BANFF SUMMARY – HEART

Dylan Miller, MD  
September 23, 2022
Kidney Concurrent

Heart Concurrent
Banff 2022 – Heart Concurrent

1. DP/AI update
2. Non-HLA Antibodies
3. ‘Mixed’ Rejection
Digital Pathology / AI

Carolyn Glass, MD PhD

- “Myocyte Damage” Algorithm
- Intra/Extra Capillary Macrophages (PU.1/CD31 IHC based)
- COVID19 myocarditis (+ donors) vs. ACR/TCMR
• After calibration amongst pathologists with >95% consensus, regions of interest representative of each category were manually annotated by the senior investigator using Leica Aperio Imagescope™ software.

• A total of 15,357 computer annotations (yellow and red boxes) used to train the algorithm:
  - 4,574 regions of healing injury
  - 3,922 regions of normal endomyocardium
  - 3,297 regions of lymphocytic infiltration with myocyte damage
  - 2,740 regions of lymphocytic infiltration without myocyte damage
  - 677 Quilty lesions
  - 147 regions of hemorrhage

Scanned at 40x
Developed a convolutional neural network that, can identify ACR with and without myocyte damage with an accuracy of 95%.

Receiver Operating Curve (ROC) and Area Under the Curve (AUC) for training set classes at the patch level (training set annotations).

ROC: TP/FP as decision threshold varies

ROC and AUC for Grade 2R diagnosis (usually triggers treatment by cardiologist) for training and test sets, respectively. TPR = true positive rate. FPR=false positive rate.

- Identifying the intravascular location of macrophages, which in part histologically defines pAMR, is difficult and associated with interobserver variability.

- Developed and performed a double IHC stain with macrophage (Pu.1) and endothelial (CD31) specificity on 25 endomyocardial cardiac transplant biopsies.

- Interpreted as positive if >10% of Pu.1-staining macrophages visualized within CD31-staining capillaries.

- The original diagnoses retrospectively reviewed; double stain altered pAMR diagnosis in 7 cases (28%); all showed >10% positivity in capillaries not originally diagnosed as pAMR-I (undercall).

- No cases were overcalled as pAMR.

- Multi-institutional validation in progress.

- PLAAK-AI, Inc. collaboration.
NON–HLA ANTIBODIES
NON–HLA Antibodies

Guillaume Coutance, MD PhD

• **Scope of the Problem:**
  
  – Have I seen it?
  
  – Can I diagnose it?
  
  – Does it affect patient outcomes?
  
  – Is there an effective treatment?
Importance of time post-transplant

B- Probability of biopsy-proven AMR

according to the time post-transplant

Coutance et al, Circulation: HF, In press
Importance of the extension of MVI

Coutance, Transplantation, 2022
• **Treatment**
  • Diuretics
  • Plasmapheresis, IVIg, CS

• **Rehospitalization for HF 2 months later**
  • EMB = ACR0, pAMR0
  • RHC = restrictive physiology
  • TTE = persistent RV dysfunction

• **Relisted for redo HTx**

• **Cardiogenic shock**
  • Brutal clinical deterioration
  • VA-ECMO
Major challenges:

- Unknown specificity and expression on allograft of non-HLA antigens
- Poorly understood pathophysiology of non-HLA Ab mediated graft injury
- Lack diagnostic criteria for transplant rejection
- Uncertainty of treatment regimens
- Lack of validated assays to understand their clinical relevance

*UCLA Patent: Non-HLA markers of transplant rejection; receives royalties
Non-HLA Abs are Classified as Alloantibodies and Autoantibodies

**Injury Derived Tissue Specific Auto-Antigens**

- Artery
- Vein
- Capillary
- Heart
- Lung
- Kidney

**Genomic Mismatches Minor Histocompatibility Antigens**

- Vimentin: *Rose ML*
- MICA: *Reed EF, Rose ML*
- Collagen V: *Wilkes DS, Mohanakumar T*
- Epithelium K-α-1 tubulin: *Mohanakumar T*
- Agrin: *Paul LC*
- LG3/Perlecan: *MJ Hebert*
- Angiotensin II type-1 Receptor/ETAR: *Dragun D*
- MICA: *Stastny P, Terasaki PI, Abramowicz D*
Non-HLA Antibody Testing at UCLA

- Anti-MICA antibodies
  - Similar to HLA antibodies (alloreactivity to foreign/non-self protein)
  - About 10% of patients
  - Almost exclusively in regrafts

- Anti-endothelial cell antibodies
  - Autoimmune/alloimmune
  - Unknown protein targets
  - About 25% of patients
  - Vasculitis

- Anti-AT1R antibodies
  - Autoimmune response
  - High prevalence >50%
  - Hypertension, endothelial dysfunction and inflammation

- Using the combination of these tests, we have been able to identify clinically relevant non-HLA antibodies associated with transplant outcomes.
Seroconversion to AT1R >10 U/ml After VAD Placement is Associated with Primary Graft Dysfunction

15/35 seroconverted AT1R >10 U/ml
11/15 seroconverted to AT1R >40 U/ml
20/35 NO seroconversion

Mean Cardiac output was significantly reduced in the immediate post transplant period in patients with >10U/ml AT1R antibody after VAD placement.

Donor risk factors for PGD were older donor age and longer recipient pre-transplant wait time

M Hickey, E DePasquale, S Shah, D Vucicevic, J Q Zhang, NM Valennzuela, M. Deng, EF Reed, unpublished, 2022
Non-HLA Antibodies Associated with Cardiac Allograft Rejection

n=18

* Rejection grading 1990 ISHLT criteria

Odds Ratio (95% CI)

Key:

UCLA newly described

Non-HLA antibodies cluster into 9 groups with 7 independently informative for predicting rejection

Butler C, Hickey M, Gjertson D, Balaz I, Ray B, Reed EF, AJT 2020
Validation Study: Independent Single Center Adult Cardiac Allograft Recipient Cohort

n=63 patients, rejection=42 no rejection=21

AUC of 0.87 (p<0.05) with 92.86% sensitivity and 66.67% specificity to predict rejection

Butler C, Hickey M, Gjertson D, Balaz I, Ray B, Reed EF, AJT 2020
Few Overlapping Non-HLA Abs with Cardiac & Renal Allograft Rejection

Key:
- UCLA newly described

Butler C, Hickey M, Gjertson D, Balaz I, Ray B, Reed EF, AJT 2020
Non-HLA Antigen Panel for Diagnostic Testing

In summary:

• Using a combination of high throughput proteomics and bioinformatics, we identified a panel of non-HLA antigens that identify non-HLA antibodies associated with cardiac transplant rejection.

• Upon validation we believe its use could be extended to other solid organ transplants.
NON–HLA Antibodies

Pathologists Perspective:

Diagnostic conundrums

Wash U experience

Chieh–Yu Lin, MD PhD
NON-HLA Antibodies

Alessia Giarraputo
PhD fellow

- Molecular Approaches:
  - Application of B-HOT to non-HLA AMR
Context of the study: Antibody mediated rejection in solid organ allograft

<table>
<thead>
<tr>
<th>Organ Transplant</th>
<th>Complement Activation</th>
<th>Acute Tissue Injury</th>
<th>Chronic Tissue Injury</th>
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<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>Microcirculation inflammation</td>
<td>Microcirculation damage</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>Macrocirculation inflammation</td>
<td>*</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td>Macrocirculation damage</td>
</tr>
</tbody>
</table>

Context of the study: current molecular gold standard

- Whole-transcriptome based profiling have taken the lead for defining molecular phenotype of rejection

- Showing promising results, perform well using a small number of genes, defining the signature of antibody mediated rejection (AMR) (Circulation, 2017;135:917–935)

- Demonstrating similar molecular profile signature in AMR challenging cases like DSA negative compared to canonical one (Am J Transplant 2022;22(8):1976-1991)

Moreover showing that AMR molecular profile is consistent across different clinical condition (DSA negativity and non-HLA situation)
Study design: Building the reference set

Reference set
> 600 Bx

- No specific diagnosis
- MIMV / IMV
- AMR
- TCMR
- Mixed rejection
- Injury

Padova

Paris

Los Angeles
We tested molecular classifier with multiple metrics with good performance for both discrimination (ROC AUC = 0.844; PR AUC = 0.742) and calibration (Brier score = 0.143)
Projection in the reference set

Diagnosis based scores represent the probability that a biopsy has an expression profile similar to reference samples with a given histology based diagnosis.
MIXED
REJECTION
Mixed Rejection

- Scope of the Problem:

Evan Kransdorf MD PhD
Mixed Rejection

• Little is known about mixed cellular (ACR) and antibody-mediated rejection (AMR) after heart transplantation
• It remains unclear whether mixed rejection (MR) has distinct clinical and pathologic features beyond those of ACR and AMR
• ISHLT recommends that each EMB specimen be evaluated and classified separately for ACR and AMR (Berry et al. JHLT 2013)
Mixed Rejection – Many Unknows

- ACR and AMR are overlapping immunologic processes.
- Remains unclear which type of rejection is the inciting event or if they evolve concurrently.
- Remains unclear whether adverse outcomes after MR resemble ACR or AMR, or if they have synergistic effect.
Cedars Experience

- 851 recipients between 2012 and 2019
- Patients
  - 69 (8%) with at least 1 biopsy with ACR
  - 44 (5%) with at least 1 biopsy with AMR
  - 18 (2%) with at least 1 biopsy with MR

Unpublished
## Epidemiology

<table>
<thead>
<tr>
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<th>No ACR/AMR</th>
<th>ACR</th>
<th>AMR</th>
<th>MR</th>
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<tbody>
<tr>
<td></td>
<td>N=720</td>
<td>N=69</td>
<td>N=44</td>
<td>N=18</td>
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<tr>
<td>Age</td>
<td>55.1 (12.9)</td>
<td>52.9 (14.5)</td>
<td>55.2 (11.6)</td>
<td>47.3 (12.3)</td>
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<tr>
<td>p</td>
<td></td>
<td>0.048</td>
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<tr>
<td>Women</td>
<td>192 (26.7%)</td>
<td>19 (27.5%)</td>
<td>25 (56.8%)</td>
<td>7 (38.9%)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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<tr>
<td>Ancestry</td>
<td></td>
<td></td>
<td></td>
<td>0.028</td>
</tr>
<tr>
<td>African American/Black</td>
<td>98 (13.6%)</td>
<td>12 (17.4%)</td>
<td>14 (31.8%)</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian/Multi</td>
<td>110 (15.3%)</td>
<td>0</td>
<td>6 (13.6%)</td>
<td>3 (16.7%)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>512 (71.1%)</td>
<td>50 (72.5%)</td>
<td>24 (54.5%)</td>
<td>10 (55.6%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td>0.355</td>
</tr>
<tr>
<td>Hispanic</td>
<td>115 (16.0%)</td>
<td>8 (11.6%)</td>
<td>3 (6.82%)</td>
<td>2 (11.1%)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>241 (33.6%)</td>
<td>16 (23.2%)</td>
<td>17 (40.5%)</td>
<td>1 (5.56%)</td>
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<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td>0.016</td>
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<tr>
<td>MCS</td>
<td>279 (38.9%)</td>
<td>19 (27.5%)</td>
<td>10 (23.3%)</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
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<td>0.048</td>
</tr>
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</table>
Cedars Experience

- **Outcomes**

<table>
<thead>
<tr>
<th></th>
<th>No ACR/AMR</th>
<th>ACR</th>
<th>AMR</th>
<th>MR</th>
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<tbody>
<tr>
<td></td>
<td>N=720</td>
<td>N=69</td>
<td>N=44</td>
<td>N=18</td>
</tr>
<tr>
<td>De Novo DSA</td>
<td>91 (17.4%)</td>
<td>18 (36.0%)</td>
<td>20 (58.8%)</td>
<td>11 (78.6%)</td>
</tr>
<tr>
<td>CAV</td>
<td>70 (9.87%)</td>
<td>10 (14.5%)</td>
<td>6 (14.0%)</td>
<td>5 (27.8%)</td>
</tr>
<tr>
<td>Death</td>
<td>129 (17.9%)</td>
<td>18 (26.1%)</td>
<td>5 (11.4%)</td>
<td>7 (38.9%)</td>
</tr>
</tbody>
</table>
Conclusions

- The incidence of MR appears to have decreased over time.
- MR appears to be a late-occurring form of rejection with clinical profile intermediate between ACR and AMR.
- Associated with an increased risk of adverse outcomes in our center’s experience.
- These findings may suggest that there is a synergistic effect of ACR and AMR in MR.
Mixed Rejection

- Immunologist Perspective:
  - Application of B-HOT to non-HLA AMR

Olivier Thaunat, MD PhD
Adaptive effector arms are inter-connected/dependant
TCMR lesions (not always full blown!) detectable in most cases of AMR

### Clinical evidence...

Lyon University Hospital AMR Cohort  
Sicard, JASN, 2015

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (n=87)</th>
<th>Patients with antibody-mediated rejection (n=69)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male, n (%)</td>
<td>60 (69)</td>
<td>42 (61)</td>
<td>0.29</td>
</tr>
<tr>
<td>Age, years</td>
<td>43.4 ± 13.7</td>
<td>39.2 ± 14.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Blood group, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type A</td>
<td>46 (53)</td>
<td>38 (55)</td>
<td>0.78</td>
</tr>
<tr>
<td>Type B</td>
<td>10 (12)</td>
<td>6 (9)</td>
<td>0.56</td>
</tr>
<tr>
<td>Type O</td>
<td>28 (32)</td>
<td>24 (35)</td>
<td>0.73</td>
</tr>
<tr>
<td>Type AB</td>
<td>3 (3)</td>
<td>1 (1)</td>
<td>0.43</td>
</tr>
<tr>
<td>Retransplantation, n (%)</td>
<td>14 (16)</td>
<td>24 (35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Deceased donor, n (%)</td>
<td>78 (90)</td>
<td>65 (94)</td>
<td>0.3</td>
</tr>
<tr>
<td>Donor age, (years)</td>
<td>43.7 ± 15.4</td>
<td>39.0 ± 17.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Delayed graft function, n (%)</td>
<td>11 (13.2)</td>
<td>14 (20)</td>
<td>0.24</td>
</tr>
<tr>
<td>Banff scores*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microvascular inflammation†</td>
<td>0.22±0.44</td>
<td>3.5 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transplant glomerulopathy</td>
<td>0.09±0.32</td>
<td>1.1 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interstitial Inflammation and Tubulitis</td>
<td>1.7±1.9</td>
<td>2.6 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interstitial Fibrosis and Tubular Atrophy</td>
<td>1.42 ± 0.72</td>
<td>1.6 ± 0.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>0.7 ± 0.9</td>
<td>1.0 ± 1.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Endarteritis (vasculitis)</td>
<td>0</td>
<td>0.25 ± 0.5</td>
<td>n/a</td>
</tr>
<tr>
<td>C4d deposition</td>
<td>0</td>
<td>1.56 ± 1.1</td>
<td>n/a</td>
</tr>
<tr>
<td>Follow-up post biopsy (months)</td>
<td>34.0 ± 17.1</td>
<td>21.6 ± 21.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Unless noted otherwise results are expressed as mean ± standard deviation. *χ² tests for comparison of proportions and unpaired t test for comparison of continuous variables. † Banff scores (0: no significant lesion, 1: mild, 2: moderate, 3: severe), † Sum of the Banff scores for glomerulitis and capillaritis.
T cell activation is both central to TCMR and required to initiate DSA generation

=> Mixed rejections are not rare!!
From Quilty “nodule” to Tertiary Lymphoid Tissue (TLT)

Lymphoid neogenesis: progressive organisation of chronic inflammatory infiltrates into functional ectopic lymphoid tissue
T cell activation is both central to TCMR and required to initiate DSA generation
=> Mixed rejection is not rare!!

The prognosis of mixed rejection is (unsurprisingly) similar to that of AMR

Chronic inflammatory infiltrates can organize themselves into functional ectopic germinal centers in which a local immune response is generated
A new kind of mixed rejection?


**METHODS**

**Antibody-mediated rejection**
- MVI: g+ptc ≥ 2
- Presence of anti-HLA DSA

- C3d-binding DSA?
- Missing self?

- NK cell recruitment by computer-assisted graft inflammation (CAGI)
- NK cell activation by transcriptomic analysis
- NK cell induced-endothelial cell lysis by real-time cell analysis system

**OUTCOME**

- NK activation by DSA
- Inhibitory KIR
- Missing self is associated with increased NK cell activation and NK cell recruitment

- NK activation by DSA + Missing self
- Graft endothelium

- Missing self is associated with increased graft failure

**Conclusion**

Assessment of missing self at the time of the diagnosis of chronic AMR identifies patients at higher risk for kidney transplant failure.

doi: 10.1681/ASN.2020040433
Mixed Rejection

• Pathologists Perspective:
UTAH Cardiac Transplant Program Experience

Cardiovascular Mortality by Rejection Pattern

**Survival (%)**

- Cellular (n = 490)
- Mixed (n = 193)
- AMR (n = 118)

**P = 0.001**

Kfoury AG et al. J Heart Lung Transplant 2009
Markov Chain Analysis – 2015 to 2022!
Mixed Rejection

Giullaume Coutance, MD PhD

• Clinical Perspective:

- Application of B-HOT to non-HLA AMR
Clinical significance - Incidence

- **2012-2020**
  - all post-HTx EMB
  - 722 patients
  - 9,108 EMB
  - **Mixed rejection**
    - ACR ≥ 1R1B + pAMR ≥ 1: n=11
    - ACR ≥ 2R + pAMR ≥ 1: n=2
    - pAMR ≥ 1: n=122, 9% with ACR ≥ 1R1B
    - ACR ≥ 1R1B: n=434, 2.5% with pAMR ≥ 1

- **2012-2018**
  - post-HTx EMB < 1-yr post-HTx
  - 732 patients
  - 6,390 EMB
  - **Mixed rejection**
    - ACR ≥ 1R1B + pAMR ≥ 1: n=39
    - ACR ≥ 2R + pAMR ≥ 1: n=2
    - pAMR ≥ 1: 6.4%, 9.5% with ACR ≥ 1R1B
    - ACR ≥ 1R1B: 3.0%, 20.5% with pAMR ≥ 1
Mixed Rejection

The term mixed rejection has been used in circumstances where EMB reveals abnormalities consistent with both cellular rejection and AMR. When hemodynamic compromise is present, aggressive therapy with high-dose IV CS and cytolytic therapy is appropriate. Additional therapies directed at AMR should be considered. In mild forms of mixed rejection without significant symptoms, therapy should in general follow the algorithm for cellular rejection.

Mixed Rejection

The term mixed rejection has been used in circumstances where EMB reveals abnormalities consistent with both cellular rejection and AMR. When hemodynamic compromise is present, aggressive therapy with high-dose IV CS and cytolytic therapy is appropriate. Additional therapies directed at AMR should be considered. In mild forms of mixed rejection without significant symptoms, therapy should in general follow the algorithm for cellular rejection with consideration for additional IVIG.
Conclusion

- Mixed rejection is important after HTx
- Important impact of patient outcomes
- Aggressive management should be considered
- Collaborative studies required!
University of Padua 2012-2022
Diagnosis on 3328 EMBs of 263 pts

Diagram showing the distribution of diagnoses:
- Negative
- ACR
- AMR
- Mixed rejection
- Other (infections, PTLD, etc.)

The percentages are not specified in the image.
MIXED REJECTION: 1.86% (62 EMBs out of 3328)  
32 pts out of 263 pts (12%)  
University of Padua 2012-2022

<table>
<thead>
<tr>
<th>pAMR /ACR</th>
<th>1R</th>
<th>2R</th>
<th>3R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(H+)</td>
<td>13/62 (20.96%)</td>
<td>4/62 (6.45%)</td>
<td>0/62 (0%)</td>
</tr>
<tr>
<td>1(I+)</td>
<td>28/62 (45.16%)</td>
<td>4/62 (6.45)</td>
<td>1/62 (1.61%)</td>
</tr>
<tr>
<td>2</td>
<td>8/62 (12.9%)</td>
<td>1/62 (1.61%)</td>
<td>0/62 (0%)</td>
</tr>
<tr>
<td>3</td>
<td>1/62 (1.61%)</td>
<td>1/62 (1.61%)</td>
<td>1/62 (1.61%)</td>
</tr>
</tbody>
</table>
Round Table – Next Steps

Banff Report on Non-HLA Antibodies in Heart Transplantation:

- Writing team organized

Review on ‘Mixed Rejection’ in Heart?

- Discussion ongoing
Shiny new project

Do you have time?

Yes

No you don't

No

Don't do it