13TH BANFF CONFERENCE ON ALLOGRAFT PATHOLOGY ï VANCOUVER 2015

Meeting report
Dr. Candice Roufosse
Imperial College Healthcare NHS Trust
Objective

To lead development and dissemination of the international Banff Classification of Allograft Pathology

To facilitate multidisciplinary, collaborative research in order to enhance the scientific basis and clinical utility of the classification, with the ultimate aim improvement in transplant patient care.
Banff Foundation for Allograft Pathology

- 1991: first meeting for a consensus classification in renal transplant pathology (Banff, Canada)
- Meetings every 2 years
- Moderated, self-organising group
- Promotes uniformity of approach to diagnosis in transplantation
- Updates to classification driven forward through consensus
- Since 10th conference: Banff Working Groups
  - Collaborative multi-centre groups for collecting and analysing data
Banff 2015 Program and Major Topics

- Summary of Working Group findings
- Molecular diagnostics
  - Pre-meeting symposium and sessions
- Antibody-mediated rejection
  - Basic/translational science afternoon (Banff)
  - Joint sessions with Canadian Society for Transplantation
BANFF WORKING GROUP PRESENTATIONS
Banff Working Groups (1)

• Implantation biopsy
• T cell-mediated rejection
• Highly sensitized
• Isolated v-lesion
• Fibrosis scoring
• Polyoma virus nephropathy
• Glomerular lesion scoring
• Molecular Pathology
• Quality assurance
Banff Working Groups (2)

- Implantation biopsy
- T cell-mediated rejection
- Highly sensitized
- Isolated v-lesion
- Fibrosis scoring
- Polyoma virus nephropathy
- Glomerular lesion scoring
- Molecular Pathology
- Quality assurance

Sis et al. JASN 2015
Farris et al. AJT 2014
Mengel et al. AJT 2013
Banff Working Groups (3)

• Implantation biopsy
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• Glomerular lesion scoring
• Molecular Pathology
• Quality assurance
• Thrombotic microangiopathy
• Recurrent glomerular disease
• Electron microscopy
• Composite and surrogate endpoints
Banff Working Groups

- Thrombotic microangiopathy (Afrouzian, Liapis)
  - Generate consensus regarding diagnostic criteria for TMA in renal allografts using histopathology/laboratory data/molecular genetics correlation

- Recurrent glomerular disease (Alachkar)
  - What are frequencies, clinical manifestations, and pathological characteristics of recurrent/de novo disease? Can any of these predict recurrence and/or graft outcomes?

- Electron microscopy (Roufosse, Singh)
  - Survey of current practices for scoring and reporting cg1a and ptcbml
  - Inter-observer variability
  - Clinical correlations - Multicenter study to develop consensus criteria for cg1a and ptcbml scoring

- Composite surrogate endpoints (Loupy, Lefaucheur, Orandi)
  - Build a validated multicenter composite scoring system integrating histopathology with other relevant allograft biomarkers to predict long-term allograft outcome
Banff Working Groups (3)

- Implantation biopsy
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- Composite and surrogate endpoints
Implantation Biopsy Working Group (H. Liapis)

**Aim:** to develop consensus criteria for interpretation and reporting of implantation biopsies

- Organ Procurement and Transplantation Network (OPTN) recommend pre-implantation biopsy for all kidneys with KDPI >85%; or at surgeon's request
- Recommend wedge biopsy with at least 25 glomeruli
- United Network for Organ Sharing has standard form, only includes % global GS; form adapted locally; need consensus form
Implantation Biopsy Working Group (H. Liapis)

- Survey of practices
- Inter-observer variability study
- Outcomes study
Implantation Biopsy Working Group

**Inter-observer variability – ICC scores**

- **Good (>0.5)**
  - Number of glomeruli
  - Number and % of globally sclerosed glomeruli
  - Interstitial fibrosis

- **Fair (0.25-0.5)**
  - Tubular atrophy
  - Interstitial inflammation
  - Arterial intimal fibrosis
  - Arteriolar hyalinosis (*paraffin*)

- **Poor (<0.25)**
  - Acute tubular injury
  - Arteriolar hyalinosis (*frozen*)
Implantation Biopsy Working Group

- Frozen vs paraffin
  - ICC scores similar except arteriolar hyalinosis
- Cores vs wedges
  - ICC scores better in wedges
Implantation Biopsy Working Group

Outcomes

- Early graft function: donor serum creatinine, AA race and % GS
- Creatinine at 1mo, 3mo, 6mo, 1yr, 2yr: variable effect of donor age, donor serum creatinine, recipient age, AA race; no effect of histology

- But n=74, all good kidneys, all implanted (median age 49, mostly caucasian)

- “It should be recognised that histological parameters may not correlate with graft outcomes in studies based on organs deemed to be acceptable after careful clinical assessment”
Implantation Biopsy Working Group

- **New score sheet**
  - Number of glomeruli
  - % global sclerosis
  - Number of arteries
  - Interstitial fibrosis
  - Tubular atrophy
  - Interstitial inflammation
  - Arterial intimal thickening
  - Arteriolar hyalinosis
  - Glomerular thrombi
  - Acute tubular injury

- **Simplified scoring system:**
  - None <5% (*Karpinski score 0 = 0%*)
  - mild 5-25% (*Karpinski score 1 = <20%*)
  - moderate 25-50% (*Karpinski score 2 = 20-50%*), severe >50%
TCMR/BL Working Group (P. Randhawa/ V. Nickeleit)

- Need to re-evaluate TCMR
  - C4d and DSA
  - Current immunosuppressive regimes

- Prospects for modifying TCMR/BL definitions
  - Acute lesions
    - Can we eliminate the borderline category?
    - Re-evaluate thresholds (t and i)
    - Add further features, e.g. oedema
    - Include molecular data (Reeve 2013; Reeve 2016)
  - Chronic lesions
    - Re-visit the definition of chronic, active TCMR
TCMR/BL Working Group - Acute lesions

- Initial data (Pittsburgh) only
  - 545 transplant biopsies explored for "pure" BL/TCMR
    - Exclude cases with any features of ABMR: histology, DSA, C4d
  - 10 cases of BL + 18 cases of TCMR = 7.8% of total bx
  - Not necessarily early post Tx
  - Follow-up
    - Doubling of creatinine: BL/TCMR > controls
    - Biopsies: more g, cg, ptc

- Multi-centre study initiated
  - Including histological features not part of current definition of BL/TCMR e.g. oedema, CCTT criteria, etc.
  - Molecular assessment
  - Outcomes include
    - Response to treatment
    - Creatinine trends
    - Development of DSA
    - Histology on follow-up biopsies
TCMR/BL Working Group ï Chronic lesions

ÂChronic, active TCMR and i-IFTA

ÂBanff 2007 â„“ chronic allograft arteriopathy â„“(arterial intimal fibrosis with mononuclear cell infiltration and formation of neo-intima)

ÂBanff 2015 â„“ lesions of transplant arteriopathy may represent chronic, active ABMR as well as TCMR; the latter may also be manifest in the tubulointerstitial compartmentâ€
TCMR/BL Working Group \ï\dagger Chronic lesions

- **i-IFTA** = interstitial inflammation in areas of atrophy

- **i-IFTA í Banff total inflammation** (ti) score (sum of inflammation in scarred and non-scarred areas of the cortex)

- **i-IFTA**
  - is associated with decreased graft survival (Mengel M et al AJT 2009, Mannon R et al AJT 2010)
  - pathogenesis unclear - ?manifestation of chronic TCMR
    - Modena et al AJT 2016
Gene Expression in Biopsies of Acute Rejection and Interstitial Fibrosis/Tubular Atrophy Reveals Highly Shared Mechanisms That Correlate With Worse Long-Term Outcomes

NIH funded, Transplant Genomics Collaborative Group (7 centres, USA)

Gene expression profiling on n=234 biopsies

Comparing:
- Biopsies with IFTA (with inflammation and without inflammation)
- Control cases, no IFTA (normal or rejection)
Most IFTA samples have molecular evidence of ongoing immune-mediated injury same as in AR samples, even if no inflammation on histology.

Molecular profiles correlate with future graft loss in IFTA samples.

AR and IFTA phenotypes are stages in the same alloimmune process. 

IFTA = chronic rejection
- Discussion around whether to include i-IFTA in the category of chronic TCMR

- i-IFTA should be included as a biopsy diagnosis, rather than within the microscopic description or as a comment

- i-IFTA should be graded as mild, moderate, or severe (10-25%, 26-50%, >50% of cortical tissue present)
Highly Sensitised Working Group Update (L. Cornell)

• To develop evidence-based recommendations for the clinical and histological assessment of highly sensitised patients
  - DSA testing
  - Protocol biopsies and indication biopsies (e.g. rise in DSA titre)
  - Service requirements for a centre to undertake this type of transplantation

• To study differences between this population and standard risk transplantation (ABMR phenotype)

• Survey
Highly Sensitised Working Group Update

- Desensitization and immunosuppressive practices are varied
- Timing of kidney allograft protocol biopsies is not uniform
- Testing and reporting of HLA antibody and DSA levels vary
Molecular Pathology (Mengel)

- Develop consensus for
  - Circumstances under which it is advisable to apply molecular analysis to renal biopsy tissue, serum and/or urine
  - Best molecular studies to perform, with the aim to generate the needed evidence for adoption of molecular diagnostics into the Banff classification
  - Standard diagnostic criteria for \textit{Molecular Microscope}
Molecular Pathology

University of Alberta ÿ Alberta Transplant Applied Genomics Centre (ATAGC)
- Pathogenesis based transcripts (PBT)
- Panels of transcripts to analyse for ABMR, TCMR, ATI

Rival microarray panels for renal transplant biopsies
- Genomics of Transplantation Co-operative Research Group (7 centres, USA, NIH funded) (Daniel Salomon) ÿ Gene Co-expression networks (GCNs)

Sarwal Lab ÿ University of California San Francisco ÿ meta-analysis of previous publications identifies a common rejection module (CRM)= 12 genes elevated in rejection across all transplanted organs
Validation of Edmonton microarray use for the diagnosis of ABMR in renal transplant biopsies

Hayde et al. AJT 2014; Gupta et al. AJT 2016

Intragraft DSA selective gene transcripts may be used as molecular markers for AMR, especially in C4d-negative biopsies
Banff Pre-meeting on Molecular Pathology

**URINE**

- Suthanthiran group (Matignon et al JASN 2014)
  - Large multicentre study (NIH-CTOT-04) of 485 kidney tx recipients, 4300 serial urine samples
  - Free cell mRNA in urine
  - 6 gene diagnostic signature to distinguish ATI from rejection
  - 5 gene diagnostic signature to distinguish TCMR from ABMR

- Aim = to refine situations where biopsy is warranted and to catch rejection at an earlier time-point
Banff Pre-meeting on Molecular Pathology

**BLOOD**

- Sarwal group
- kSORT = kidney solid organ response test = 17 gene-set
- Serial blood sample measurement
- Predicts AR up to 3 months before current gold standard (biopsy)
Gartner Hype Cycle
Molecular Pathology

• When do we perform molecular analysis?
• What tissue do we look in?
• What markers do we study?
• What platform do we use?
• How do we use the result?
<table>
<thead>
<tr>
<th>Potentially equivocal situations for precision diagnosis</th>
<th>Informations provided By complementary approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borderline lesions</td>
<td>Eliminate Borderline category</td>
</tr>
<tr>
<td></td>
<td>Diagnose AKI</td>
</tr>
<tr>
<td></td>
<td>Wound healing process</td>
</tr>
<tr>
<td></td>
<td>Diagnose rejection, TCMR</td>
</tr>
<tr>
<td>Minimal microcirculation inflammation not qualifying for Banff</td>
<td>Diagnose ABMR</td>
</tr>
<tr>
<td>ABMR +/- DSA</td>
<td>Exclude ABMR</td>
</tr>
<tr>
<td></td>
<td>Prompt third parties Ab testing</td>
</tr>
<tr>
<td>TCMR (Banff 1a or greater) with microcirculation inflammation</td>
<td>Rule out superimposed ABMR</td>
</tr>
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<td></td>
<td>Diagnose mixed rejection</td>
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<tr>
<td>ABMR (Banff 2013) with TCMR features</td>
<td>Rule out superimposed TCMR</td>
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<tr>
<td></td>
<td>Diagnose mixed rejection</td>
</tr>
<tr>
<td>Isolated C4d (ABO compatible/ DSA+)</td>
<td>Rule out early onset ABMR</td>
</tr>
<tr>
<td>Isolated cg≥1 with microcirculation injury/C4d below Banff thresholds for chronic, active ABMR</td>
<td>Exclude / Diagnose ABMR</td>
</tr>
<tr>
<td></td>
<td>Information of activity</td>
</tr>
<tr>
<td>Isolated v lesions, with or without DSA, C4d</td>
<td>Diagnose ABMR, TCMR</td>
</tr>
<tr>
<td></td>
<td>Diagnose AKI</td>
</tr>
<tr>
<td>BKVN with histologic findings meeting Banff criteria for ABMR and/or TCMR</td>
<td>Rule out superimposed ABMR</td>
</tr>
<tr>
<td></td>
<td>Rule out superimposed TCMR</td>
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</table>

Courtesy A. Loupy
Molecular Pathology

What molecular panels do we use?
- In graft biopsy, for diagnosis
- In blood or urine, to predict rejection

What platform do we use?
- Microarray
- RT-PCR
- Novel - e.g. nanotring n-counter

Establish a consensus on panel of markers
Multicentre studies, including inter-centre assay reproducibility, to establish clinically relevant thresholds
Antibody-mediated rejection

- Heterogeneity of ABMR
- Requirement for DSA in diagnosis of C4d+ ABMR (M Haas)
- Non HLA ABMR \(\rightarrow\) anti-endothelial antibodies
- Mixed rejection
  - TCMR appears to predispose to de novo DSA (Mannitoba group)
  - Late post transplant/with de novo DSA and non adherence
- Treatment of ABMR
  - How to treat - targeting antibody, complement, proteosome or ADCC/NK cells?
  - When to treat? Acute, subclinical/indolent, chronic
- HLA antibodies - IgG subtypes and complement fixation
Heterogeneity of ABMR

Is there a difference between sensitised and non-sensitised patients? See Highly sensitised Working Group

Clinical and subclinical

In presensitised patients
- Lefaucheur C AJT 2007; Loupy A AJT 2009 and 2011; Loupy A JASN 2015; Cornell L 2015; Orandi 2015
- Haas M AJT 2007; Kraus E AJT 2009

In conventional risk transplantation
- Papadimitriou et al 2012
Subclinical ABMR

Wiebe et al (Mannitoba) - More evidence for subclinical ABMR in conventional risk patients

- 70% of patients with de novo DSA have subclinical ABMR (<25% variation in creatinine)
- Typical case is mixed rejection
- About 50% C4d+

- Can remain subclinical for 2 years after bx
- Progressive loss of function (less than if clinical ABMR)
- Loose their graft to cg and ptcbml

- Histological predictors of poor outcome: cg and t/i
- Probably need to treat both T cells and antibody
Requirement for DSA in C4d+ biopsies

- Banff 2013 requires the presence of "Serologic evidence of DSA against HLA or other antigens" (criterion 3) for diagnosis of both acute/active and chronic, active ABMR

- C4d deposition in ptc is highly specific for DSA and potentially picks up antibodies against antigens currently not tested for in some labs (HLA DP, non-HLA antigens)

- Some publications show similar outcomes for C4d or DSA versus C4d and DSA (Gaston et al 2010, Lessage et al 2015)

- Poll for waiving requirement for DSA if morphological evidence + C4d - rejected
Banff 2013 Classification of Antibody-Mediated Rejection (ABMR) in Renal Allografts, Revised 2015

Acute/Active ABMR; all 3 features must be present for diagnosis

1. Histologic evidence of acute tissue injury
   including at least one of the following:
   - Microvascular inflammation (g > 0 and/or ptc > 0)
   - Intimal or transmural arteritis (v > 0)
   - Acute thrombotic microangiopathy, in the absence of any other cause
   - Acute tubular injury, in the absence of any other apparent cause

2. Evidence of current/recent antibody interaction with vascular endothelium,
   including at least one of the following:
   - Linear C4d staining in peritubular capillaries
   - At least moderate microvascular inflammation ([g + ptc] ≥2)
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated

3. Serologic evidence of donor-specific antibodies (HLA or other antigens)
   - Biopsies meeting the above histologic criteria and showing diffuse or focal linear peritubular capillary C4d staining on frozen or paraffin sections (are highly suspicious for ABMR and) should prompt expedient DSA testing
Chronic, Active ABMR; all three features must be present for diagnosis

1. Morphologic evidence of chronic tissue injury, including 1 or more of the following:
   - Transplant glomerulopathy, if no evidence of chronic TMA
   - Severe peritubular capillary basement membrane multilayering
   - Arterial intimal fibrosis of new onset, excluding other causes

2. Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following:
   - Linear C4d staining in peritubular capillaries
   - At least moderate microvascular inflammation ([g + ptc] >2)
   - Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated

3. Serologic evidence of donor-specific antibodies (HLA or other antigens)
   - Biopsies meeting the above histologic criteria and showing diffuse or focal linear peritubular capillary C4d staining on frozen or paraffin sections (are highly suspicious for ABMR and) should prompt expedient DSA testing
Do we need to change the terminology for ABMR? 
Should we replace current ABMR categories with 

Activity indices:
- Possibly predictive of response to treatment
- g, ptc, C4d, molecules, EMé

Chronicity
- Possibly predictive of outcome
- cg, ptcbml, cv, ci, ct, molecules, EMé
Non Anti-HLA antibodies

Bridging alloimmunity and autoimmunity (Dragun et al 2016)
Non Anti-HLA antibodies

Angiotensin II type 1 receptor (AT\textsubscript{1}R)

- Superficial endothelial antigen
- Receptor-mediated selective signaling leading to changes in gene expression instrumental for organ damage and C4d negative phenotype
70 renal transplant patients
recipient serum tested for
- anti-AT1R antibodies using ELISA - 3 groups AT1R-Ab levels >17, 10-17, and <10 U/ml
- endothelial flow cytometric cross match (ECXM) in 35 patients

The data show an association between non-HLA antibodies detected in the ECXM and AT1R ELISA and microvascular injury observed in ABMR
BUT

44% of patients tested have ABMR (and contains protocol biopsies)
   high percentage of sera with DSA to HLA makes it difficult to determine the impact of HLA vs the non-HLA

ECXM was only performed on 35 patients without DSA

Not tested if present pre-transplant or de novo

Clinical relevance needs to be confirmed and validated at population level
test its independency from antiHLA
test its additional prognostic value
A) Biopsy scores and AT1R-Ab concentration for patients with HLA-DSA

B) Biopsy scores and AT1R-Ab concentration for patients with no HLA-DSA

Glomerulitis  Interstitial inflammation  Peritubular capillaritis
Thanks to Mark Haas and Alexandre Loupy for sharing their slides

Banff 2017 - Barcelona, March 27-31
Paris Transplant Group - Ibox (integrative box)

- Statistical programme for predicting allograft loss integrating:
  - Clinical parameters
  - All pathology parameters
  - DSA data
  - Molecular data (genes; transcripts)
  - Etc.

- Diagnosis, Activity, Risk, Response to treatment

- Personalised medicine; tool for stratification in clinical trials; end point measurements

- Important prognostic factors in 1st analysis: Mi score, C4d, IFTA, GFR, proteinuria, DSA
Identifying Subphenotypes of Antibody-Mediated Rejection in Kidney Transplants

ABMR presents distinct sub-phenotypes
- early pg-dominant
- late cg-dominant
- combined pgcg phenotype

These differ in time post tx, molecular features, accompanying TCMR, HLA antibody, and probability of nonadherence.

Halloran et al 2016
Table 5: Summarizing the discrepancies between the histologic ABMR subclasses and the molecular phenotype

<table>
<thead>
<tr>
<th>Histologic ABMR subclasses</th>
<th>Pure ABMR</th>
<th>Mixed</th>
<th>Pure TCMR</th>
<th>No rejection</th>
<th>Discrepancies (% of n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure ABMR† (n=132)</td>
<td></td>
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<tr>
<td>pgABMR (n = 35)</td>
<td>20†</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>15/35 (43%)</td>
</tr>
<tr>
<td>pgcgABMR (n = 73)</td>
<td>68†</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5/73 (7%)</td>
</tr>
<tr>
<td>cgABMR (n = 24)</td>
<td>21†</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3/24 (13%)</td>
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<tr>
<td>Mixed (n=28)</td>
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<td>pgMixed (n = 10)</td>
<td>3</td>
<td>6†</td>
<td>0</td>
<td>1</td>
<td>4/10 (40%)</td>
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<tr>
<td>pgcgMixed (n = 17)</td>
<td>13</td>
<td>4†</td>
<td>0</td>
<td>0</td>
<td>13/17 (76%)</td>
</tr>
<tr>
<td>cgMixed (n = 1)</td>
<td>1</td>
<td>0†</td>
<td>0</td>
<td>0</td>
<td>1/1 (100%)</td>
</tr>
</tbody>
</table>
IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury

Carmen Lefaucheur,*† Denis Viglietti,*† Carol Bentlejewski,* Jean-Paul Duong van Huyen,*‡ Dewi Vernerey,*† Olivier Aubert,* Jérôme Verine,*† Xavier Jouven,*† Christophe Legendre,*** Denis Glotz,* Alexandre Loupy,*†** and Adriana Zeevi‡

647 Kidney Transplant Recipients 01/2008-12/2009

Excluded: Recipients with desensitization protocols (N=12)

635 Recipients screened for DSA in the first year post-transplant

Recipients with DSA (N=125)

MFI > 500

41% Acute ABMR (N=51)

29% sABMR (N=36)

30% No ABMR (N=38)

Biopsies at time of clinical rejection OR At 1 year post transplant

Lefaucheur C et al JASN 2

Negative B and T CDC

Circulating DSA characteristics: class, specificity, MFI
• DSA IgG subclasses
• C1q-binding DSA
• Allograft histology
IgG4 and class II DSA distinguishes sABMR
Later rejection
More TG and IFTA

<table>
<thead>
<tr>
<th>Target complement?</th>
<th>IgG3 DSA distinguishes aABMR</th>
<th>Early rejection</th>
<th>High MI</th>
<th>C4d+</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td></td>
<td></td>
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<tr>
<td>YES</td>
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</tbody>
</table>
Electron Microscopy working Group
(Candice Roufosse, Sharan Singh)

- **Current use**: double contours (including ultrastructural only, cg1a) and severe ptcbml have been established as diagnostic features of chronic antibody-mediated rejection and are part of the Banff classification

- **Prospects**: reproducibility and clinically relevant cut-offs have not been validated

- **Future applications**:
  - Early ultrastructural findings may provide evidence of recent interaction of antibody with endothelium and may predict future risk of chronic antibody-mediated injury
  - Ultrastructural findings need to be evaluated for added value compared to histological and molecular parameters
Em working group

Group objectives:
Part 1 - development of consensus criteria and guidelines for cg1a and ptcbml, based on current practice amongst transplant pathologists; investigate the inter-observer variability on consensus criteria
Part 2 - multicentre study of the natural history, associations and predictive value of cg1a and ptcbml as established using consensus criteria

Findings/plans:
Survey of current practice - completed June 2016
Circulation of images for inter-observer reproducibility variability - autumn 2016
Results of Part 1 presented to Banff Conference 2017
2017 - 2018: multicentre study
Mannon R et al. AJT 2010
(Dekaf - Long term deterioration of kidney allograft function)
Samples with IFTA separated into:
- IFTA, no inflammation (n=42)
- IFTA, inflammation in areas of scarring (n=10)
- IFTA, with acute rejection (n=29)

Histology shows no alternative explanation for pathogenesis (excluded BK, recurrent disease)
Modena et al AJT 2016
C

AT1R-Ab and microcirculation inflammation (MCI) scores without HLA-DSA

[Graph showing MCI scores for different AT1R-Ab levels, with p = 0.07]
A

Correlation between endothelial cell crossmatch and AT1R-Ab levels

B

Correlation between endothelial cell crossmatch and microcirculation inflammation (MCI) scores
Anti-endothelial cell antibodies (AECA)

Endothelial cell cross match (ECXM)

- Angiopoietin receptor (Tie2) positive EC precursors isolated from donor blood according
- Positive IgG ECXM test defined by a ratio of the median fluorescence of test serum to negative control serum ≥1.3
- Several antibodies eluted from serum of patients with +ve ECXM (but known ones such as AT1R and ETaR not in there!)
Anti-Angiotensin II Type 1 Receptor and Anti-Endothelial Cell Antibodies: A Cross-Sectional Analysis of Pathological Findings in Allograft Biopsies

Mary Carmelle Philogene, PhD, Serena Bagnasco, MD, Edward S. Kraus, MD, Robert A. Montgomery, MD, DPhil, Duska Dragun, MD, Mary S. Leffel, PhD, Andrea A. Zachary, PhD, and Annette M. Jackson, PhD

- 70 renal transplant patients
- recipient serum tested for
  - anti-At1R antibodies using ELISA - 3 groups AT1R-Ab levels >17, 10-17, and <10 U/ml
  - endothelial flow cytometric cross match (ECXM) in 35 patients

- Patients with a positive ECXM had higher AT1R-Ab levels (P = 0.005)
- Patients with higher levels of anti-AT1R have more ABMR and more MI, even if anti-HLA negative
- G and ptc scores independently correlated with increased AT1R-Ab concentrations in the presence or absence of HLA-DSA

- The data show an association between non-HLA antibodies detected in the ECXM and AT1R ELISA and microvascular injury observed in ABMR

Philogene et al. Transplantation 2016
TCMR/BL Working Group ĭ Acute lesions

Molecular pathology in the diagnosis of TCMR

Reeves AJT 2016 ĭ How to improve diagnosis in BL/TCMR?
- Rigorous application of Banff TCMR diagnostic algorithms for t and i
- Isolated v-lesions are not all TCMR, particularly in first 6 months
- The different subclasses (IA, IB, IIA, IIB and III) may not be relevant
- Molecular assessment may help improve accuracy of diagnosis
- The diagnosis of TCMR could be improved by using a probabilistic estimate
<table>
<thead>
<tr>
<th>Sum (n)</th>
<th>Number of TCMR scores &gt;0.1 (%)</th>
<th>Mean TCMR classifier score</th>
<th>Sum (n)</th>
<th>Number of TCMR scores &gt;0.1 (%)</th>
<th>Mean TCMR classifier score</th>
<th>Predicted probability (n)</th>
<th>Number of TCMR scores &gt;0.1 (%)</th>
<th>Mean TCMR classifier score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (356)</td>
<td>8 (2)</td>
<td>0.02</td>
<td>0 (346)</td>
<td>7 (2)</td>
<td>0.02</td>
<td>0.0-0.2 (515)</td>
<td>25 (5)</td>
<td>0.03</td>
</tr>
<tr>
<td>1 (77)</td>
<td>8 (10)</td>
<td>0.05</td>
<td>1 (79)</td>
<td>8 (11)</td>
<td>0.04</td>
<td>0.2-0.4 (69)</td>
<td>17 (25)</td>
<td>0.12</td>
</tr>
<tr>
<td>2 (86)</td>
<td>12 (14)</td>
<td>0.06</td>
<td>2 (92)</td>
<td>11 (12)</td>
<td>0.06</td>
<td>0.4-0.6 (35)</td>
<td>18 (51)</td>
<td>0.27</td>
</tr>
<tr>
<td>3 (57)</td>
<td>15 (26)</td>
<td>0.13</td>
<td>3 (58)</td>
<td>14 (24)</td>
<td>0.12</td>
<td>0.6-0.8 (26)</td>
<td>19 (73)</td>
<td>0.37</td>
</tr>
<tr>
<td>4 (49)</td>
<td>23 (47)</td>
<td>0.29</td>
<td>4 (43)</td>
<td>18 (42)</td>
<td>0.23</td>
<td>0.8-1.0 (23)</td>
<td>22 (96)</td>
<td>0.75</td>
</tr>
<tr>
<td>5 (21)</td>
<td>16 (76)</td>
<td>0.45</td>
<td>5 (25)</td>
<td>18 (72)</td>
<td>0.44</td>
<td>0.57</td>
<td>7 (7)</td>
<td>0.89</td>
</tr>
<tr>
<td>6 (22)</td>
<td>19 (86)</td>
<td>0.66</td>
<td>6 (20)</td>
<td>16 (80)</td>
<td>(0.92)^2</td>
<td>0.89</td>
<td>8 (0)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

1i, i-lesion (interstitial inflammation) score; t, t-lesion (tubulitis) score; TCMR, T cell-mediated rejection; v, v-lesion (arteritis) score.

1The regression equation is presented in Table 1.

2By interpolation; no data available for sum (i-t-v) = 8.