Impact of Donor-Specific HLA Antibodies on Lung Allograft

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Frequency of human leukocyte antigen antibodies post-transplant by organ, n = 5219.
Impact of Preformed HLA-Ab

Panel Reactive Antibody (PRA) detected by cell based methods (pre 2010)

• Influence of PRA on post-transplant outcomes in LTx recipients  
  Lau et al Ann Thorac Surg 2000 (n=200 LTx, method CDC)  
  – Sensitized LTx experienced more acute and chronic complications after LTx (BOS 56% vs. 23%).

• Pretransplant PRA in LTx is associated with significantly worse post-transplant survival in a multicenter study  
  Hadjiliadis et al JHLT 2005 (n=656 LTx, method CDC)  
  – Patients with PRA >25% had decreased median survival at 1 month, 1 and 5 years (at 5 years 31% vs. 50%).

• Pretransplant PRA in human LTx: an analysis of over 10,000 patients.  
  – UNOS database from 1995-2008, PRA >25% associated with increased mortality, this effect was not seen in more recent era.

Based on PRA using PBMC: only class I HLA-Ab were considered and no information on donor-specific HLA antibodies
Impact of Preformed HLA-Ab
PRA and DSA detected by solid-phase methods
(after 2010)

• Lung transplantation in patients with pre-transplantation donor-specific antibodies (DSA) detected by luminex assay Brugiere et al Transplantation 2013 (n=56)
  – Freedom from BOS and survival was lower in patients with pre-formed HLA class II DSA vs. patients with HLA class I DSA or without DSA.

• Impact of pre-transplant anti-HLA antibodies on outcomes in LTx candidates. Kim et al Am J of Resp and Crit. Care 2014 (n=224)
  – The presence of HLA-Ab at >3000 MFI was associated with lower transplant rate and higher AMR rate as compared with patients with lower threshold HLA-Abs.

• Pre-transplant donor HLA-specific antibodies: characteristics causing detrimental effects on survival after lung transplantation. Smith et al JHLT 2014 (n=425)
  – Complement fixing pre-formed DSA and high MFI were associated with poor survival within the first year post LTx.

**determination by single antigen bead assay correlated with poor outcome**
Key criteria:

Presence of DSA

Lung Histology
HLA-Ab Testing

Single Antigen Bead

Risk Assessment for AMR
Persistent DSA- Monitoring
Increase Titer - Ab Burden
Complement Binding - IgG Subtype

Specificity
DSA
DSA bead 6 and 8 show the same neat MFI- during AMR very different titers and required treatment- the kinetics of response to removal therapy different

Tambur et al 2016
C1q Screen

New approaches for detecting complement-fixing antibodies

Dolly B. Tran

Histocompatibility

KEY POINTS
- Complement-fixing capability of HLA antibodies can be determined using C1q and C4d solid phase assays.
- The C1q assay has high sensitivity and specificity.
- The IgG mean fluorescence intensity (MFI) cannot be used to predict which antibodies can fix complement.
- C1q+ donor-specific antibody correlates with antibody mediated rejection and graft loss in kidney and heart transplant recipients.
- The C1q assay can be used to predict and monitor resolution of antibody mediated rejection (AMR) in heart transplant patients.
- The C1q assay can be used to predict and monitor desensitization by intravenous immunoglobulin (IVIG).

Figure. The total antibody burden present in a sensitized patient is composed of non-complement fixing antibodies (Non-CFAs) and complement fixing antibodies (CFAs).
### HLA-Ag T IgG C1q IgG1 IgG2 IgG3 IgG4

<table>
<thead>
<tr>
<th>HLA-Ag</th>
<th>T IgG</th>
<th>C1q</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
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<tbody>
<tr>
<td>B53</td>
<td>14522</td>
<td>1247</td>
<td>5280</td>
<td>2023</td>
<td>1022</td>
<td>19999</td>
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<tr>
<td>B51</td>
<td>13778</td>
<td>949</td>
<td>4239</td>
<td>2195</td>
<td>1079</td>
<td>20023</td>
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<tr>
<td>DQ5</td>
<td>16026</td>
<td>20787</td>
<td>14030</td>
<td>5668</td>
<td>26</td>
<td>8066</td>
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<tr>
<td>DQ6</td>
<td>16639</td>
<td>22113</td>
<td>14577</td>
<td>6045</td>
<td>20</td>
<td>9009</td>
</tr>
<tr>
<td>A32</td>
<td>13967</td>
<td>11</td>
<td>5498</td>
<td>1615</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A23</td>
<td>11440</td>
<td>89</td>
<td>4733</td>
<td>1413</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>DR12</td>
<td>11741</td>
<td>30</td>
<td>3864</td>
<td>89</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

**C1q Reactivity was not predicted by total IgG MFI**

Table 1: Correlation between different approaches currently used to assign antibody strength, for different HLA loci

<table>
<thead>
<tr>
<th>Correlation</th>
<th>HLA-A</th>
<th>HLA-B</th>
<th>HLA-C</th>
<th>HLA-DR</th>
<th>HLA-DQ</th>
<th>HLA-DP</th>
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</thead>
<tbody>
<tr>
<td>C1q vs. Neat</td>
<td>0.395</td>
<td>0.529</td>
<td>0.484</td>
<td>0.788</td>
<td>0.344</td>
<td>0.197</td>
</tr>
<tr>
<td>C1q vs. Peak</td>
<td>0.820</td>
<td>0.779</td>
<td>0.750</td>
<td>0.856</td>
<td>0.660</td>
<td>0.689</td>
</tr>
<tr>
<td>C1q vs. Titers</td>
<td>0.709</td>
<td>0.830</td>
<td>0.911</td>
<td>0.891</td>
<td>0.870</td>
<td>0.973</td>
</tr>
</tbody>
</table>
Complement Binding HLA-Ab Characteristics

C1q Screen Positive

Relative Ratio of IgG subtype:
CF: IgG1 and IgG3
NCF: IgG2, IgG4

Level of IgG:
Titer > 1:16 to 1:32

<table>
<thead>
<tr>
<th>COMPLEMENT FIXING ABILITY OF DSA</th>
<th>IgG SUBCLASS OF DSA</th>
<th>MICROVASCULAR INFLAMMATION</th>
<th>INFLAMMATORY CELLS PRESENT</th>
<th>TIME TO INDUCE GRAFT DAMAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q NEG</td>
<td>IgG4</td>
<td>+</td>
<td>MONOCYTES</td>
<td>LONG</td>
</tr>
<tr>
<td></td>
<td>IgG2</td>
<td>+</td>
<td>MONOCYTES</td>
<td>LONG</td>
</tr>
<tr>
<td>C1q POS</td>
<td>IgG1</td>
<td>+++</td>
<td>NK CELLS, MONOCYTES</td>
<td>MODERATE</td>
</tr>
<tr>
<td></td>
<td>IgG3</td>
<td>+++</td>
<td>NK CELLS, MONOCYTES</td>
<td>SHORT</td>
</tr>
</tbody>
</table>
Pretransplant DSA Characteristics Causing Detrimental Effects on Survival after Lung Transplantation:
Effect of Complement-Fixing DSA on Patient Survival

Conclusion: Complement-fixing DSA had significantly lower 1-year survival (11.1%) than DSA that do not fix complement (72.2%).

Smith et al, JHLT 33(10): 1074, October 2014

Courtesy Dr Reinsmoen
The Histopathology of Lung Allograft Dysfunction Associated with the Development of DSA
Yousem and Zeevi, American Journal of Surgical Pathology. 2012; 36:987

Post LTx de-novo DSA is 72% DQ-specific (DQB, DQA and DQB/DQA pairs)

<table>
<thead>
<tr>
<th>Lung Phenotype</th>
<th>ACR</th>
<th>DSA</th>
<th>Complement Fixing (C1q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR/AMR</td>
<td>Refractory/Persistent post IST*</td>
<td>De-novo Rising Titer</td>
<td>Positive Persistent</td>
</tr>
<tr>
<td>ACR</td>
<td>Response to IST*</td>
<td>Transient</td>
<td>Negative/Transient</td>
</tr>
</tbody>
</table>

* IST- Immunosuppression Treatment

Donor-Specific Class II HLA-DQ Complement Binding Antibody Are Associated with Severe Rejection in Lung Transplantation

Lobo et al JHLT 2013: DSA are associated with AMR, ACR, BOS after LTx
Link between serum MFI, biopsy fragment size and gDSA

- 7/11 > 10,000 MFI
- 8/11 > size 6 mm³

- 28 LTx sDSA (50 DSAs)
  - 28% Class I (6A, 2B, 6C)
  - 72% Class II (8DR, 22DQ, 4DP)

- 15 sDSA C1q + (1DR, 14 DQ)
- 15 gDSA (4 Class I, 1DR, 10 DQ)

- 1/15 gDSA in the biopsy only

- Only 1 LTx had C4d+ biopsy
  - 45.5% had ACR
  - 36.4% had BOS (prior biopsy)
De novo DSA are associated with early and high grade BOS and death after LTx

- **445 LTx**
- **Follow-up 3.3±1.9y**
- **14.8% had DSA**
- **41/58 with DSA Developed BOS**

Safavi et al JHLT 2014: de novo DSA predict development of BOS after LTx
Ius et al JHLT 2014: DSA in Ltx risk factors and impact on survival
Witt et al JHLT 2013: Acute antibody mediated rejection after LTx
The impact and temporal relationship of dnDSA and DQ specific DSA on chronic allograft dysfunction (CLAD)
Implications for Human Leukocyte Antigen Antibodies After Lung Transplantation
A 10-Year Experience in 441 Patients

• HLA antibodies after lung transplant are associated with
  – increased risk for BOS and worse survival,
  – DSA is associated with worse survival.

• HLA antibodies appear to be an integral part of the immune response to the allograft both preceding graft dysfunction and after allograft dysfunction.

• A key question to address in further analysis is if the decrease or elimination of these antibodies correlates with improved outcomes.

*Laurie D Snyder et al Chest 2013: 144(1) 226-233*
Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation

BOS was more likely to develop in recipients who had persistent DSA than in those who cleared the DSA.

Proteasome Inhibitor Carfilzomib-Based Therapy for Antibody-Mediated Rejection of the Pulmonary Allograft: First Use and Short-Term Findings

Ensor, Zeevi, McDyer AJT 2017

16pts (23 iDSA) : 69% DQ, 19% DQ+DR, 12% DR
6 patients had C4d+ AMR, 10 patients C4d- probably AMR

CFZ-based therapy resulted in profound depletion of circulating iDSA, removal of DSA C1q-fixing ability in vitro, a high degree of responsiveness, and stabilized or recovered lung allograft function.
Carfilzomib Responder: Loss of DSA C1q Reactivity
Pt1 DSA DQB1*04:02/DQA1*04:01

1. Total IgG MFI Persisted (yellow bar)
2. Loss of C1q Reactivity (red bar)
3. Drop in level of IgG Subtypes (IgG1 blue, IgG2 pink and IgG4 green)
DSA Titer Pre AMR Treatment

Immuno-dominant DSA

DQB1*04:02/DQA1*04:01

MFI

Pre CFZ Drop DSA (dilution)

iDSA was <2000 MFI at 1:2048 titer
LTx Non Responder
High Titer DSA Pre-AMR
Persisted post Treatment

iDSA was >10,000 MFI at 1:2048 titer
PFT Recovery after Carfilzomib-Based AMR Therapy

PFTs mean change

PFTs in C1q responders

PFTs in C1q non-responders

FEV1 \( p=0.4 \)

AMR

AMR

AMR

Courtesy of Chris Ensor PharmD
Impact of DSA in LTx

Antibody rejection in lung transplantation: Myth or reality?

AMR

Graft Dysfunction

ACR

Consensus Report AMR in LUNG Tx

Allan Glanville JHLT 2010; 29

Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation

Deborah J. Levine, MD, Allan R. Glanville, MBBS, MD, Christina Aboyoun, BA, MBA, John Belperio, MD, Christian Benden, FCCP, Gerald J. Berry, MD, Ramsey Hachem, MD, Don Hayes Jr., MD, MS, Desley Neil, MBBS, PhD, Nancy L. Reimn基金份额，PhD, D(ABHI), Laurie D. Snyder, MD, Stuart Sweet, MD, PhD, Dolly Tyan, PhD, Geert Verleden, MD, PhD, Glen Westall, MBBS, PhD, Roger D. Yusen, MD, MPH, Martin Zamora, MD, and Adriana Zeevi, PhD.
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