Cardiovascular Pathology
Evening Specialty Conference
A Tale of Two Tickers: Pre- and Post-Transplant Pathology
Case 3
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Dr. Carmela Tan declares she has no conflict(s) of interest to disclose.
Adult and Pediatric Heart Transplants
Kaplan-Meier Survival
(Transplants: January 1982 – June 2013)

Median survival = 11 years
Median survival conditional on surviving 1st year = 13 years

N = 112,521
N at risk at 30 years = 16

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N = 112,521
N at risk at 30 years = 16
So, going back to our case.....
- Biopsy was performed on routine follow-up at 18 months
- Complaints of abdominal bloating, dyspnea on exertion and weight gain
- Maintenance immunosuppression: prednisone, cyclosporine, mycophenolate mofetil
- Physical exam: HR 118, BP 138/76, bilateral lower extremity edema
- EMB performed

Right heart catheterization:
- CVP 20 cm H2O = 15.4 mmHg
- RA mean 18
- RV 38/18
- PA 38/24
- PCWP mean 24
- Fick CO 4.82 L/min
- Fick CI 2.35 L/min/m²

Elevated filling pressures, low normal cardiac index. Suspicion of rejection.
Admitted to the ICU after a diagnosis of AMR on biopsy

Echocardiogram
- LV normal in size, EF of 50%
- mild RV dysfunction, 3+ tricuspid regurgitation

Therapy:
- corticosteroid 1 g IV daily for 3 days
- plasmapheresis
- IV diuretic therapy

Discharged after 7 days

Immunosuppression: cyclosporine → tacrolimus
Antibody-mediated rejection (AMR)
- clinical presentation has no pathognomonic features
- suspected in a patient with low-grade acute cellular rejection and hemodynamic compromise
- risk factors: pregnancy, transfusion, previous transplantation, LVAD
- early vs late rejection
- associated with inferior survival
2013 ISHLT-Working formulation for diagnosis of AMR

- **pAMR 0**: Negative for pathologic AMR: both histological and immunopathological studies are negative
- **pAMR 1 (H+)**: Histopathological AMR alone: histopathological findings present and immunopathological findings absent
- **pAMR 1 (I+)**: Immunopathologic AMR alone: Immunopathological findings present and histological findings absent
- **pAMR 2**: Pathologic AMR: both histological and immunopathological findings present
- **pAMR 3**: Severe pathologic AMR: Rare cases of severe AMR with histopathological findings of IS hemorrhage, capillary fragmentation, mixed inflammation, endothelial cells pyknosis, karyorrhexis, marked edema

Pathologic diagnosis of AMR: H&E evaluation

- **1990**: vasculitis, severe edema in absence of cellular infiltrate
- **2005**: endothelial swelling or denudation; macrophages and neutrophils in capillaries; interstitial edema or hemorrhage
- **2013**: “activated mononuclear cells” to accommodate both swollen endothelial cells and intravascular macrophages; interstitial edema; hemorrhage, necrosis, and vascular thrombosis
Histopathologic features of AMR

- presence of activated mononuclear cells should trigger immunostaining (IHC or IF)
- “Neither endothelial swelling (sensitivity 63%, specificity 80%) nor vascular adherence of macrophages (sensitivity 30% specificity 99%) indicated sufficiently high sensitivity to serve as a screening tool before further diagnostic investigation for AMR.”

Hammond et. al. J Heart Lung Transplant 2005: 24, 2015-2021

Pathologic diagnosis of AMR: Immunostains

- 2005: Immunofluorescence / immunoperoxidase staining – Ig(G,M,A) + C3d and/or C4d or C1q; CD68 and/or C4d; fibrin
- 2013:

<table>
<thead>
<tr>
<th></th>
<th>Primary/ mandatory panel</th>
<th>Secondary / optional panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>C4d and CD68</td>
<td>CD3, CD20, C3d, CD31 or CD34, and others</td>
</tr>
<tr>
<td>IF</td>
<td>C4d, C3d Anti-HLA-DR (to identify capillary structures)</td>
<td>Fibrin, IgG and M, and others</td>
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</tbody>
</table>

General classification of humoral responses

- Table 3: Putative stages of humoral response to an organ graft
  I. Latent humoral response
     Circulating antibody (alone) but without biopsy findings or graft dysfunction
  II. Silent humoral reaction (accommodation vs. pre-rejection state)
     Circulating antibody + C4d deposition (but without histologic changes or graft dysfunction)
  III. Subclinical humoral rejection?
     Circulating antibody + C4d deposition + tissue pathology (but without graft dysfunction)
  IV. Humoral rejection
     Circulating antibody + C4d deposition + tissue pathology + graft dysfunction

1Circulating antibody to HLA or other antigens expressed on donor endothelial cells.
2May differ among organs, as the ability to detect particularly mild degrees of graft dysfunction varies among organs.

Value of C4d and C3d
- Correlates with the presence of DSA and hemodynamic compromise
  - Positive predictive value: 84.21%
  - Negative predictive value: 99.68%
  - Tan et al, AJT 2009; 9:1-10
- Correlates with increased cardiovascular mortality
  - C4d + C3d: $R^2 = 0.0930$
  - C4d + C3d + HLADR + fibrin: $R^2 = 0.0988$
  - Revelo et al, J Heart Lung Transplant 2011:30:144-50
One month later, C3d was negative, C4d was focal

Evolving or resolving?
2005 ISHLT Criteria for Diagnosis of AMR

1. Clinical evidence of acute graft dysfunction
2. Histologic evidence of acute capillary injury – endothelial swelling or denudation, macrophages and neutrophils in capillaries, interstitial edema or hemorrhage
3. Immunofluorescence / immunoperoxidase staining – IgG,M,A + C3d and/or C4d or C1q, CD68 and/or C4d; fibrin
4. Serologic evidence of anti-HLA class I and/or II antibodies or other anti-donor antibody

Reed EF et al, J Heart Lung Transplant 2006; 25:153-9

General classification of humoral responses

<table>
<thead>
<tr>
<th>Subclinical AMR</th>
<th>Clinical AMR</th>
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<td>Silent humoral rejection (accommodation vs. pretransplant state)</td>
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General classification of humoral responses

**Subclinical AMR**

1. Silent humoral reaction (accommodation vs. preinjection state)
   - Circulating antibody\(^1\) alone (but without biopsy findings or graft dysfunction)
   - Circulating antibody\(^1\) + C4d deposition (but without histologic changes or graft dysfunction)

**Clinical AMR**

1. Humoral reaction
   - Circulating antibody\(^1\) + C4d deposition + tissue pathology (but without graft dysfunction)

\(^1\) Circulating antibody to HLA or other antigens expressed on donor endothelial cells.


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**General classification of humoral responses**

**Subclinical AMR**

1. Silent humoral reaction (accommodation vs. preinjection state)
   - Circulating antibody\(^1\)

**Clinical AMR**

1. Humoral reaction
   - Circulating antibody\(^1\) + C4d deposition + tissue pathology + graft dysfunction

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General classification of humoral responses

Table 2: Putative stages of humoral response to an organ graft

<table>
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<tr>
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<tbody>
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<td>I</td>
<td>Latent humoral response</td>
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Conclusions & Future Directions

- Concomitant evaluation of C4d and C3d increases the positive predictive value of the IF diagnosis of AMR
- Deposition of C4d and C3d in capillaries correlates highly with clinical AMR
- Refinement of criteria for pathologic AMR
- Need to define a therapeutic strategy
  - Who to treat
  - When to treat
  - How to treat
Thank you