMR (n=3)
- Preformed antibodies/PRAs > 30% (n=1)
- Primary non-function (n=1)

Study patients (n=125)

DSA (n=30) 20%

No DSA (n=95) 80%
HLA Class I and II distribution in patients with DSAs

N = 30
P < 0.0001
DSA is Associated with Medication Non-Aderence (MNA) by Patient Self-report

n=125  p<0.001
DSA is a Predictor of MNA by Patient Self-report
Distributions of FK-CV% by Rejection Status

$P < 0.003$

(Hsaiu et al, Transplantation 2011)
DSA is Associated with CV%
CV% is a Predictor of the Development of DSA
Post-transplant Antibody Monitoring

- DSA monitored in all patients:
  - Monthly for 0-12 months
  - Quarterly 1-3yr
  - Biannually >3yr
  - Clinical suspicion of rejection
  - Clinical suspicion of non-adherence
- All patients protocol biopsies 6m, 1yr, 2yr
- Biopsies
  - If DSA MFI>1000, suspected non-adherence, or clinical suspicion of rejection
Pathology of Antibody-Mediated Rejection in the Renal Allograft

M. Fernando Palma Diaz, MD
Department of Pathology & Laboratory Medicine
UCLA Medical Center

Outline

1. Pathologic manifestations of AMR and diagnostic criteria
2. Current limitations in diagnosis of AMR
3. Expanding clinicopathologic spectrum of AMR
Diagnosis of AMR and potential limitations

Diagnostic criteria according to Banff

1. Morphologic evidence of tissue injury:
   - Microvascular injury
   - Early/acute vs. late/chronic

2. Immunopathologic evidence of antibody acting on the microcirculation: diffuse C4d staining of peritubular capillaries

3. Serologic evidence of circulating DSA

* If only 2 criteria met → Suspicious for AMR
Morphologic manifestations of acute AMR – transplant glomerulitis (g > 0)

Quantitative criteria for glomerulitis (g) score

- **g0**: No glomerulitis
- **g1**: Glomerulitis in <25% of glomeruli
- **g2**: Glomerulitis in 25-75% of glomeruli
- **g3**: Glomerulitis in >75% of glomeruli

Morphologic manifestations of acute AMR – peritubular capillaritis (ptc > 0)
Morphologic manifestations of acute AMR – peritubular capillaritis (ptc > 0)

Quantitative criteria for peritubular capillaritis (ptc) score

<table>
<thead>
<tr>
<th>ptc 0</th>
<th>No significant cortical ptc, or &lt;10% of PTCs with inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ptc 1</td>
<td>≥10% of cortical peritubular capillaries with capillaritis, with 3 to 4 luminal inflammatory cells</td>
</tr>
<tr>
<td>ptc 2</td>
<td>≥10% of cortical peritubular capillaries with capillaritis, with 5 to 10 luminal inflammatory cells</td>
</tr>
<tr>
<td>ptc 3</td>
<td>≥10% of cortical peritubular capillaries with capillaritis, with &gt;10 luminal inflammatory cells</td>
</tr>
</tbody>
</table>

Morphologic manifestations of acute AMR – thrombosis
Morphologic manifestations of **acute AMR** – arteritis (v3)

Quantitative criteria for arteritis (v) score

<table>
<thead>
<tr>
<th>v0</th>
<th>No arteritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>v1</td>
<td>Mild-to-moderate intimal arteritis</td>
</tr>
<tr>
<td>v2</td>
<td>Severe intimal arteritis comprising &gt;25% of the luminal area</td>
</tr>
<tr>
<td>v3</td>
<td>Transmural arteritis and/or arterial fibrinoid necrosis of medial smooth muscle cells</td>
</tr>
</tbody>
</table>

Morphologic manifestations of **chronic AMR** – transplant glomerulopathy (cg > 0)

Quantitative criteria for transplant glomerulopathy (cg) score

<table>
<thead>
<tr>
<th>cg0</th>
<th>No significant glomerulopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg1</td>
<td>Double contours involving &lt;25% of capillary walls in the most affected nonsclerotic glomeruli</td>
</tr>
<tr>
<td>cg2</td>
<td>Double contours involving 26-50% of capillary walls in the most affected nonsclerotic glomeruli</td>
</tr>
<tr>
<td>cg3</td>
<td>Double contours involving &gt;50% of capillary walls in the most affected nonsclerotic glomeruli</td>
</tr>
</tbody>
</table>
Morphologic manifestations of chronic AMR – transplant glomerulopathy

Podocyte foot processes
Original basement membrane
New basement membrane
Endothelial cell

Morphologic manifestations of chronic AMR – peritubular capillary BM multilayering (PTCML)
Other morphologic manifestations attributed to AMR

Limitations of histology

- Specificity
  - Glomerulitis: TCMR, glomerulonephritis
  - PTCitis: ATN or TCMR
  - Thrombosis: multiple causes
  - Double contours: immune complex-mediated MPGN, chronic TMA
  - Improves when other features are present

- Reproducibility
  - Definition of glomerulitis is somewhat vague
  - Thresholds for transplant glomerulopathy
Pathways to C4d deposition

Immunofluorescence

Detection of C4d

Immunoperoxidase

Banff 2007
Guidelines for Interpretation of PTC C4d

- IF: higher reproducibility and sensitivity than IHC

<table>
<thead>
<tr>
<th>% biopsy area (cortex and/or medulla)</th>
<th>IF</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4d0 Negative: 0%</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>C4d1 Minimal 1&lt;10%</td>
<td>Neg</td>
<td>Unknown</td>
</tr>
<tr>
<td>C4d2 Focal 10-50%</td>
<td>Unknown</td>
<td>? Pos</td>
</tr>
<tr>
<td>C4d3 Diffuse &gt;50%</td>
<td>Pos</td>
<td>Pos</td>
</tr>
</tbody>
</table>

C4d Pitfalls

- Fairly good specificity but limited sensitivity
  - Misses up to 40% of AAMR and more than 50% of CAMR

- Focal C4d (+) by IF → unknown clinical significance

- C4d deposition is transient

- Areas of scarring – ptc vs. atrophic tubules
  - IHC is superior
The expanding spectrum of AMR

C4d Positive Without Graft Pathology

- Minority of HLA incompatible grafts
- Common in ABO incompatible grafts – possibly innocuous (‘accommodation’)
- Uncertain long-term clinical significance
- Banff: ‘C4d staining without morphologic evidence of active rejection’
Subclinical AMR

One study from Johns Hopkins reported subclinical AMR, defined as glomerulitis and/or peritubular capillaritis, diffuse C4d staining in PTCs by IF, and anti-HLA DSAs in 12/83 sensitized pts with stable SCr who underwent protocol biopsies at 1,3,6, and 12 months.

- Grafts with subclinical AMR on 1 or more protocol biopsies showed more chronic changes including TG at 1 year than 24 positive cross-match grafts with no evidence of AMR during the 1st year post-txp.

| Table 1. Findings 1 year after transplantation based on results of a 3-month protocol biopsy |
|---------------------------------|-----------------|-----------------|
| 3-Month biopsy result          | GFR (ml/min/1.73m²) | Percentage with TA/IF | Percentage with TG |
| Subclinical AMR (n=14) (C4d+, g > 0, ptc score > 0) | 39 ± 14' | 100* | 43' |
| Suspicious (n=22) (C4d-, g > 0, ptc score > 0) | 46 ± 18" | 77" | 18 |
| No AMR (n=9) (C4d-, g = 0, ptc score = 0) | 62 ± 19 | 33 | 0 |

Am J Transplant 2009; 9:2561–2570

Ultrastructural evidence of early glomerular endothelial cell injury on protocol biopsies predicts development of TG
Identified 119 endothelial-associated transcripts (ENDATs) from the literature, and studied their expression by microarrays in 173 renal transplant biopsies performed for cause (acute or persistent graft dysfunction, proteinuria)

Ten ENDATs predicted graft loss

ENDATS are associated with transplant glomerulopathy, even in the absence of C4d
C4d-Negative AMR

- Defined as microvascular injury plus DSAs in the absence of peritubular capillary C4d deposition, may occur early or late and may be overt or subclinical
- C4d-negative AMR is characterized by:
  - High within-graft endothelial and NK cell associated transcripts
  - The presence of alloantibodies and histology reflecting chronic AMR (and, less frequently, acute AMR)
  - Poor outcomes
- C4d-negative AMR is noted twice as often as C4d-positive AMR
  - Recognition of this new phenotype reveals C4d-positive or C4d-negative AMR to be the most common cause of late kidney transplant loss
Intimal arteritis (v1 or v2) as a manifestation of AAMR

Antibody-mediated vascular rejection of kidney allografts: a population-based study
CONCLUSIONS

- Demonstration of morphologic evidence of injury remains a crucial component in the diagnosis of AMR.
- AMR of renal allografts may occur in the presence or absence of clinical evidence of graft dysfunction, and in both cases is associated with development of chronic graft injury.
- C4d-negative AMR, defined as microvascular injury plus DSA in the absence of peritubular capillary C4d deposition, may occur early or late and may be overt or subclinical, and has the potential to progress to graft failure.
- Intimal arteritis, currently classified as a lesion of CMR, may in some cases be, at least in part, humorally mediated, and therefore warrants treatment strategies targeting antibody/B-cells.
- AMR highlights the importance of a multidisciplinary approach in arriving to a better understanding of complex disease processes and improving their diagnosis and treatment strategies.
Defining Antibody-Mediated Rejection:
A UCLA Cross-Disciplinary Workshop to improve diagnosis and treatment of Acute and Chronic AMR

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Advanced Heart Failure/Mechanical Support/Heart Transplantation
Ronald Reagan Medical Center
Division of Cardiology
Department of Medicine
David Geffen School of Medicine at UCLA
University of California, Los Angeles
USA
heart transplantation surgery
**heart transplants reported per year**

NOTE: This figure includes only the heart transplants that are reported to the ISHLT Transplant Registry. As such, the presented data may not mirror the changes in the number of heart transplants performed worldwide.

ISHLT  
survival by era

1982-1991 vs. 2002-6/2009: p < 0.0001
1992-2001 vs. 2002-6/2009: p < 0.0001


ISHLT

UCLA Htx 1 year Survival

Log rank

$p = 0.931$
UCLA's heart transplant program ranked best in the U.S.

MINI MED SCHOOL
The course offers an introduction into basic science as it relates to medicine, disease, health, and aging. Although you will not receive a medical degree or be able to practice medicine, you will get a better understanding of the human brain and body.

UCLA LYMPHOMA PROGRAM
A Celebration of Survivorship, On Track for a Cure. Come join us on Oct 9th at UCLA Drake Stadium. Together we can help fight for a cure for Lymphoma Cancer!

HEALTH NEWS
- UCLA center announces student winners of first ‘business venture in science’...
- Researchers devise index for predicting long-term survival after liver...
- UCLA geneticists develop promising mouse model for testing new autism therapies
- Big Tobacco knew radioactive particles in cigarettes posed cancer risk but...

MEET OUR PHYSICIANS
Martin, Neil MD
Neurological Surgery
Find More Physicians »
Deaths: January 1992 - June 2008

<table>
<thead>
<tr>
<th>CAUSE OF DEATH</th>
<th>0-30 Days (N = 3,531)</th>
<th>31 Days – 1 Year (N = 2,716)</th>
<th>&gt;1 Year – 3 Years (N = 2,356)</th>
<th>&gt;3 Years – 5 Years (N = 5,335)</th>
<th>&gt;5 Years – 10 Years (N = 5,335)</th>
<th>&gt;10 Years (N = 3,677)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARDIAC ALLOGRAFT VASCULOPATHY</td>
<td>62 (1.8%)</td>
<td>163 (4.6%)</td>
<td>383 (14.1%)</td>
<td>369 (15.7%)</td>
<td>767 (14.4%)</td>
<td>520 (14.1%)</td>
</tr>
<tr>
<td>ACUTE REJECTION</td>
<td>227 (6.4%)</td>
<td>427 (12.2%)</td>
<td>274 (10.1%)</td>
<td>104 (4.4%)</td>
<td>88 (1.6%)</td>
<td>33 (0.9%)</td>
</tr>
<tr>
<td>LYMPHOMA</td>
<td>11 (0.3%)</td>
<td>66 (1.9%)</td>
<td>163 (6.1%)</td>
<td>103 (4.4%)</td>
<td>246 (4.6%)</td>
<td>145 (3.9%)</td>
</tr>
<tr>
<td>MALIGNANCY, OTHER</td>
<td>4 (0.1%)</td>
<td>78 (2.2%)</td>
<td>301 (11.1%)</td>
<td>440 (18.7%)</td>
<td>999 (18.7%)</td>
<td>690 (18.8%)</td>
</tr>
<tr>
<td>CMV</td>
<td>4 (0.1%)</td>
<td>43 (1.2%)</td>
<td>17 (0.6%)</td>
<td>4 (0.2%)</td>
<td>6 (0.1%)</td>
<td>1 (0.0%)</td>
</tr>
<tr>
<td>INFECTION, NON-CMV</td>
<td>458 (13.0%)</td>
<td>1,066 (30.3%)</td>
<td>343 (12.6%)</td>
<td>229 (9.7%)</td>
<td>570 (10.7%)</td>
<td>361 (9.8%)</td>
</tr>
<tr>
<td>GRAFT FAILURE</td>
<td>1,452 (41.1%)</td>
<td>626 (17.8%)</td>
<td>636 (23.4%)</td>
<td>473 (20.1%)</td>
<td>965 (18.1%)</td>
<td>609 (16.6%)</td>
</tr>
<tr>
<td>TECHNICAL</td>
<td>253 (7.2%)</td>
<td>38 (1.1%)</td>
<td>19 (0.7%)</td>
<td>17 (0.7%)</td>
<td>41 (0.8%)</td>
<td>33 (0.9%)</td>
</tr>
<tr>
<td>OTHER</td>
<td>209 (5.9%)</td>
<td>303 (8.6%)</td>
<td>272 (10.0%)</td>
<td>220 (9.3%)</td>
<td>531 (10.0%)</td>
<td>364 (9.9%)</td>
</tr>
<tr>
<td>MULTIPLE ORGAN FAILURE</td>
<td>451 (12.8%)</td>
<td>386 (11.0%)</td>
<td>135 (5.0%)</td>
<td>122 (5.2%)</td>
<td>369 (6.9%)</td>
<td>293 (8.0%)</td>
</tr>
<tr>
<td>RENAL FAILURE</td>
<td>23 (0.7%)</td>
<td>34 (1.0%)</td>
<td>43 (1.6%)</td>
<td>86 (3.7%)</td>
<td>309 (5.8%)</td>
<td>308 (8.4%)</td>
</tr>
<tr>
<td>PULMONARY</td>
<td>150 (4.2%)</td>
<td>137 (3.9%)</td>
<td>105 (3.9%)</td>
<td>112 (4.8%)</td>
<td>218 (4.1%)</td>
<td>165 (4.5%)</td>
</tr>
<tr>
<td>CEREBROVASCULAR</td>
<td>237 (6.7%)</td>
<td>146 (4.2%)</td>
<td>95 (3.5%)</td>
<td>77 (3.3%)</td>
<td>226 (4.2%)</td>
<td>155 (4.2%)</td>
</tr>
</tbody>
</table>

allograft rejection

Recipient immune response

T-cell activating signals
#1 T-cell receptor
#2 CD28
#2a CD40L etc
#3 TAC, ICAM, etc

TCR

Allo Ag
allo MHC

self Ag
self MHC

allo APC

self APC

Cardiac allograft

monocyte
B-cell

CD3+ T-cell

CD8+

CD4+

CD8+

Cytotoxic

CD8+28-suppr

CD4+25+

CD4+/45RO+

Rejection

Tolerance

Allomap (leukocytes) quiescence monitoring

Histology rejecton monitoring

level
clinical
proteome
transcriptome
genome

organ
Echo, RHC, ECG
histology, Cd4
RT-PCR, and array (myocytes)
DNA-sequencing

systemic
clinical signs/symptoms
biomarkers
Allomap (leukocytes)
DNA-sequencing
pathways of immunosuppression

• Calcineurin Inhibitors
  • Tacrolimus
  • Cyclosporin

• Inhibitors of Purine Metabolism
  • Mycophenolate Mofetil
  • Azathioprine

• Proliferation Inhibitors
  • Rapamycin/Sirolimus
  • Everolimus

In 1990, an international grading system for cardiac allograft biopsies was adopted by the International Society for Heart Transplantation. This system has served the heart transplant community well, facilitating communication between transplant centers, especially with regard to patient management and research. In 2004, under the direction of the International Society for Heart and Lung Transplantation (ISHLT), a multidisciplinary review of the cardiac biopsy grading system was undertaken to address challenges and inconsistencies in its use and to address recent advances in the knowledge of antibody-mediated rejection.

The revised (R) categories of cellular rejection are as follows:

Grade 0 R—no rejection (no change from 1990);
Grade 1 R—mild rejection (1990 Grades 1A, 1B and 2);
Grade 2 R—moderate rejection (1990 Grade 3A);
Grade 3 R—severe rejection (1990 Grades 3B and 4).

Because the histologic sub-types of Quilty A and Quilty B have never been shown to have clinical significance, the “A” and “B” designations have been eliminated.

Stewart S et al. The Journal of Heart and Lung Transplantation
Volume 24, Issue 11, November 2005, Pages 1710–1720
Recommendations are also made for the histologic recognition and immunohistologic investigation of acute antibody-mediated rejection (AMR) with the expectation that greater standardization of the assessment of this controversial entity will clarify its clinical significance. Technical considerations in biopsy processing are also addressed. This consensus revision of the Working Formulation was approved by the ISHLT Board of Directors in December 2004.

Acute humoral rejection is recognized as a clinical entity in the grafted heart ... Acute antibody-mediated rejection is associated with worse graft survival and is observed in allosensitized patients, including those with previous transplantation, transfusion or pregnancy and previous ventricular assist device use. The incidence may be up to 15% in the first year post-transplantation and the clinical presentation has no pathognomonic features.

Pathologically, it can be recognized by myocardial capillary injury with endothelial-cell swelling and intravascular macrophage accumulation (Figures 19, 20 and 21). Interstitial edema and hemorrhage can be present together with neutrophils in and around capillaries. Intravascular thrombi and myocyte necrosis without cellular infiltration can also be identified. When these features are seen in the presence of unexplained cardiac dysfunction, typically early onset of hemodynamic compromise and myocardial dysfunction, it is proposed that immunostaining can be performed by immunofluorescence or immunohistochemistry.

• Immunoglobulin (IgG, IgM and/or IgA) plus complement deposition (C3d, C4d and/or C1q) in capillaries by immunofluorescence on frozen sections (Figures 22 and 23); and/or

• CD68 staining of macrophages within capillaries (CD31- or CD34-positive) by immunohistochemistry (Figure 24); and C4d staining of capillaries by paraffin immunohistochemistry (Figure 25).

• It is recommended that patients with hemodynamic compromise undergo assessment for circulating antibodies. The consensus meeting recommended that screening should not be advocated at this time.

BACKGROUND: The problem of AMR remains unsolved because standardized schemes for diagnosis and treatment remains contentious. Therefore, a consensus conference was organized to discuss the current status of antibody-mediated rejection (AMR) in heart transplantation. METHODS: The conference included 83 participants (transplant cardiologists, surgeons, immunologists and pathologists) representing 67 heart transplant centers from North America, Europe, and Asia who all participated in smaller break-out sessions to discuss the various topics of AMR and attempt to achieve consensus. RESULTS: A tentative pathology diagnosis of AMR was established, however, the pathologist felt that further discussion was needed prior to a formal recommendation for AMR diagnosis...

One of the most important outcomes of this conference was that a clinical definition for AMR (cardiac dysfunction and/or circulating donor-specific antibody) was no longer believed to be required due to recent publications demonstrating that asymptomatic (no cardiac dysfunction) biopsy-proven AMR is associated with subsequent greater mortality and greater development of cardiac allograft vasculopathy. It was also noted that donor-specific antibody is not always detected during AMR episodes as the antibody may be adhered to the donor heart. Finally, recommendations were made for the timing for specific staining of endomyocardial biopsy specimens and the frequency by which circulating antibodies should be assessed. Recommendations for management and future clinical trials were also provided.

Graft dysfunction (GD) after heart transplantation (HTx) is a major cause of morbidity and mortality. The impact of different pathophysiologic mechanisms on outcome is unknown. In this large, single-center study we aimed to assess the incidence of GD and compare the outcomes with different histopathologic mechanisms of rejection. We analyzed a data set of 1,099 consecutive patients after their HTx at Columbia University Medical Center between January 1994 and March 2008, and identified all patients hospitalized with new-onset GD. Based on the histopathologic data, patients were divided into GD-unexplained (Group-GD-U), GD-antibody-mediated rejection (Group-GD-AMR), GD-cardiac allograft vasculopathy (Group-GD-CAV) and GD-acute cellular rejection (Group-GD-ACR) groups.

Shahzad K …Deng MC. J Heart Lung Transplant. 2011;30:194
…Of 126 patients (12%) identified with GD, complete histology data were available for 100 patients. There were 21, 20, 27 and 32 patients identified in Group-GD-U, Group-GD-AMR, Group-GD-CAV and Group-GD-ACR, respectively. The in-hospital mortality rates were 52%, 20%, 15% and 6%, respectively. The in-hospital mortality rate was significantly higher in Group-GD-U compared with all other groups (p = 0.0006). The 3-, 6- and 12-month survival rate was also significantly lower in Group-GD-U compared with all other groups. A significant proportion of patients presenting with new-onset GD have unexplained histopathology. Unexplained GD is associated with a significantly higher mortality rate. New diagnostic tools are necessary to better understand and detect/predict this malignant phenotype.

Shahzad K …Deng MC. J Heart Lung Transplant. 2011;30:194
Since 2005, a number of studies have raised the question of the presence of an asymptomatic but clinically relevant form of AMR, questioned the sensitivity and specificity of the histologic features of AMR, shown evidence for C4d deposition in biopsy specimens without a positive histology for AMR, and even raised the scenario (in the renal allograft) of positive AMR histology in the absence of C4d staining. Combined forms of AMR and ACR have been recognized, that may also increase susceptibility to cardiac allograft vasculopathy.

Consensus #1: While debate continues about the make-up of the primary recommended panel for Paraffin IC, it is recommended that at a minimum, the panel should include C4d. Centers currently using CD68 or other macrophage markers in their IC panel will continue and other centers are encouraged to consider using a multi-antibody approach.

Consensus #2: Agreement was reached to limit the panel of antibodies in the recommended primary panel for IF to C3d and C4d with HLA staining for capillaries as needed.

Consensus #3: There was agreement that, for C4d staining, only the interstitial capillaries should be assessed in the evaluation of AMR.

Consensus #4: The group recommended that immunostaining for C4d is avoided in the first 2 weeks after transplant, because there are a number of perioperative issues that may confound staining and interpretation.

Consensus #5: the recommended surveillance protocol includes 2 biopsies in the first month (eg, at 2 and 4 weeks) and then according to the center’s circulating antibody monitoring schedule (eg, at 1, 3, 6, and 12 months).

Consensus #6: After a positive biopsy, the group recommended that subsequent biopsies should be studied by immunostaining until a negative result is achieved.

Consensus #7: The group reached consensus that, for IF methodologies, the intensity of immunostaining of 2+ or 3+ staining was required for a positive result. For IC staining, 3 categories were enumerated: 0 = negative, 1 = faint positive, and 2 = strong positive. For both IF and IC, only multifocal or diffuse staining constitutes a positive result.

ISHLT CONSENSUS 2011

PRELIMINARY nomenclature scheme:

pAMR 0: Negative for Pathologic AMR: both histologic and immunopathologic studies are negative.
pAMR 1 (H+): Histopathologic AMR Alone: histological findings present and immunopathological findings negative.
pAMR1 (I+): Immunopathologic AMR Alone: histological findings negative and immunopathological findings positive.
pAMR 2: Pathologic AMR: Both histologic and immunopathologic findings are present.

PRELIMINARY nomenclature scheme:

pAMR 3: Severe Pathologic AMR: This category recognizes the rare cases of severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis and/or karyorrhexis and marked edema.

Cases of combined ACR and AMR arise in clinical practice, but the frequency is unknown. The diagnosis and grading of ACR should be made by established ISHLT 2005 criteria. Some centers use CD3 or other antibodies in their AMR panel to address this problem. Further study to determine the incidence and significance of mixed ACR and AMR will be required.

Dr Hammond submitted a prototype template to the group and suggested that a de-identified central registry for standardized collection of histopathologic and immunophenotypic data and digital images be established.... Given the wide diversity of pathologists who report endomyocardial biopsy specimens (including academic and smaller hospital centers) and to encourage compliance and active participation, the registry should be simple to use and comprehensive at the same time. If digital images of classic and problem cases are added, this resource could serve for evaluation of reproducibility of methodology and interpretation, and thus serve to improve consistency of the pathologic diagnosis of AMR.

Considerable progress has been made in achieving consensus amongst pathologists and in refining the ISHLT 2005 working formulation criteria for the pathologic diagnosis of AMR. Several technical and interpretative issues have been raised. Although some were resolved and a new grading system proposed, further work is required to standardize and validate the consistency and reproducibility of a proposed scoring system for capillary C4d deposition in EMB specimens. Further additional inquiry is needed to address a number of unresolved and essential issues, such as the early histopathologic features of AMR. This can be achieved by establishing a central ISHLT-supported database.

Mobilized dendritic cells carry antigen to lymph nodes to prime high affinity naïve T cells.

Rejection-associated inflammation:
- Endothelial activation
- Mobilization of dendritic cells
- Expression of inflammatory mediators (e.g. IL-6)
CARGO clinical study summary

- **Overview**
  - **Cardiac Allograft Rejection** Gene expression Observational study = “CARGO”
  - 8 center, 4-year observational study initiated in 2001 (22% of US HTx).
  - 629 patients, 4917 post-transplant encounters

- **Hypothesis**
  - Gene expression profiling of peripheral blood mononuclear cells can discriminate ISHLT grade 0 rejection (quiescence) from moderate/severe (ISHLT grade ≥ 3A) rejection

- **Design & Result**
  - Prospective, blinded validation study of 20 gene algorithm demonstrated ability to distinguish Grade 3A rejection from quiescence

---

- **Candidate gene selection**
  - 285 Leukocyte microarray
  - Database / literature mining
  - 252 candidate genes

- **Algorithm development**
  - Real-time PCR
  - 20-gene algorithm to distinguish rejection from quiescence (AlloMap molecular testing)

- **Validation**
  - Prospective, blinded, statistically-powered (n = 270)
  - Additional samples tested to further define performance (n > 1000)

Deng/Eisen/Mehra et al. Am J Transplant 2006;6:150
Study Design
- Prospective
- Multi-center
- Non-blinded
- Randomized
- Non-inferiority

Patients
- 2-5 years post-Tx
- ≥ 18 years old
- Stable outpatients

Hypothesis
To determine whether the monitoring of acute rejection using GEP is not inferior compared to the use of the EMB with respect to the event-free survival

- Decrease in LV function, defined as LVEF change ≥ 25% compared with the baseline, or enrollment value, as measured by echocardiography
- Development of clinically overt rejection (heart failure, hemodynamic compromise)
- Death from any cause/Re-transplant

Pham/Deng/Kfoury et al. J Heart Lung Transplant 2007;26:808
2-year incidence of the composite primary outcome was similar between gene profiling and biopsy.

IMAGE patient satisfaction

**UCLA HTx surveillance protocol 7/1/11**

| Year 1 | Clinic Echo | Clinic RHC/Bx | Clinic Echo | Clinic Echo | Clinic Echo | Clinic Echo | Clinic Echo | Clinic Echo | Clinic Echo | First Annual Clinic Echo | LHC/IVUS/RHC/Bx*** AlloMap |
|--------|-------------|---------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------------------|---------------------------|
|        |             | RHC/Bx***     | RHC/Bx****  | AlloMap1    | AlloMap2    | AlloMap***  | AlloMap***  | AlloMap***  | AlloMap***  | clinical               |                           |

<table>
<thead>
<tr>
<th>Noninvasive phase</th>
<th>invasive phase</th>
<th>overlap phase</th>
<th>noninvasive phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Week 1, 2, 3, 4, 6</td>
<td>2 mo</td>
<td>3 mo</td>
<td>4 mo</td>
</tr>
<tr>
<td></td>
<td>5 mo</td>
<td>6 mo</td>
<td>7 mo</td>
</tr>
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<th>RHC/Bx*** AlloMap</th>
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| Noninvasive phase | 15 mo | 18 mo | 21 mo | 24 mo |

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| Noninvasive phase | +3 mo | +6 mo | +9 mo | +12 mo |

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*single-antigen-based antibody testing (or flow crossmatch) in suspected rejection/graft dysfunction (frequency determined by clinical suspicion)

**RHC/Bx including C4D/CD68 (do also single-antigen-based antibody testing & cylex)

***AlloMap (do also single-antigen-based antibody testing & cylex)

**** earliest timepoint 2 mo (day 56) post transplant if clinician & patient feel comfortable. Echo preferentially included

***** if LO REJECTION RISK, if HI REJECTION RISK BX until stabilization, if INTERMEDIATE RISK> alternate BX and ALLOMAP

****** DSE/radionuclide and LHC/IVUS alternating years if no CAV and clinician & patient comfortable
...[The Carolinas Medical Center Team] analyzed ...experience from 2009-2012 evaluating 711 Allomap scores in 126 patients. Beginning at month 5, patients underwent alternating GEP/EMB procedures until one year post transplant. At one year, surveillance monitoring was based on GEP alone except in patients with concern for acute cellular rejection, antibody mediated rejection or history of an infiltrative/inflammatory process. Decisions regarding scores >34 (reference threshold) were made based on patient test results during the first 5 months, standard clinical assessment and echocardiographic testing. Prediction values were based on IMAGE end points (graft dysfunction, hemodynamic deterioration or death). Technical and professional charges were obtained for the EMB/pathological interpretation arm and GEP/echocardiography for the Allomap strategy.

A.M. Thomley et al. J Heart Lung Transplant 2013:32
… No patients with a GEP score equal to or less than 34 developed any IMAGE end points or evidence of grade 2R acute cellular rejection on subsequent EMB resulting in a negative predictive value of 100%. Our economic analysis revealed a savings of over $5,000 utilizing GEP in lieu of EMB.

Conclusions: GEP is a novel, non-invasive approach that is economically attractive, positively associated with a high level of patient satisfaction and very high negative predictive values. GEP should replace EMB as the routine surveillance tool to monitor for acute cellular rejection in all clinically stable patients 6 months post cardiac transplantation.

A.M. Thomley et al. J Heart Lung Transplant 2013:32
HLA Class I & II Ab MFI – 36 m
HLA Class I & II Ab MFI – 28 m

HTx 10/18/2012

Donor ZJQ365, No DSA before 7/18/13

DQ7 and Cw2 are C1q binding antibodies on 9/26/13 and 9/30/13
Deng MC, Cadeiras M, Reed E. Curr Opin Organ Transplant 2013
Development of genomic algorithms, supervised by intragraft gene expression may lead to improvements in the accuracy of non-invasive molecular testing and provide further insights into the pathophysiology of heart transplant rejection.

Eleanor Chang; Amanda Russell; Maral Bakir; Charlotte Starling; Helya Azadmanesh-Samimi; Nick Wisniewski; Galyna Bondar; Yael Korin; Elaine Reed; X'avia Chang; Peipei Ping; José Tallaj; Mario C. Deng, and Martin Cadeiras (AHA grant; DOM RESEARCH DAY POSTER PRIZE 2013)
AMR in Pediatric Heart Transplant

October 25, 2013

UCLA
Pediatric Heart

• “Epidemiology”
  – Incidence Antibodies and AMR
  – Effects of Ab/AMR

• Diagnosing Rejection/AMR
Pediatric Heart

- Antibodies
  - At-risk for developing Ab
    - VAD 35%, Neonatal surgery 44%
  - PRA longer wait times/death while listed
  - Higher PRA, worse outcomes
Pediatric Heart

• High prevalence of AMR- Utah 2012
  – pAMR 2 or higher in 18% biopsies/ 59% patients.

• Increased risk of CV mortality and CAV

• (UCLA 2007, 35%)

UNOS DATA:

• 3535 patients:
  – 12% PRA 1- 10%
  – 11% PRA > 10%.

• Patients with PRA >10% were:
  – more likely to have a longer wait list time (P = .006).
  – significantly worse graft survival and patient survival (P < .05).

Pediatric Heart Transplant Study Group

- 1904 pts:

- PRA ≥ 50%: only 57% were transplanted by 1 year vs. 76% with PRA <10%

- Waitlist mortality (≥ PRA 50%) was 19% by 12 months.

- Survival at 1 year post significantly lower with PRA ≥ 50% versus PRA <10% (73% vs 90%)

Pediatric Heart

- Pre-transplant:
  - Development of Ab
    - Eval and 2wks post event
  - AB characterization- MFI, C1q test
Pre-Transplant Antibodies

“Virtual Crossmatch”

• Heart- rare prospective XMTC
• SAg MFI 5000 avoided based on expected +XMTC
• MFI cutoffs vary 1000-7000
Pre-Transplant Antibodies

SAB-C1q Assay
“Ab Function”

– Correlated with:
– CDC-XM
– early post-transplant biopsy findings
– early antibody-mediated rejection

Persistent strong anti-HLA antibody at high titer is complement binding and associated with increased risk of antibody-mediated rejection in heart transplant recipients.
Post-Transplant

- Symptoms/Signs: CHF
- Asymptomatic AMR
- Labs: BNP (not specific for AMR)
- Echocardiogram: systolic fxn, diastolic fxn
Abnormal Diastolic and Tissue Doppler Indices in AMR
Post-Transplant

• **AB:**
  - SAg DSA MFI
  - Each clinic visit (3mo) and with rejection episodes
  - $\text{DSA}^- / \text{AMR}^+$ (graft adsorption)
  - $\text{DSA}^+ / \text{AMR}^-$ (follow closely!)
Catheterization: TCAD
Post-Transplant

• Biopsy:
  - catheterization
  - AMR incidence depends on path techniques/stains/schedule
  - Differs btwn institutions

Schedule
1\textsuperscript{st} yr: wk 1, 3, 12, 26, 52
2\textsuperscript{nd} yr: 18, 24 mo
Yearly to 4 yrs
Q 2 yrs
Figure 2  Time from transplantation to first HR-positive biopsy.

True Incidence vs Protocol?

Tim W. Casarez, Gregory Perens, Ryan J. Williams, Erin Kutay, Michael C. Fishbein, Elaine F. Reed, Juan C. ...
(i) pAMR 0: Negative for pathologic AMR—both histologic and immunopathologic studies are negative.

(ii) pAMR 1: Histopathologic AMR alone—histologic findings present and immunopathologic findings negative.

(iii) pAMR 1: Immunopathologic AMR alone—histological findings negative and immunopathologic findings positive.

(iv) pAMR 2: Pathologic AMR—both histological and immunopathologic findings are present.

(v) pAMR 3: Severe pathologic AMR—this category recognizes the rare cases of severe AMR with histopathologic findings of interstitial hemorrhage, capillary fragmentation, mixed inflammatory infiltrates, endothelial cell pyknosis, and/or karyorrhexis and marked edema.
AMR Pathologic Diagnosis: Clinical Questions

- Differences in published AMR incidence
  - UCLA 35%
  - Utah 59%
  - Pitt 50% (if DSA MFI >4000)

- How often should immunostaining be performed?
- What should be tested for? C4d, CD68, C3d, IgG.
- Biopsy how often?
Recommendations?

• ISHLT

  Immunostaining:
  – ? Avoid C4d in first 2 weeks
  – Perform at 2, 4 weeks and with protocol bx’s first year
  – Follow up bx after AMR treatment – until negative
  – Late: based on DSAs, clinical, histological findings
• Thank you
Utility of C4d immunostaining in the first year after pediatric and young adult heart transplantation.

- Twenty-six of 406 first-year EMBs (6%) were C4d(+) in 6 (12%) patients.
- 5 of 10 patients with pre-transplant DSA ≥ 4,000 MFI and/or a positive DSXM were C4d(+) compared with only 1 of 41 without

Donor-specific antibodies: Can they predict C4d deposition in pediatric heart recipients?
• Ventricular assist device-associated anti-human leukocyte antigen antibody sensitization in pediatric patients bridged to heart transplantation.

• VAD-associated sensitization developed in 35% of recipients

• Persistence of anti-human leukocyte antibodies in congenital heart disease late after surgery using allografts and whole blood.

• Significant class I anti-HLA antibodies were seen in 44% (8 of 18) of the neonatal group, 25% (1 of 4) of the infant group, and 14% (1 of 7) of controls;

• class II anti-HLA antibodies were seen in 44% (8 of 18) of the neonatal group, 25% (1 of 4) of the infant group, and 29% (2 of 7) of controls.

• All patients received fresh whole blood

Questions:

• Does potential high incidence of asymptomatic AMR mean we should do more biopsies or should find other ways to diagnose it?

• Based on protocols, path techniques are we under or overdiagnosing AMR?

• SAg, DSA: MFI 5000 cutoff, C1q
Figure 3  Percent freedom from death among patients surviving at least 1 year, stratified by recipient PRA (n = 1,354).

William T. Mahle, Margaret A. Tresler, R. Erik Edens, Paolo Rusconi, James F. George, David C. Naftel, Rober...

Allosensitization and outcomes in pediatric heart transplantation

The Journal of Heart and Lung Transplantation Volume 30, Issue 11 2011 1221 - 1227

http://dx.doi.org/10.1016/j.healun.2011.06.005
Figure 5  Survival after transplantation, stratified by prospective crossmatching. This analysis includes only those subjects who at the time of listing were specified to require a prospective crossmatch (n = 148, with 5 missing DSC).

William T. Mahle, Margaret A. Tresler, R. Erik Edens, Paolo Rusconi, James F. George, David C. Naftel, Rober...

Allosensitization and outcomes in pediatric heart transplantation

The Journal of Heart and Lung Transplantation Volume 30, Issue 11 2011 1221 - 1227

http://dx.doi.org/10.1016/j.healun.2011.06.005
Antibody depletion for the treatment of crossmatch-positive pediatric heart transplant recipients

HD-AMR during the first post-transplant year was more frequent in the CM+ patient population, occurring in 50% of these patients compared with 2% of CM− patients (Table 3)
CV-related deaths or CAV was more common in patients with > 1 episode of pAMR3 (p < 0.0001).
AMR Diagnosis
Pediatric Heart

• No signs/symptoms:
  – routine protocol clinic/biopsy
  – Biopsy and lab schedule
  – Diagnosing aAMR depends on incidence and biopsy sensitivity
  – Does clinical schedule and path methods account for true incidence of AMR?
• Antibody mediated rejection (AMR) is a diagnostic and therapeutic challenge.
• NO evidence based guidelines to guide management.
• Increasingly recognized as cause of graft failure.
• Associated with development of allograft pathology and inferior survival.
• NO reliable data to guide diagnosis, monitoring, timing of intervention and choice of therapy → suboptimal outcomes.

**AMR: Adult Liver**
• Result of preformed antibodies reactive against class I MHC antigens expressed on graft.
• Risk higher in pts with a high panel reactive antibody, BUT logistical problems with allocation outweigh the benefits of prospective crossmatching.
• AMR is uncommon in ABO-compatible liver grafts.
• Liver is resistant to humoral rejection.
  • Secretion of soluble HLA class I Ag, Kupffer cell phagocytosis of cytotoxic Abs and complement.
  • Complement-mediated lysis of target cells may be less effective if target cell and complement are derived from the same source.
  • Liver provides syngeneic complement and protects itself against complement-mediated lysis.

**AMR: Adult Liver**
• Early injury is often unrecognized.
• No clear clinical, histological, and immunohistological criteria for diagnosis.
• No crossmatch in liver transplant.
• Prevalence of positive crossmatch or high PRA predict reduced 1-year graft and patient survival.
Clinical Manifestation

**Cholestasis**
- IRI
- initial graft dysfunction
- Sepsis
- HAT
- ACR
- Viral infection
- Recurrence

**Biliary obstruction**
- nonanastomotic biliary strictures
- ABO incompatibility
- Preformed non-HLA Ab leading to biliary injury and rejection.
Histological findings:

- Proliferation of small bile ducts
- Centrilobular hepatocyte swelling
- Single cell necrosis
- Sinusoidal accumulation of neutrophils
- Hepatocanalicular cholestasis
- Non-specific and can also be found in IRI.

Additional evidence needed to make unequivocal diagnosis

- DSAs and linear C4d staining
Clinical Definition

• Who has it?
  • Clinical Graft Dysfunction
  • High DSA
  • Histological Evidence of Rejection
  • Linear deposition of C4d (?)
• NOT routine in the first year of transplant.
  • Few centers perform routine monitoring at 2 weeks, 1, 3, 6 and 12 months post transplant.
  • Patients more than a year post transplant and without a history of AMR or DSA, most perform no routine DSA and very few do annual surveillance.
  • For patients with a prior history of AMR, majority perform more frequent monitoring.

DSA Monitoring
197 LTs
- 19 pts w/ positive T and B cell flow crossmatch before transplantation.
- 15 pts converted to negative crossmatches early after transplantation and displayed normal liver function while they were on routine immunosuppression.
- 4 pts maintained positive crossmatches.
- 3 met criteria for AMR and showed evidence of graft dysfunction:
  - DSA
  - Morphological tissue destruction
  - Positive C4d linear staining on graft sinusoidal endothelium
  - Improved function with attempts to eliminate DSAs.
• IV Pulse Steroids
• Thymoglobulin
• Plasmapharesis (7 sessions minimum)
• Rituximab (4 x 375 g/m²) – CD20 Ab
• IVIG
• Bortezomib – proteasome inhibitor
• Cyclophosphamide
• Photopheresis
• Total lymphoid Irradiation
• Eculizumab – C5 Ab
• 65 y.o. man with hepatitis C cirrhosis
  • OLT 10/18/2012
• Path –
  • Explant: Cirrhosis c/w chronic hepatitis
  • Donor post-reperfusion: Minimal lobular inflammation consistent with surgical manipulation. No steatosis.
• Somewhat protracted post-operative course due to hydrothorax and severe malnutrition.
• Discharged 12/7/12 – POD 50

Adult UCLA Pt
Adult UCLA Pt

[Graph showing liver function tests (AST, ALT, TBili) from 10/8/12 to 9/23/13]
- Developed AKI post-OLT requiring HD.
- Came off HD as outpatient.
- To preserve renal fxn CNI was decreased.
- Presented in April with obstructive type numbers and graft dysfunction (ascites).
- Imaging studies showed no anatomic issues.

**Adult UCLA Pt**
• Bx (4/22/13)
  • Moderate acute allograft rejection
  • No significant fibrosis or steatosis
  • Almost all portal tracts are involved by a mixed inflammatory infiltrate of lymphocytes, occasional plasma cells, and frequent eosinophils.
  • Portal inflammation is largely confined to portal zones, although there is some spillover into surrounding hepatocyte parenchyma with accompanying hepatocyte injury.
  • There is extensive duct injury and endotheliitis in several portal tracts.
  • Across lobules, there are scattered sinusoidal lymphocytes and patchy areas of pericentral hepatocellular dropout.
  • Bile present within hepatocytes in pericentral region. There is no bile duct loss.
  • There is convincing central vein endotheliitis along with patchy perivenulitis.

Adult UCLA Pt
• Steroid Pulse x 2
• Bx (4/29/13)
  • Ongoing moderate acute allograft rejection
• Thymoglobulin, Plasmapharesis, & Rituximab
• Bx (5/13/13)
  • Histologic improvement
  • Moderate cholestasis
    • A component of recurrent hepatitis C cannot be excluded, but is not the prevailing pattern of injury.
    • May be at risk for chronic ductopenic rejection but does not meet the criteria at this time.
Bx (5/30/13)
- Consistent with on-going acute rejection
- Moderate pericentral cholestasis and hepatocellular damage
- Periportal and lobular fibrosis
- There is mixed portal inflammation, prominent bile duct damage and portal vein endotheliitis which are consistent with on-going acute rejection.
Adult UCLA Pt
• ReLT (6/9/13)
  • Ongoing acute and chronic rejection
  • Recurrent hepatitis C viral infection with mild activity
  • Periportal fibrosis (stage 2 of 4)
  • Focal bile duct infarct with cyst formation (3.5 cm)
  • Mixed features of acute rejection (extensive bile duct infiltration with lymphocytes) and chronic rejection (extensive bile duct injury, infiltration and injury of large bile ducts, perivenular inflammation, arterial foam cell deposition and marked pericentral cholestasis).

• Basiliximab induction

Adult UCLA Pt
Adult UCLA Pt
Adult UCLA Pt
Graft Pathology of AMR in the liver

Bita V. Naini, M.D.
Department of Pathology and Laboratory Medicine
David Geffen School of Medicine
AMR in the liver – Histologic features

• Histologic features reported in the literature vary with the time of tissue examination after revascularization.
# AMR – Histologic features

<table>
<thead>
<tr>
<th>Time</th>
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<tbody>
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<td>0-24 hr</td>
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</tr>
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Explant suspicious for AMR showing massive hemorrhage and fibrinoid arteritis
# AMR – Histologic features

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AMR – Histologic features

• No characteristic or specific histologic features

• Histologic findings cannot be reliably distinguished from:
  – ischemic injury
  – preservation injury
  – bile duct obstruction
AMR – Histologic features

• What is the histology of AMR in the context of co-existent ACR?
  – We know based on the other organs that they can happen simultaneously.
  – No studies in the liver yet
C4d pattern of deposition

• Clinical utility of C4d staining in liver allografts for diagnosis of AMR is not established.

• Data about the location and character of C4d deposits in allograft and native liver tissues are inconsistent.

• Most studies use an IHC method on FFPE tissue:

  – The most frequent and specific pattern described: diffuse staining of portal venules and capillaries (>50% of portal tracts).
    • Some studies describe portal stroma or sinusoidal staining.
C4d immunostain
C4d pattern of deposition

• Many studies have demonstrated that these IHC staining patterns lack specificity for AMR.

• C4d staining has also been observed in ACR, chronic ductopenic rejection, recurrent hepatitis B and C, preservation injury, AIH and GVHD.

Bellamy CO. Liver Transpl. 2011.
C4d pattern of deposition

- Kozlowski, et al. performed C4d immunofluorescence (IF) on 141 fresh-frozen liver allograft biopsy samples and native livers

- Correlated the findings with the presence of DSAs.

C4d pattern of deposition

- A linear/granular sinusoidal pattern of C4d IF was seen in 19/28 patients with (pos-XM/DSA).
- None of the 59 biopsy samples from patients with (neg-XM/DSA) were C4d-positive.
- No such association was seen in other non-sinusoidal liver compartments such as portal vein vasculatures.

C4d pattern of deposition

• To compare the results of sinusoidal C4d staining with IF and IHC, C4d IHC was performed on 19 biopsies in which a sinusoidal pattern of C4d IF had been noted.

  – 17/19 biopsy samples had negative sinusoidal C4d IHC

  – 2/19 showed weak and focal staining

A) Linear sinusoidal endothelial C4d deposits

B) Negative C4d IHC findings for the same specimen

C4d pattern of deposition

- Diffuse sinusoidal C4d deposits detected by IF in frozen tissue samples correlates with the presence of DSAs and an AMR allo-response.

- Further validation of IHC techniques for C4d detection in liver allograft tissue is required.

C4d pattern of deposition

• C4d staining in isolation has very limited diagnostic value

• C4d can be useful in specific situations such as:
  – morphology suspicious for acute AMR
  – persistent ACR
  – un-resolving ischemia-like changes

• Needs to be correlated with DSA

Bellamy CO. Liver Transpl. 2011.
International Banff meeting 2013

• Significance of different methods for C4d deposition and significance of different patterns of staining is uncertain.
  – IF vs IHC?
  – Sinusoidal vs portal?

• Work required to validate C4d staining and standardize method
  – Identify positive liver cases
  – Send unstained sections to all labs
  – Assess reproducibility
International Banff meeting 2013

• A consensus document is being prepared, which will include:
  – tissue sampling recommendations
  – consensus criteria
  – areas requiring further development
Our experience at UCLA

- Similar to what has been reported in the literature.
  - Non-specific staining seen in transplant cases and native biopsies.

- C4d immunostain in patients with DSA+
  - Clinical follow-up?

- C4d IF ?:
  - Twice as much tissue needed as usual
  - Separate from the tissue submitted in formalin for histologic evaluation, a separate core of tissue needs to be sent in a special fixative for IF
  - Radiology must be informed (not dried out)
Questions/Comments?
Clinical definition of AMR in small bowel transplantation

Hugo Kaneku, MD
Post-Doctoral Research Fellow
Department of Surgery
Division of Liver and Pancreas Transplantation

AMR workshop, 10/25/2013
The Impact of Positive T-Cell Lymphocytotoxic Crossmatch on Intestinal Allograft Rejection and Survival


From the Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA.

Transplantation Proceedings, 32, 1197–1198 (2000)

- 51 isolated SBT, 79 MVT
- 18% had a +XM
- No difference in graft survival
- Allograft rejection: 91% in XM+ vs. 84% in XM-
- # of rejection episodes: 5.4 vs. 4.0
- Use of OKT3: 43% vs. 33%
• 6/28 (21%) CDC-XM+
• 3 developed clinically and endoscopically severe mucosal congestion, cyanotic discoloration, and focal hemorrhage within the first 10 days
• Biopsy showed severe congestion, neutrophil margination, fibrin-platelet thrombi within lamina propria microvasculature, along with focal hemorrhage
FIGURE 1. Gross appearance of the ileostomy in a patient who received isolated intestinal graft against a strongly positive crossmatch. The stromal mucosa became severely congested with a dusky appearance 2 days after transplantation. The cyanotic appearance became less prominent after OKT3 treatment and gradually returned to be pink at the end of the eighth dose of OKT3 treatment.
Figure 3. Daily postoperative platelet counts ($\times 10^3/mm^3$) in crossmatch-positive and crossmatch-negative recipients.
28 cases, 126 serum samples
All negative CDC-XM
3 cases with high %PRA post-tx developed rejection around the time of Ab detection
<table>
<thead>
<tr>
<th>Patient #</th>
<th>Postoperative Day</th>
<th>Flow PRA Class I</th>
<th>Flow PRA Class II</th>
<th>Rejection Episodes</th>
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<tbody>
<tr>
<td>#1 Modified multivisceral</td>
<td>Pre</td>
<td>20%</td>
<td>19%</td>
<td>Numerous rejection episodes (days 11, 25, 57, 74, 102, 114, 136, 154, 182, 191). Patient lost the graft due to rejection. DSA was positive posttransplant and negative pretransplant.</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>91%</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>85%</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>85%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>57%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>87%</td>
<td>68%</td>
<td></td>
</tr>
<tr>
<td>#2 Modified multivisceral</td>
<td>15</td>
<td>76%</td>
<td>46%</td>
<td>Rejections at days 8 and 57 successfully treated with OKT3. DSA positive.</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>21%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>#3 Multivisceral</td>
<td>7</td>
<td>2%</td>
<td>3%</td>
<td>Single episode of rejection at day 37. Rejection treated successfully. DSA negative.</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>20%</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>53%</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16%</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>6%</td>
<td>2%</td>
<td></td>
</tr>
</tbody>
</table>

DSA; donor-specific antibody.
Association Between Panel Reactive Antibodies and Acute Small Bowel Rejection: Analysis of a Series of 324 Intestinal Transplants


From the Department of Surgery (I.M.G.-P.), Hospital Universitario Central de Asturias, Universidad de Oviedo, Grant Proext-MICINN A, Spain; Department of Surgery (I.M.G.-P., A.G.T., H.-L.T., J.-W.C., P.T., S.N., E.I., G.S., A.T., J.M., D.L., P.R.), Division of Transplantation, University of Miami School of Medicine, Miami, Florida; National Yang-Ming University and Taipei Veterans General Hospital (H.-L.T., J.-W.C.), Taipei, Taiwan.

Transplantation Proceedings, 42, 4269–4271 (2010)

![Bar chart showing the percentage of rejection and non-rejection in patients with and without Panel Reactive Antibodies (PRA).](chart.png)
Graft loss

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preformed DSA</td>
<td>7.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Isolated ITx (vs. liver inclusive)</td>
<td>4.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Patient death

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preformed DSA</td>
<td>4.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Recipient splenectomy</td>
<td>3.3</td>
<td>0.05</td>
</tr>
</tbody>
</table>
39F, Crohn’s disease, SBT 5/2008, XM+

Hypoxia, hypotension and acidosis developed on reperfusion

Mucosal biopsies after reperfusion showed severe vascular congestion with thrombi, neutrophils margination, hemorrhage and focal arteritis

Immunofluorescence: vascular and parenchymal IgG, IgM, C4d and C3 deposition

Treatment: campath, rituximab, plasmapheresis

POD7: patient and allograft were stable
• 15 allografts, 33% pre-tx DSA, 27% de novo DSA
• Biopsies:
  • 191 protocol : 6% DSA+
  • 100 clinically indicated : 59% DSA+
• DSA associated with severe AR grading
Table 2. Relationship between clinical rejection episode occurrence and the presence of DSA and analysis in paired DSA-biopsy sample

<table>
<thead>
<tr>
<th>DSA status</th>
<th>Presence of DSA</th>
<th>Absence of DSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical rejection episode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graft experienced clinical</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>rejection episode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graft never had clinical</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>rejection episode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejection status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rejection positive biopsies</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Rejection negative biopsies</td>
<td>39</td>
<td>189</td>
</tr>
</tbody>
</table>

The patients had DSA were significantly associated with clinical rejection episode ($P=0.041$). Positive DSA showed correlated to acute rejection (kappa=0.30, $P<0.001$). The positive predictive value and negative predictive value were 43.48% and 85.14%, respectively.

DSA, donor-specific antibodies.
Abstracts from 2011 ISBTS

- Wozniak et al: DSA, PRA, and XM are associated with increased rates of rejection and worse graft and patient survival
- Suh et al: case report of accelerated AMR in a highly sensitized patient leading to graft loss
- Gerlach et al: DSA are often associated with acute rejection episodes
- Romano et al: 43% of biopsies showing AR were DSA+. Resolution of rejection correlated with DSA levels drop.
• Hyperacute rejection: disseminated thrombosis, necrosis of allograft
• Acute AMR: still challenging (requires a high index of suspicion in the presence of DSA)
Graft Pathology of AMR in the Small Intestine

Bita V. Naini, M.D.
Department of Pathology and Laboratory Medicine
David Geffen School of Medicine
AMR in the small intestine

• AMR is poorly characterized in small intestine allografts.

• The presence of DSA has been reported to increase the incidence and severity of allograft rejection and to worsen the overall prognosis for graft and patient.

• Consensus criteria are not yet identified.

• Only a small number of publications on AMR in intestinal allografts.

Histology of AMR in the small intestine

- Histologic changes are not well described

- Hyperacute and acute AMR in animal models and in patients with pre-transplant DSA:
  - Severe intestinal injury
  - Diffuse mucosal hemorrhage, congestion
  - Vascular thrombosis
  - Necrosis and mixed acute inflammation

- Vascular injury is partially associated with humoral pre-sensitization

AMR in the small intestine

• Studied allograft pathology in pts with preformed IgG antibodies

• +Xmatch: Immediately post perfusion, allograft spasm, cyanotic discoloration and serosal petechial hemorrhage

• Those with strong +Xmatch developed more severe mucosal injury within 10 days.
  – Mucosal biopsies: severe congestion, hemorrhage, neutrophilic inflammation, fibrin platelet thrombi in microvasculature of LP

C4d deposition

- Role of C4d in SBTx is unclear and its use has not yet been validated.

- Normal: C4d+ restricted to small arterial branches, but be negative in capillaries and venules.

- The significance of diffuse C4d capillary staining in the LP of intestinal allografts is unclear with respect to AMR or ACR.
  - Two studies to date have shown lack of sensitivity and specificity of C4d IHC for diagnosing AMR or with regard to clinical outcome.

Literature Review

- 36 small intestine were stained with C4d IHC stain including:
  - native specimens
  - allografts with and without features of ACR
  - explants
C4d staining of the small intestine:

A-C, Native normal small intestine.

D-F, Allografts with AR

G-I, Allografts with no rejection
Diagnosis | C4d staining of capillaries (intensity scale of 0 to 3, extent as focal, patchy, extensive). Positive when 2+ staining was seen more than focally
---|---
Allografts with no rejection | 27% (n=15)
Acute rejection | 36% (n=14)
Native normal small bowel | 28% (2 of 7)

Conclusion: C4d is unlikely to have utility in small intestine allograft biopsy specimens.
• Prospectively evaluated 12 children undergoing SBTx for histologic changes (vascular lesions), DSA and C4d deposition by IF.
C4d deposition of capillaries and venules was positive in 37% of allograft biopsies with or without AR.

No evidence that C4d deposition was of any clinical relevance to the outcome of SBtx.
Current status

- Histopathologic features of possible AMR is not well described

- C4d staining in intestine biopsies is unreliable
UCLA experience

• C4d stain performed on a few biopsies.

• C4d highlighted a few capillaries in the mucosa, but similar positive staining was also seen in a control non-transplant small bowel biopsy.

• The staining difficult to interpret and the significance of the staining is unclear.
Future direction

• Devise a way to examine SBTx patients for AMR

• Which patients have DSAs and have the clinical characteristics for these patients

• Look for histopathologic findings and correlate with C4d deposition in this group versus control
Questions/Comments?