13TH BANFF CONFERENCE ON ALLOGRAFT PATHOLOGY ï VANCOUVER 2015

Meeting report

Dr. Candice Roufosse

Imperial College Healthcare NHS Trust

Banff Foundation for Allograft Pathology

- 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 ï 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 ï Programme 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 — Sis et al. JASN 2015
- Fibrosis scoring — Farris et al. AJT 2014
- Polyoma virus nephropathy
- Glomerular lesion scoring
- Molecular Pathology
- Quality assurance — Mengel et al. AJT 2013

Banff Working Groups (3)

- Implantation biopsy
- T cell-mediated rejection
- Highly sensitized
- Isolated v-lesion
- Fibrosis scoring
- Polyoma virus nephropathy
- 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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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 surgeonôs 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 ï donor serum creatinine, AA race and % GS
  - Creatinine at 1mo, 3mo, 6mo, 1yr, 2yr ï 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 ï Acute lesions

- Initial data (Pittsburgh) only
  - 545 transplant biopsies explored for ñpureò 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

- Chronic, active TCMR and i-IFTA
  - Banff 2007 ï ñchronic allograft arteriopathyò (arterial intimal fibrosis with mononuclear cell infiltration and formation of neo-intima)
  - Banff 2015 - ñlesions of transplant arteriopathy may represent chronic, active ABMR as well as TCMR; the latter may also be manifest in the tubulointerstitial compartmentò

TCMR/BL Working Group ï 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

B. D. Modena[1], S. M. Kurian[1,2], L. W. Gaber[3], J. Waalen[1], A. I. Su[1], T. Gelbart[2], T. S. Mondala[2], S. R. Head[2], S. Papp[2], R. Heilman[4,5], J. J. Friedewald[6], S. M. Flechner[4,7], C. L. Marsh[4,8], R. S. Sung[4,9], H. Shidban[4,10], L. Chan[4,11], M. M. Abecassis[6] and D. R. Salomon[1,2,4,*]

Am J Transplantation 2016

- 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 ñMolecular Microscopeò

Molecular Pathology

- University of Alberta ï Alberta Transplant Applied Genomics Centre (ATAGC)
  - ñPathogenesis based transcriptsò(PBT)
  - Panels of transcripts to analyse for ABMR, TCMR, ATI
- ñRivalòmicroarray panels for renal transplant biopsies
  - Genomics of Transplantation Co-operative Research Group (7 centres, USA, NIH funded)(Daniel Salomon) ï Gene Co-expression networks (GCNs)
  - Sarwal Lab ï University of California San Francisco ï meta-analysis of previous publications identifies a common rejection module (CRM)= 12 genes elevated in rejection across all transplanted organs

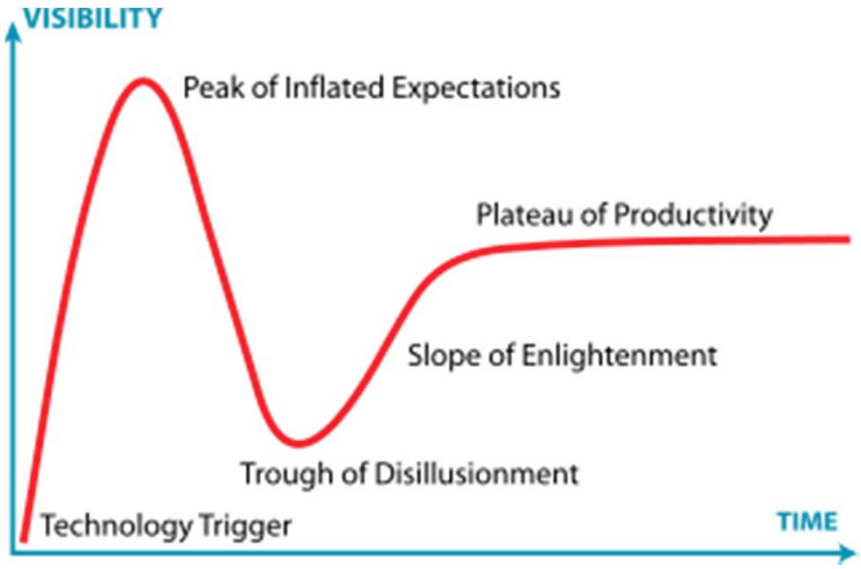
- Validation of Edmonton microarray use for the diagnosis of ABMR in renal transplant biopsies
  - Hayde et al. AJT 2014; Gupta et al. AJT 2016
    - Intragraft DSAï selective gene transcripts may be used as molecular markers for AMR, especially in C4d-negative biopsies

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?

Potentially equivocal situations for precision diagnosis	Informations provided By complementary approaches
Borderline lesions	• Eliminate Borderline category • Diagnose AKI • Wound healing process • Diagnose rejection, TCMR
Minimal microcirculation inflammation not qualifying for Banff ABMR +/- DSA	• Diagnose ABMR • Exclude ABMR • Prompt third parties Ab testing
TCMR (Banff 1a or greater) with microcirculation inflammation	• Rule out superimposed ABMR • Diagnose mixed rejection
ABMR (Banff 2013) with TCMR features	• Rule out superimposed TCMR • Diagnose mixed rejection
Isolated C4d (ABO compatible/ DSA+)	• Rule out early onset ABMR
Isolated cg≥1 with microcirculation injury/C4d below Banff thresholds for chronic, active ABMR	• Exclude / Diagnose ABMR • Information of activity
Isolated v lesions, with or without DSA, C4d	• Diagnose ABMR, TCMR • Diagnose AKI
BKVN with histologic findings meeting Banff criteria for ABMR and/or TCMR	• Rule out superimposed ABMR • Rule out superimposed TCMR

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 ï 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 ï 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? ï 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 ï 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 at 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

Banff 2013 Classification of Antibody-Mediated Rejection (ABMR) in Renal Allografts, Revised 2015 (continued)

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)[i]**
   - **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 expediant 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

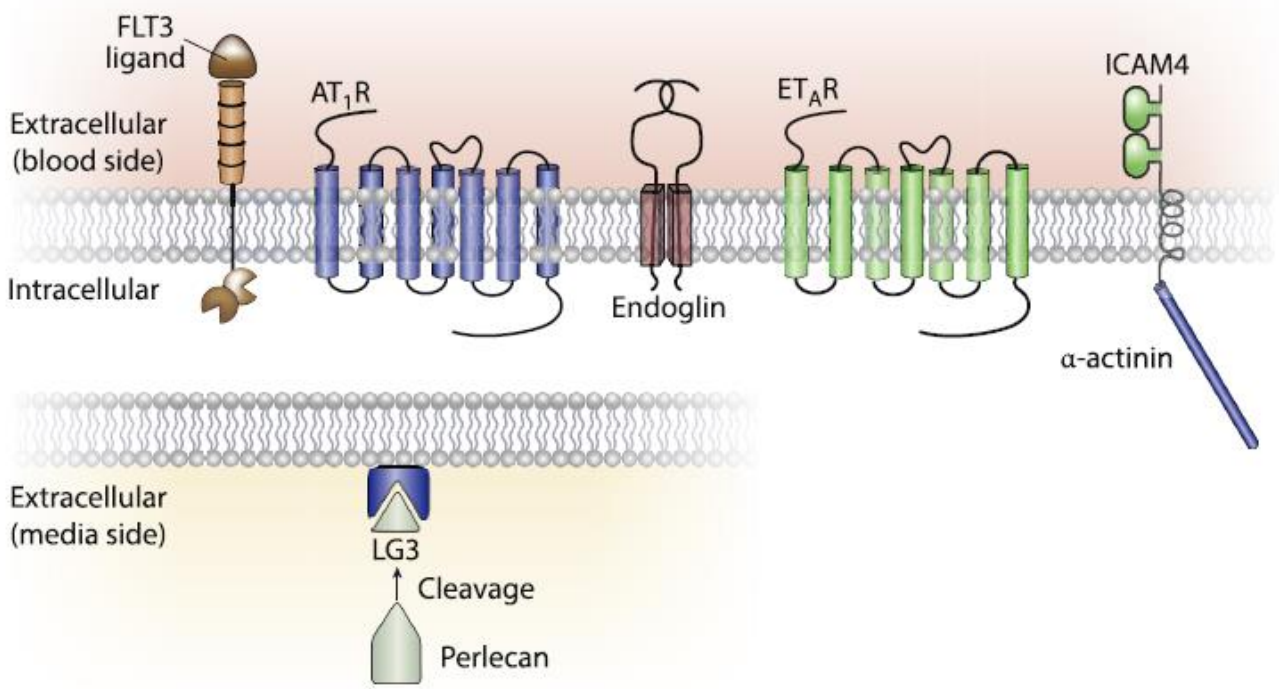
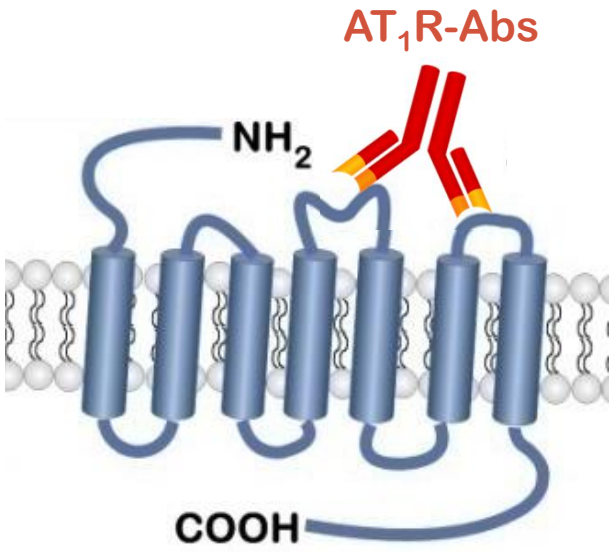


Figure 1 | Overview of nonhuman leukocyte antigen antibodies directed against endothelial targets. AT_1R, angiotensin II type 1 receptor; ET_AR, endothelin-1 type A receptor; FLT3, Fms-like tyrosine kinase-3; ICAM4, intercellular adhesion molecule 4.

Bridging alloimmunity and autoimmunity (Dragun et al 2016)

Non Anti-HLA antibodies

Angiotensin II type 1 receptor (AT_1R)



- Superficial endothelial antigen
- Receptor-mediated selective signaling leading to changes in gene expression instrumental for organ damage and C4d negative phenotype

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,[1] Serena Bagnasco, MD,[2] Edward S. Kraus, MD,[3] Robert A. Montgomery, MD, DPhil,[3] Duska Dragun, MD,[4] Mary S. Leffell, PhD,[1] Andrea A. Zachary, PhD,[1] and Annette M. Jackson, PhD[1]

Transplantation 2016

- 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**

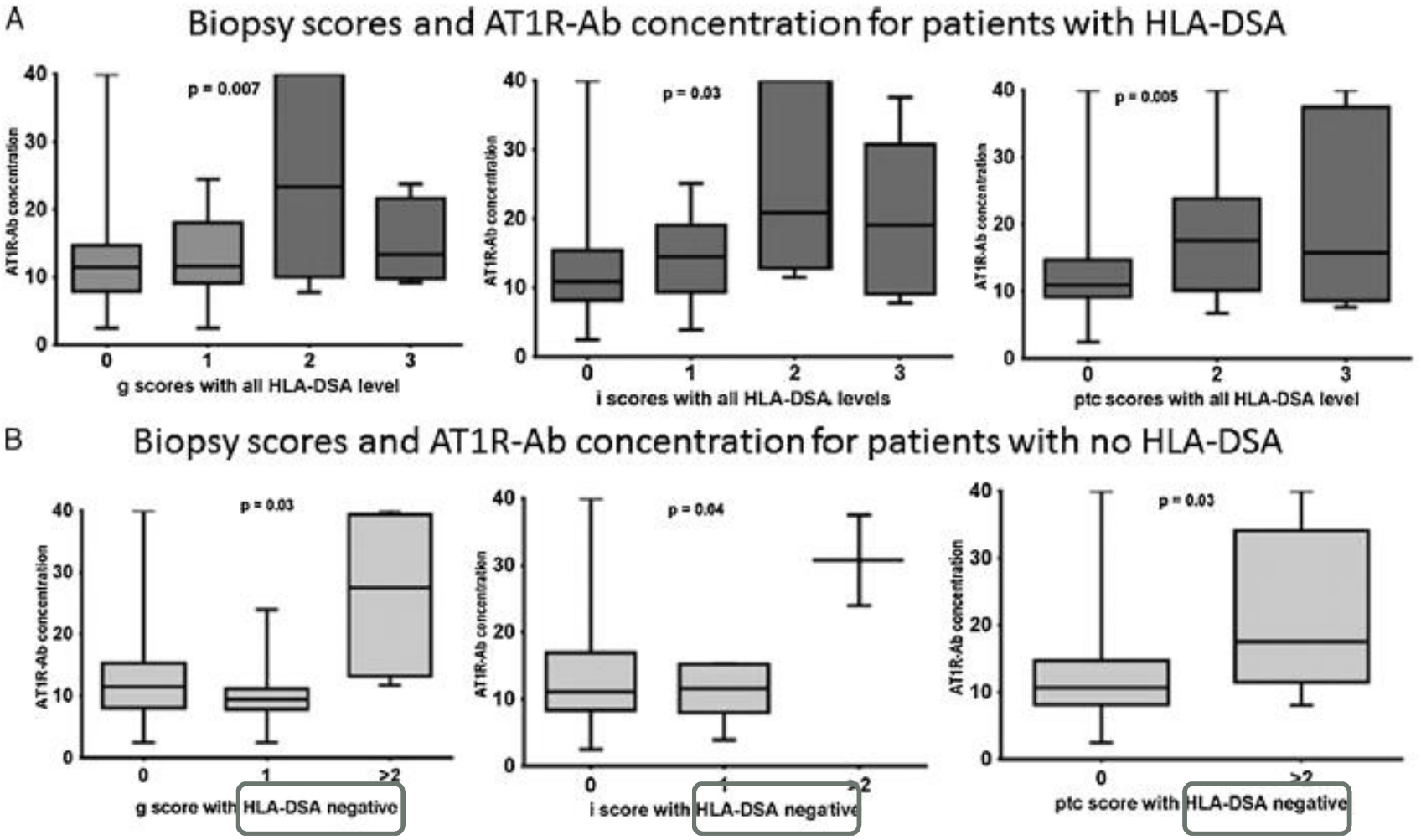
- **BUT**

- 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



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 ï genes; transcriptsé
    - 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

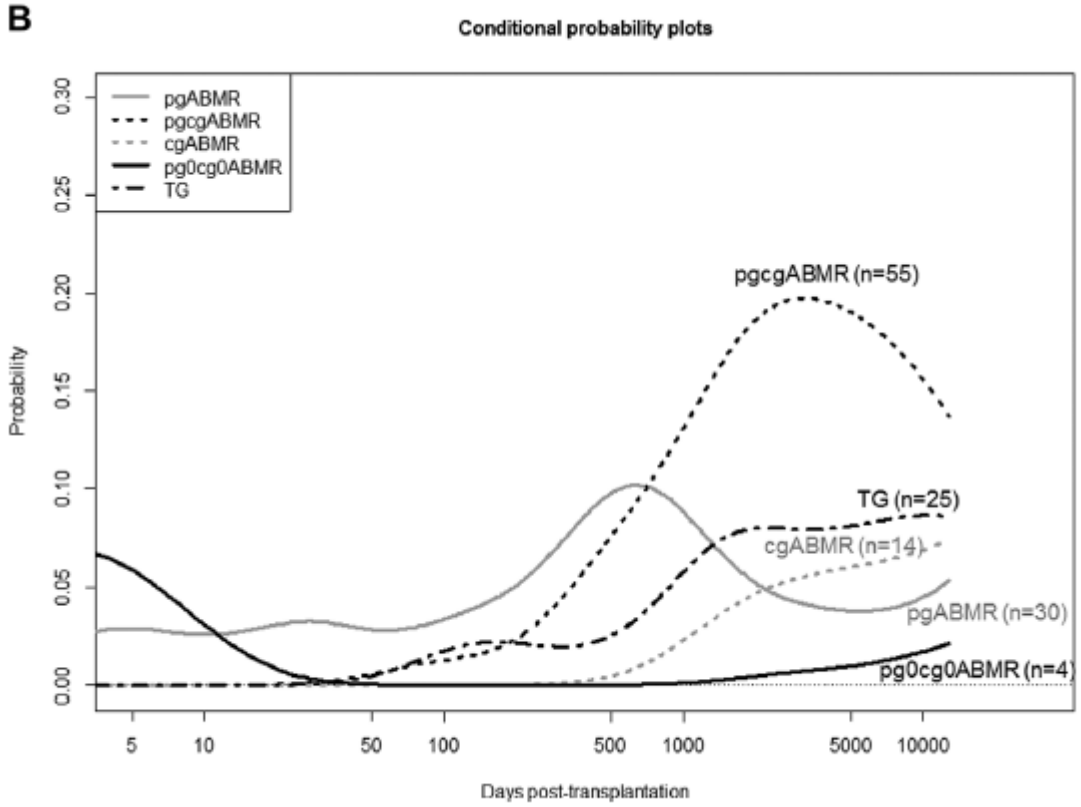
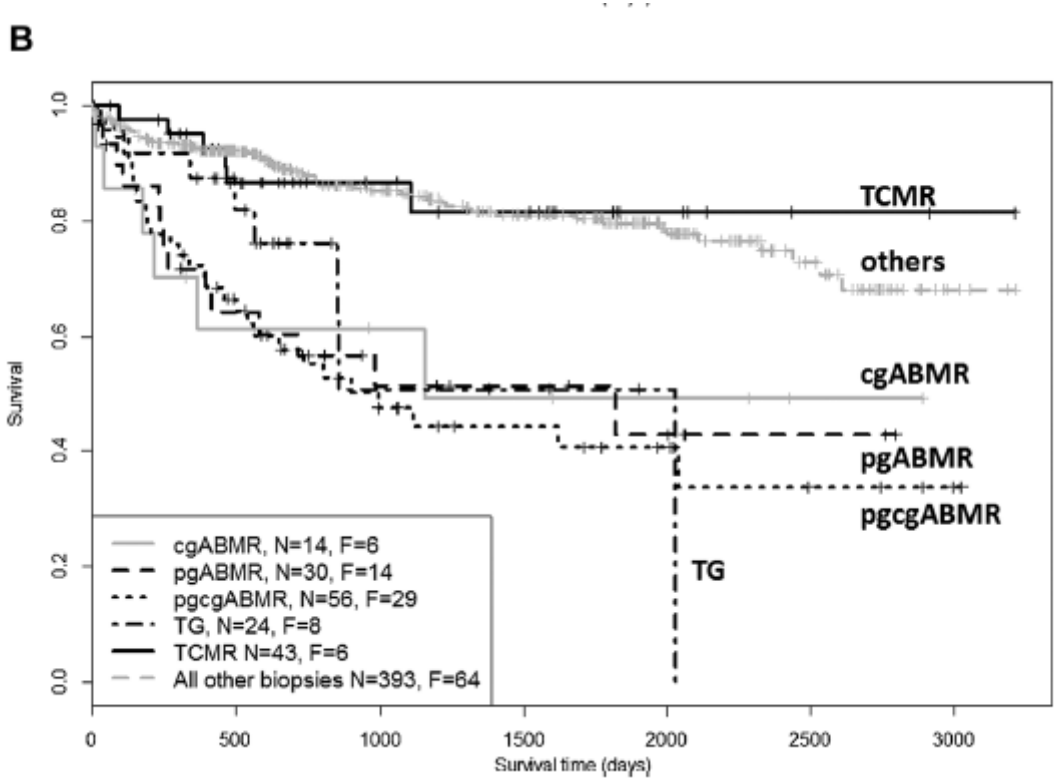

B
Conditional probability plots
pgABMR
pgcgABMR
cgABMR
pg0cg0ABMR
TG
pgcgABMR (n=55)
TG (n=25)
cgABMR (n=14)
pgABMR (n=30)
pg0cg0ABMR (n=4)
Probability
0.00
0.05
0.10
0.15
0.20
0.25
0.30
5
10
50
100
500
1000
5000
10000
Days post-transplantation

Table 5: Summarizing the discrepancies between the histologic ABMR subclasses and the molecular phenotype

Histologic ABMR subclasses		Molecular classes*				
		Pure ABMR	Mixed	Pure TCMR	No rejection	Discrepancies (% of n)
Pure ABMR† (n = 132)	pgABMR (n = 35)	*20*†	7	1	7	15/35 (43%)
	pgcgABMR (n = 73)	*68*†	1	0	4	5/73 (7%)
	cgABMR (n = 24)	*21*†	0	0	3	3/24 (13%)
Mixed (n = 28)	pgMixed (n = 10)	3	*6*†	0	1	4/10 (40%)
	pgcgMixed (n = 17)	13	*4*†	0	0	13/17 (76%)
	cgMixed (n = 1)	1	*0*†	0	0	1/1 (100%)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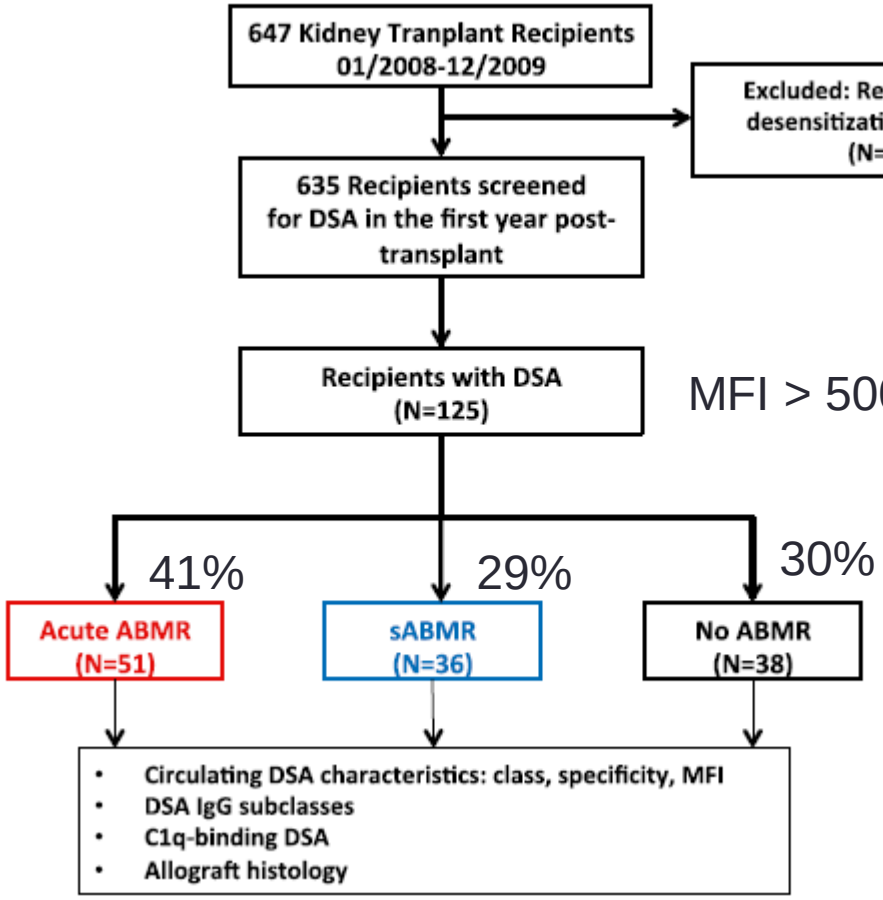
Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
Survival time (days)
TCMR
others
cgABMR
pgABMR
pgcgABMR
TG
cgABMR, N=14, F=6
pgABMR, N=30, F=14
pgcgABMR, N=56, F=29
TG, N=24, F=8
TCMR N=43, F=6
All other biopsies N=393, F=64

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‡



Negative B and T CDC

MFI > 500

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

Lefaucheur C et al JASN 2

Unsupervised PC analysis

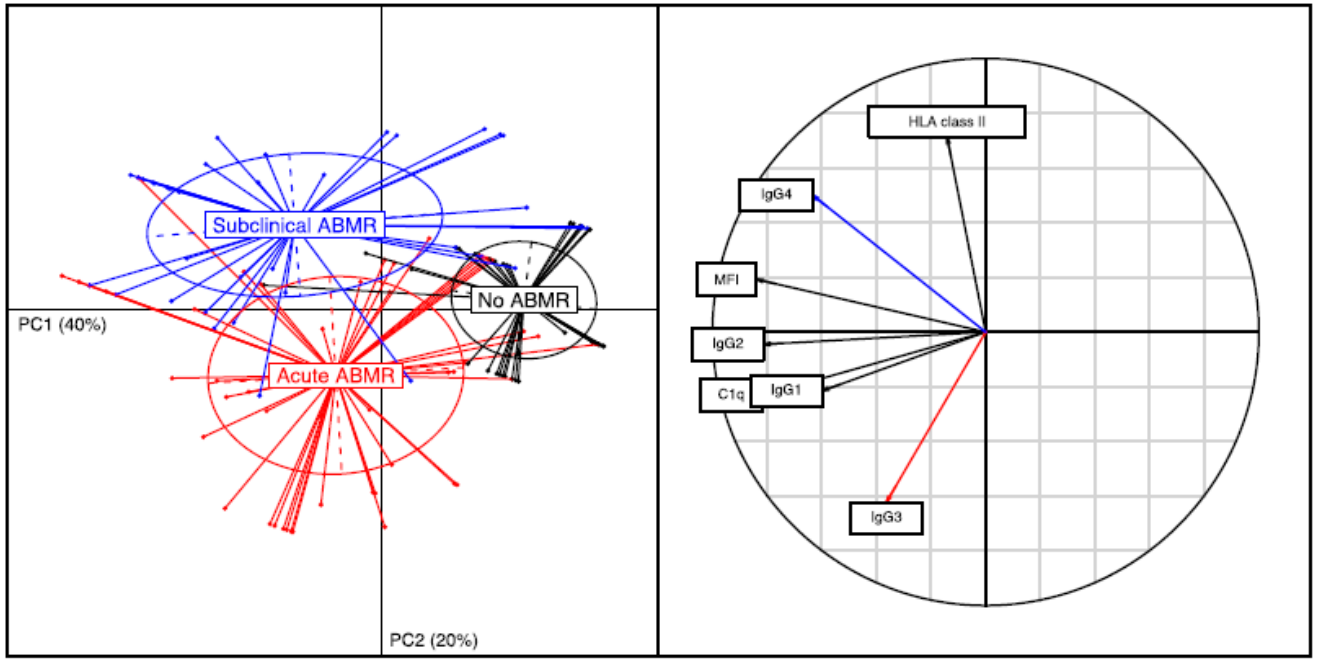
IgG4 and class II DSA distinguishes sABMR
Later rejection
More TG and IFTA

↑ NO

Target complement?

↓ YES

IgG3 DSA distinguishes aABMR
Early rejection
High MI
C4d+



Lefaucheur C et al JASN 2

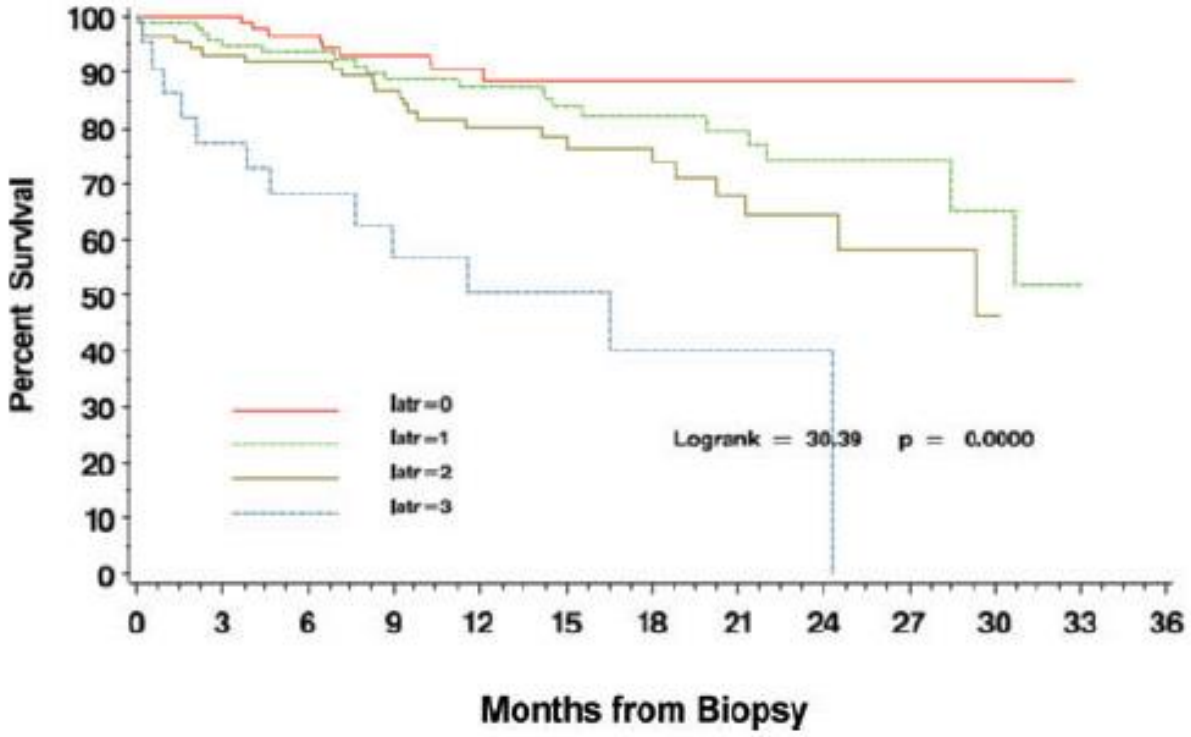
Electron Microscopy working Group

(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

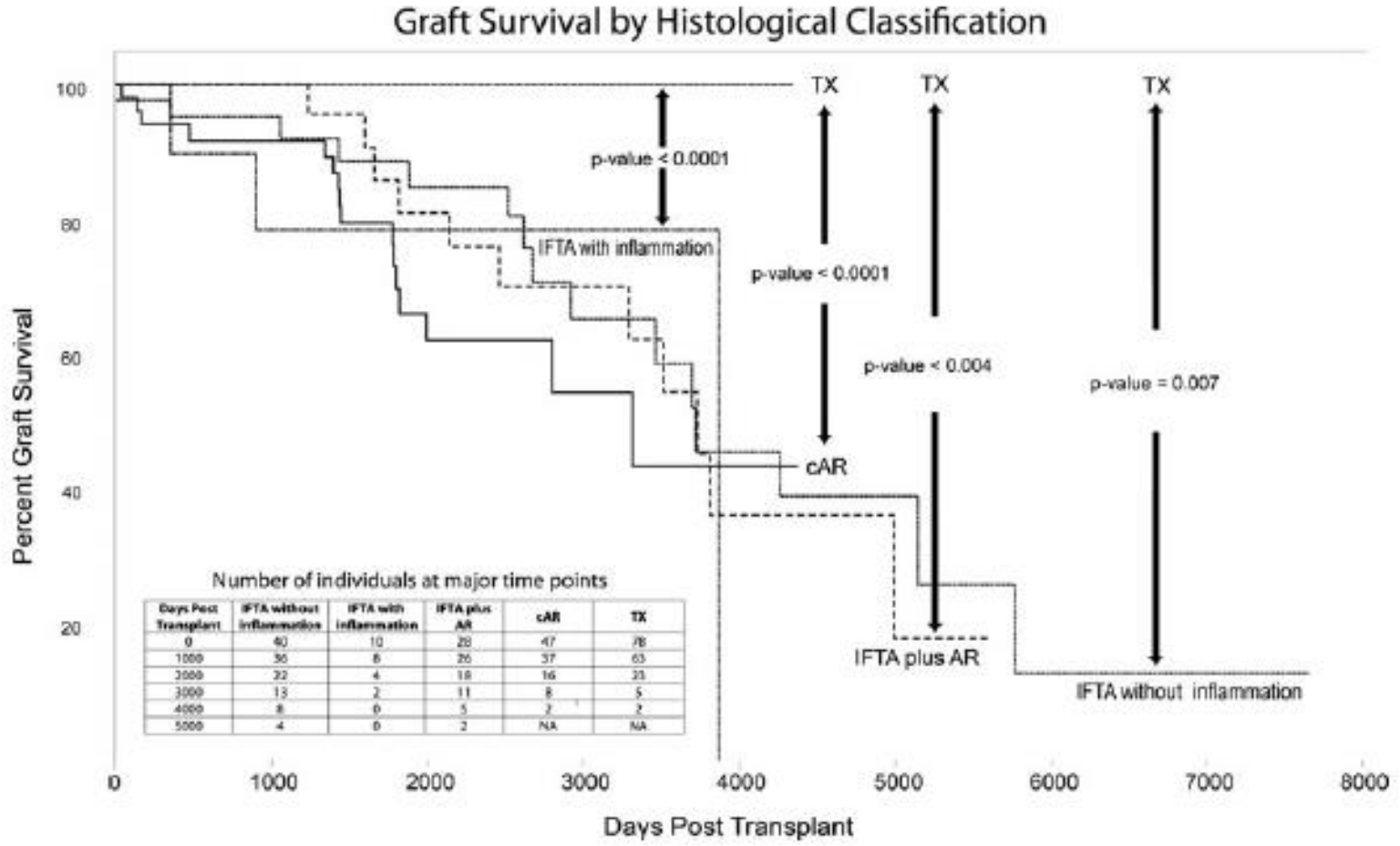
Em working group

- 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 ï 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 ï completed June 2016
- Circulation of images for inter-observer reproducibility variability ï autumn 2016
- Results f Part 1 presented to Banff Conference 2017
- 2017 ï 2018: multicentre study

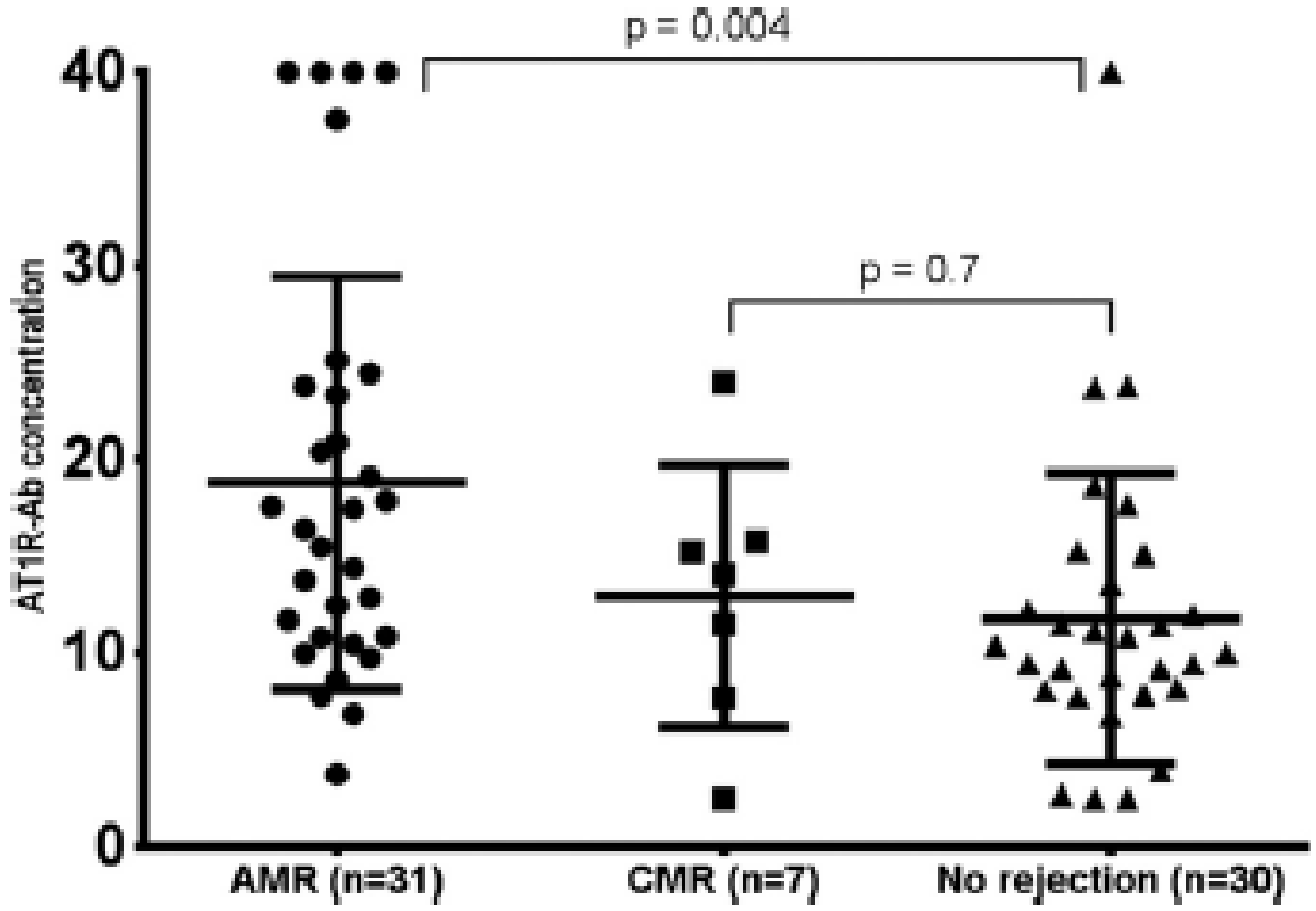


Mannon R et al. AJT 2010
(Dekaf - Long term deterioration of kidney allograft function)

- 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 diseaseé)

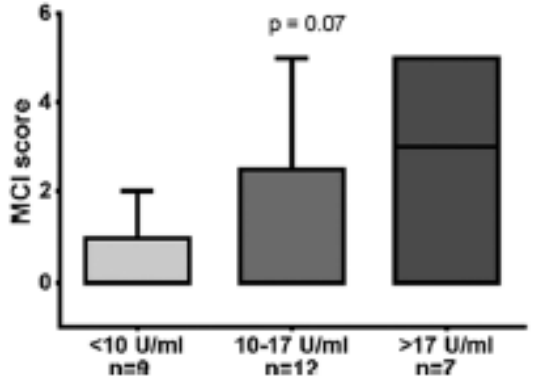


Modena et al AJT 2016


p = 0.004
p = 0.7
AT1R-Ab concentration
40
30
20
10
0
AMR (n=31)
CMR (n=7)
No rejection (n=30)

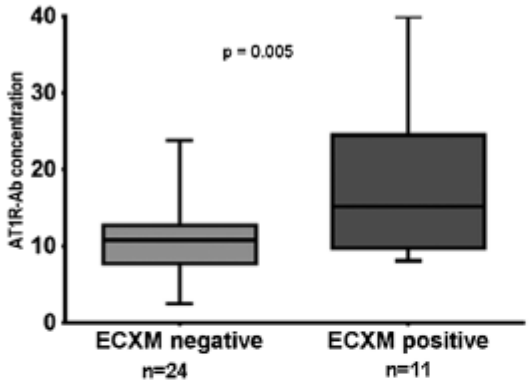
C

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



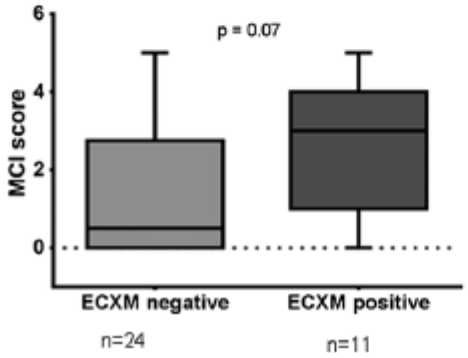
A

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 Ó1.3
    - 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,[1] Serena Bagnasco, MD,[2] Edward S. Kraus, MD,[3] Robert A. Montgomery, MD, DPhil,[3] Duska Dragun, MD,[4] Mary S. Leffell, PhD,[1] Andrea A. Zachary, PhD,[1] and Annette M. Jackson, PhD[1]

- 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 ï Acute lesions

- Molecular pathology in the diagnosis of TCMR
  - Reeves AJT 2016 ï 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 4: Conversion table for using TCMR lesion scores to predict molecular TCMR scores without categories

Sum i + t			Sum i-t-v			Logistic regression		
Sum (n)	Number of TCMR scores >0.1 (%)	Mean TCMR classifier score	Sum (n)	Number of TCMR scores >0.1 (%)	Mean TCMR classifier score	Predicted probability[1] range (n)	Number of TCMR scores >0.1 (%)	Mean TCMR classifier score
0 (356)	8 (2)	0.02	0 (346)	7 (2)	0.02	0.0–0.2 (515)	25 (5)	0.03
1 (77)	8 (10)	0.05	1 (75)	8 (11)	0.04	0.2–0.4 (69)	17 (25)	0.12
2 (86)	12 (14)	0.06	2 (92)	11 (12)	0.06	0.4–0.6 (35)	18 (51)	0.27
3 (57)	15 (26)	0.13	3 (58)	14 (24)	0.12	0.6–0.8 (26)	19 (73)	0.37
4 (49)	23 (47)	0.29	4 (43)	18 (42)	0.23	0.8–1.0 (23)	22 (96)	0.75
5 (21)	16 (76)	0.45	5 (25)	18 (72)	0.44			
6 (22)	19 (86)	0.66	6 (20)	16 (80)	0.57			
			7 (7)	7 (100)	0.89			
			8 (0)	–	(0.92)[2]			
			9 (2)	2 (100)	0.95			

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

[1]The regression equation is presented in Table 1.

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