Antibody-Mediated Rejection in the Lung Allograft

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I have no financial relationships with commercial interests to disclose.

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

• To define the current histopathologic criteria for the diagnosis of lung AMR
• To review the immunophenotypic findings in AMR
• To identify potential avenues of investigation in the pathology of AMR
TEMPORAL PARADIGM FOR LUNG TRANSPLANT PATHOLOGY

PERIOPERATIVE AND EARLY POST-TRANSPLANT PERIOD (UP TO 1 MONTH)
- Primary Graft Dysfunction/failure
- Hyperacute Rejection
- Anastomotic Complications
- Infections

INTERMEDIATE COMPLICATIONS (1 MONTH – 1 YEAR)
- Acute Cellular Rejection (ACR)
- Airway Inflammation
- Antibody-Mediated Rejection (AMR)
- Infections
- Post-transplant Lymphoproliferative Disorder (PTLD)
- Drug Toxicity
- Aspiration Changes

LATE COMPLICATIONS (AFTER 1 YEAR)
- Obliterative Bronchiolitis
- Chronic Vascular Rejection
- Restrictive Allograft Syndrome/Pleuropulmonary Fibroelastosis
- Post-transplant Lymphoproliferative Disorder and other EBV-related disorders
Hyperacute Rejection

• Rare but potentially fatal complication caused by presence of pre-circulating anti-donor antibodies in recipient
• Pts. with high titer of “panel reactive antibodies (PRA) or anti-endothelial Ab are at highest risk
• Pulmonary edema, progressive respiratory failure and pleural effusions develop within minutes to hours after transplantation
• Histologic findings include DAD, interstitial neutrophilia, fibrin thrombi, vasculitis
• IF or IHC shows immunoglobulin and complement deposition in alveolar septa
Hyperacute Rejection (HAR)

- HAR reported in series of case reports (5)
- Risk factors include ABO mismatch, circulating anti-HLA Ab (anti-B8, -A2, -DR11)
- Hadjiliadis et al JHLT 2005; 24:S249 reported correlation between pretransplant high PRA and survival
- 30% of pts with high PRA died of acute lung injury within 30 days of transplantation
Hyperacute Rejection
Antibody-Mediated Rejection

• Numerous studies over last decade have reported association of \textit{de novo} donor HLA-specific antibodies and development of persistent/recurrent ACR, LB, and CLAD

• True incidence/prevalence unknown as consensus definitions, diagnostic criteria and management protocols have been lacking until recently

• AMR has been reported in combined heart-lung, single & bilateral lung and living-donor lobar transplant adult & pediatric recipients

• Prior to 2012 variety of morphologic terms applied to AMR: “septal capillary necrosis”, “capillary injury”
Histopathological Patterns in AMR

• “Neutrophilic Capillaritis”: patchy or diffuse process composed of dense neutrophilic septal infiltrates associated with neutrophilic karyorrhetic debris & fibrin +/- platelet-fibrin thrombi in microvasculature, alveolar hemorrhage and flooding of PMNs into airspaces

• “Neutrophilic Margination”: neutrophilic infiltrates in interstitial capillaries and septa in absence of karyorrhetic changes and fibrinous accumulations

Neutrophilic Margination
Neutrophilic Capillaritis
Lung: Spectrum of AMR Pathology
• New onset DSA (anti-HLA); graft dysfunction, histopathology; biopsy at time of onset - 23 patients.

N=17 Coexistent ACR – A2 (2), A3 (14), A4 (1)
- 18% had coexistent neutrophilic capillaritis
- When compared to matched group of ACR patients, only capillaritis was distinct.
- C4D positive in 13/17 (76%); matched group 24%.

N=5 Acute/organizing lung injury.
- 80% C4D positive; historical controls 50%.

N=1 Lymphocytic bronchiolitis
C4D -ve

- Microvascular inflammation as septal neutrophilia (Gr 2-4) and/or DAD as markers of AMR
- 41 biopsies (16 DSA+ve; 9/25 control gr developed de novo anti-HLA Ab (not DSA))
- 17/41 had suspicious histology (11/16 vs 6/25)
- C4d and C3d not more common in DSA+ vs control group

- 21 pts with AMR (+triple test)
- Median time to onset 258 days; 7/21 developed AMR within 45 days of Tx
- 15/21 treated and improved clinically but all developed CLAD
- 6 pts died of refractory AMR
Where are we in 2017?

• Series of individual center and Banff 2016 studies confirm lack of specific histopathologic findings in AMR
• Acute lung injury +/- DAD, neutrophilic margination or capillaritis exhibit variable sensitivity/specificity
• C4d appears to be quite specific but insensitive
Banff study of pathologic changes in lung allograft biopsy specimens with donor-specific antibodies

William Dean Wallace, MD, a Ning Li, PhD, b Claus B. Andersen, DMSc, c A. Valeria Arrossi, MD, d Medhat Askar, MD, PhD, d Gerry J. Berry, MD, e Matthew M. DeNicola, MD, f Desley A. Neil, MBBS, PhD, FRCPATH, f Elizabeth N. Pavlisko, MD, g Elaine F. Reed, PhD, h Myriam Remmelink, MD, h S. Sam Weigt, MD, i Birgit Weynand, MD, j Jennifer Q. Zhang, PhD, a Marie M. Budev, DO, k and Carol F. Farver, MD d

METHODS: We asked 9 pathologists with experience in lung transplantation to evaluate 161 lung transplant biopsy specimens for various histologic parameters. The findings were correlated with antibody status positive for DSAs, positive for non-DSAs, and no antibodies (NABs) present. The significance of each histologic variable was reviewed.

RESULTS: We found no statistically significant association with acute cellular rejection, airway inflammation, or bronchiolitis obliterans and the presence or absence of antibodies. However, biopsy specimens with DSAs had a statistically significant difference vs NABs in the setting of acute lung injury, with or without diffuse alveolar damage (p = 0.0008), in the presence of capillary neutrophilic inflammation (p = 0.0014), and in samples with endotheliitis (p = 0.0155). In samples with complement 4d staining, there was a trend but no statistically significant difference between specimens associated with DSAs and specimens with NABs.

CONCLUSIONS: Capillary inflammation, acute lung injury, and endotheliitis significantly correlated with DSAs. The infrequently observed diffuse staining for complement 4d limits the usefulness of this stain. J Heart Lung Transplant 2016;35:40–48
<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-DSA (n = 288)</th>
<th>DSA (n = 495)</th>
<th>NAB (n = 657)</th>
<th>p-value Non-DSA vs DSA</th>
<th>p-value Non-DSA vs NAB</th>
<th>p-value DSA vs NAB</th>
<th>p-value Non-DSA vs DSA</th>
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<tbody>
<tr>
<td>ACR</td>
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<tr>
<td>0 (none)</td>
<td>217 (76%)</td>
<td>387 (79%)</td>
<td>531 (81%)</td>
<td>0.68</td>
<td>0.47</td>
<td>0.69</td>
<td>0.54</td>
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<td>1</td>
<td>30 (10.5%)</td>
<td>54 (11%)</td>
<td>75 (12%)</td>
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<td>2</td>
<td>30 (10.5%)</td>
<td>31 (6%)</td>
<td>33 (5%)</td>
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<td>3, 4</td>
<td>8 (3%)</td>
<td>20 (4%)</td>
<td>13 (2%)</td>
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<td>Airway inflammation</td>
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<tr>
<td>B0 (none)</td>
<td>144 (84%)</td>
<td>270 (82%)</td>
<td>330 (85%)</td>
<td>0.90</td>
<td>0.84</td>
<td>0.68</td>
<td>0.82</td>
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<tr>
<td>B1R (low grade)</td>
<td>24 (14%)</td>
<td>48 (14%)</td>
<td>47 (12%)</td>
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<td>B2R (high grade)</td>
<td>3 (2%)</td>
<td>13 (4%)</td>
<td>13 (3%)</td>
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<tr>
<td>Obliterative bronchiolitis</td>
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<td>C0 (none)</td>
<td>162 (95%)</td>
<td>318 (96%)</td>
<td>380 (97%)</td>
<td>0.82</td>
<td>0.37</td>
<td>0.95</td>
<td>0.63</td>
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<tr>
<td>C1 (present)</td>
<td>9 (5%)</td>
<td>12 (4%)</td>
<td>10 (3%)</td>
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<tr>
<td>ALI</td>
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<tr>
<td>None</td>
<td>247 (87%)</td>
<td>359 (74%)</td>
<td>568 (88%)</td>
<td>0.0019</td>
<td>0.91</td>
<td>0.0008</td>
<td>0.0272</td>
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<tr>
<td>ALI</td>
<td>36 (12%)</td>
<td>97 (20%)</td>
<td>75 (11%)</td>
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<tr>
<td>ALI with DAD</td>
<td>2 (1%)</td>
<td>30 (6%)</td>
<td>5 (1%)</td>
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<td>Endothelitis</td>
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<tr>
<td>No</td>
<td>262 (92%)</td>
<td>448 (91%)</td>
<td>628 (96%)</td>
<td>0.0535</td>
<td>0.15</td>
<td>0.0155</td>
<td>0.61</td>
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<tr>
<td>Yes</td>
<td>22 (8%)</td>
<td>43 (9%)</td>
<td>24 (4%)</td>
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<tr>
<td>Capillary inflammation grade</td>
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</tr>
<tr>
<td>0</td>
<td>235 (83%)</td>
<td>354 (72%)</td>
<td>544 (83%)</td>
<td>0.0050</td>
<td>0.99</td>
<td>0.0014</td>
<td>0.0230</td>
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<tr>
<td>1</td>
<td>33 (11%)</td>
<td>86 (17%)</td>
<td>74 (11%)</td>
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<tr>
<td>2, 3</td>
<td>16 (6%)</td>
<td>53 (11%)</td>
<td>35 (6%)</td>
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<tr>
<td>C4d by IHC</td>
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</tr>
<tr>
<td>Negative</td>
<td>89 (83%)</td>
<td>131 (71%)</td>
<td>183 (85%)</td>
<td>0.1196</td>
<td>0.38</td>
<td>0.0322</td>
<td>0.67</td>
</tr>
<tr>
<td>Positive &lt; 50%</td>
<td>13 (12%)</td>
<td>29 (16%)</td>
<td>27 (13%)</td>
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<tr>
<td>Positive &gt; 50%</td>
<td>5 (5%)</td>
<td>25 (13%)</td>
<td>5 (2%)</td>
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</tbody>
</table>

ACR, acute cellular rejection; ALI, acute lung injury; C4d, complement 4d; DAD, diffuse alveolar damage; DSA, donor-specific antibodies; IHC, immunohistochemistry; NAB, no antibodies of any kind.

*aThe p-value for significance is < 0.0167.

- Retrospective study of 206 pts classified into DSA+ AMR+ (11%), DSA+ AMR- (40%), DSA limited AMR- (6%), DSA- AMR- (43%) groups
- Higher incidence of ACR in DSA+ AMR+ group
- Multivariate analysis showed AMR as risk factor for CLAD and graft loss
### Definitions

**Table 1: Criteria for AMR-DSA status categorization**

<table>
<thead>
<tr>
<th>AMR patients ($\text{DSA}<em>{\text{pos}}$, $\text{AMR}</em>{\text{pos}}$)</th>
<th>DSA$^{\text{pos}}$ AMR$^{\text{neg}}$</th>
<th>DSA$^{\text{lim}}$</th>
<th>DSA$^{\text{neg}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DSA positivity:</strong> DSA MFI &gt; 1000, or MFI of 500–1000 with more than two specificities and/or detected more than once</td>
<td>DSA positivity: DSA MFI &gt; 1000, or MFI of 500–1000 with more than two specificities and/or detected more than once</td>
<td>DSA detected only once and having only one specificity with an MFI of 500–1000</td>
<td>All single-antigen tests with DSA MFI &lt; 500</td>
</tr>
<tr>
<td><strong>AMR C4d$^{\text{pos}}$</strong></td>
<td>AMR C4d$^{\text{neg}}$</td>
<td>Clinical dysfunction and DSA positivity and C4d positive staining with or without histological patterns suggestive of AMR</td>
<td>Clinical dysfunction and DSA positivity and negative C4d staining with histological patterns suggestive of AMR: neutrophilic capillaritis,‡ acute lung injury‡</td>
</tr>
</tbody>
</table>
Antibody-Mediated Rejection in Lung Transplantation: Clinical Outcomes and Donor-Specific Antibody Characteristics

A

Graft Survival

Number of subjects at risk

- DSA^{pos}AMR^{pos} 22 15 11 6 3
- DSA^{pos}AMR^{neg} 84 70 43 16 7
- DSA^{lim} 13 13 8 5 2
- DSA^{neg} 87 68 44 22 9

B

Freedom from CLAD

Number of subjects at risk

- DSA^{pos}AMR^{pos} 15 12 8 5 2
- DSA^{pos}AMR^{neg} 45 44 41 15 7
- DSA^{lim} 8 8 8 5 2
- DSA^{neg} 49 49 40 20 8
Stanford Adult Pulmonary AMR Experience

• Retrospective cohort study
• Inclusion Criteria:
  • Lung transplantation at Stanford University in the period June 2008 to March 2013
  • Tested for DSA based on clinical indication, mainly evaluation of allograft dysfunction
    – Transbronchial biopsy specimens (within 30 days of serum collection for DSA) grouped according to presence or absence of complement-fixing donor-specific antibody
    – HLA antibody evaluation
      • Tested for HLA antibody by IgG and C1q methods
      • MFI > 1000 was MFI cut-off
• 116 serum samples (obtained from 60 lung transplant recipients) were tested for DSA based on clinical indication with concurrent transbronchial biopsies performed within 30 days
• Of these, 37 samples showed C1q+ DSA and 79 samples showed no DSA by C1q

Chhatwani L et al. J Heart Lung Transplant 2016; 35:S108
## Histopathologic Findings

<table>
<thead>
<tr>
<th></th>
<th>Serum samples with C1q DSA positive n = 37 (%)</th>
<th>Serum samples with no C1q DSA n = 79 (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A grade rejection ≥ A2</strong></td>
<td>13 (35.0)</td>
<td>11 (13.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>B grade rejection</td>
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</tr>
<tr>
<td>B1R</td>
<td>1 (2.7)</td>
<td>3 (3.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>B2R</td>
<td>2 (5.4)</td>
<td>1 (1.3)</td>
<td></td>
</tr>
<tr>
<td><strong>C grade rejection</strong></td>
<td></td>
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</tr>
<tr>
<td>C1</td>
<td>3 (8.1)</td>
<td>2 (2.53)</td>
<td>0.16</td>
</tr>
<tr>
<td>C4d Positive</td>
<td>1 (2.7)</td>
<td>4 (5.1)</td>
<td>0.48</td>
</tr>
<tr>
<td>Negative</td>
<td>30 (81.0)</td>
<td>67 (84.85)</td>
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</tr>
<tr>
<td>Not done</td>
<td>6 (16.2)</td>
<td>8 (10.1)</td>
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</tr>
<tr>
<td><strong>Neutrophilic margination</strong></td>
<td></td>
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<tr>
<td>Present</td>
<td>2 (5.4)</td>
<td>7 (8.9)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Acute capillaritis</strong></td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td><strong>Acute lung injury</strong></td>
<td></td>
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<tr>
<td>ALI present</td>
<td>0</td>
<td>1 (1.3)</td>
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</tr>
<tr>
<td>DAD</td>
<td>0</td>
<td>2 (2.53)</td>
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<tr>
<td><strong>Other</strong></td>
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<tr>
<td>Organizing pneumonia</td>
<td>0</td>
<td>2 (2.53)</td>
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<tr>
<td>Aspiration</td>
<td>0</td>
<td>1 (1.3)</td>
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</tbody>
</table>
Issues in AMR

- Is it a technical issue?
- Are there more sensitive markers than C4d
- Biomarkers of endothelial activation: mTOR effectors p70S6K, pS6R
- Are there more specific histopathologic features that might be useful; European Pulmonary Pathology digital microscopy study
- Pathology Council plans to reconvene at ISHLT 2017 to reassess histopathologic and immunophenotypic aspects
Issues in AMR

• Is it a technical issue?
• Are there more sensitive markers than C4d
• Biomarkers of endothelial activation: mTOR effectors p70S6K, pS6R have shown utility in cardiac AMR but not well studied in lung AMR
• Are there more specific histopathologic features that might be useful; European Pulmonary Pathology digital microscopy study raises new histopathologic targets
Summary & Future Directions

- Diagnosis of pulmonary AMR requires multidisciplinary approach
- Histopathological findings are nonspecific patterns of injury; patterns should trigger clinical, serologic and immunophenotypic evaluation
- Qualified terminology should be used in pathology report with final clinical diagnosis incorporating all modalities
- Centers are encouraged to develop protocols that will promote investigations addressing issues of time to onset of AMR, incidence, prevalence, spectrum of temporal, morphological and immunopathologic changes, clinical outcomes and risk for chronic allograft dysfunction
- Novel approaches to the histopathologic, immunophenotypic and molecular components of pulmonary AMR are required
Case 1

- 15-year old female s/p BSSLTx for CF
- Prior episodes of ACR and AMR
- Presents with new & rising DSA (C1q-binding Class II AB) and severe lung dysfunction (fever, dyspnea)
CASE #2

- 18-year old male s/p bilateral SSLTx in 2002 for CF
- Biopsy at 1 week ACR-A2
- Subsequent TBBx A1 or AX
- In 2008 he presented with drop in PFTs
- Underwent FOB with TBBx on 10/2/08
Antibody-Mediated Rejection in Lung Transplantation: Clinical Outcomes and Donor-Specific Antibody Characteristics

Transplanted Patients 2010-2013 (n=209)

Excluded
Deceased before first DSA, n=2
Lost to follow up, n=1

Excluded
Deceased before first DSA, n=2
Lost to follow up, n=1
Deceased<6 months, n=21
Follow up<2 years if no CLAD, n=61
Bronchial issues, n=7

Descriptive and Survival Analysis (n=206)

Non AMR population (n=184)

DSAPos AMRPos (n=22)
- AMRPos C4dPos (n=19)
- AMRPos C4dNeg (n=3)

DSAPos AMRneg (n=83)

DSALim (n=13)

DSAneg (n=88)

CLAD Analysis (n=117)

DSAPos AMRPos (n=15)
- AMRPos C4dPos (n=13)
- AMRPos C4dNeg (n=2)

Non AMR population (n=102)

DSAPos AMRneg (n=44)

DSALim (n=8)

DSAneg (n=50)