Do We Always Need Donor-Specific Antibodies to Diagnose ABMR?

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Brief review of current Banff diagnostic criteria for acute/active and chronic, active ABMR

Can we use one or more surrogate markers to diagnose ABMR in the absence of detectable DSA?

- C4d
- Molecular markers
  - DSA – specific transcripts (DSASTs)
  - Molecular ABMR classifier

Statement of Disclosure

Mark Haas serves as a paid consultant on pathology adjudication committees for two industry-sponsored clinical trials:

Shire ViroPharma – Treatment of Acute ABMR

AstraZeneca – Treatment of Proliferative Lupus Nephritis

Neither represents a conflict of interest relevant to any of the material presented in this talk.

Banff 2013 Classification of Antibody-Mediated Rejection (ABMR) in Renal Allografts

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

1. Histologic evidence of acute tissue injury, *including one or more of the following*:
   - Microvascular inflammation (g > 0[b] and/or ptc > 0)
   - Intimal or transmural arteritis (v > 0)[c]
   - 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 (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ([g + ptc] ≥2)[d]
   - 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)

[a] These lesions may be clinically acute, smoldering, or subclinical. Biopsies showing two of the 3 features may be designated as "suspicious" for acute/active ABMR.

[b] Recurrent/de novo glomerulonephritis should be excluded

[c] These lesions may be indicated of ABMR, TCMR, or mixed ABMR/TCMR

[d] In the presence acute T cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc ≥2 alone is not sufficient to define moderate microvascular inflammation and g must be ≥1.

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

Chronic, Active ABMR; all three features must be present for diagnosis[f]

1. Morphologic evidence of chronic tissue injury, *including 1 or more of the following*:
   - Transplant glomerulopathy (cg >0)[g], if no evidence of chronic TMA
   - Severe peritubular capillary basement membrane multilayering (requires EM)[h]
   - 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 (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections)
   - At least moderate microvascular inflammation ([g + ptc] $\geq$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)

[f] In the absence of evidence of current/recent antibody interaction with the endothelium (those features in section 2), the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time post-transplantation.

[g] Includes GBM duplication by electron microscopy only (cg1a) or GBM double contours by light microscopy

[h] $\geq$7 layers in 1 cortical peritubular capillary and $\geq$5 in 2 additional capillaries, avoiding portions cut tangentially

[i] In the presence acute T cell-mediated rejection, borderline infiltrates, or evidence of infection, ptc $\geq$2 alone is not sufficient to define moderate microvascular inflammation and g must be $\geq$1.

Comparison of Predictive Value of Banff 2013 vs. Banff 2007 Criteria for Chronic, Active ABMR

De Serres et al (Quebec), Am J Transplant 16: 1515-25, 2016

123 patients, single center, indication bx Jan 2006 – Oct 2014
45 reached combined endpoint of graft loss or doubling of SCr

	Banff 2007	Banff 2013
% with CAABMR	18%	36%
HR of CAABMR for combined endpoint	1.6 [0.7-3.8]	2.5 [1.2-5.2]

1. What to do with a biopsy showing (g + ptc) ≥1, C4d+, ± TG, and NO DSA?

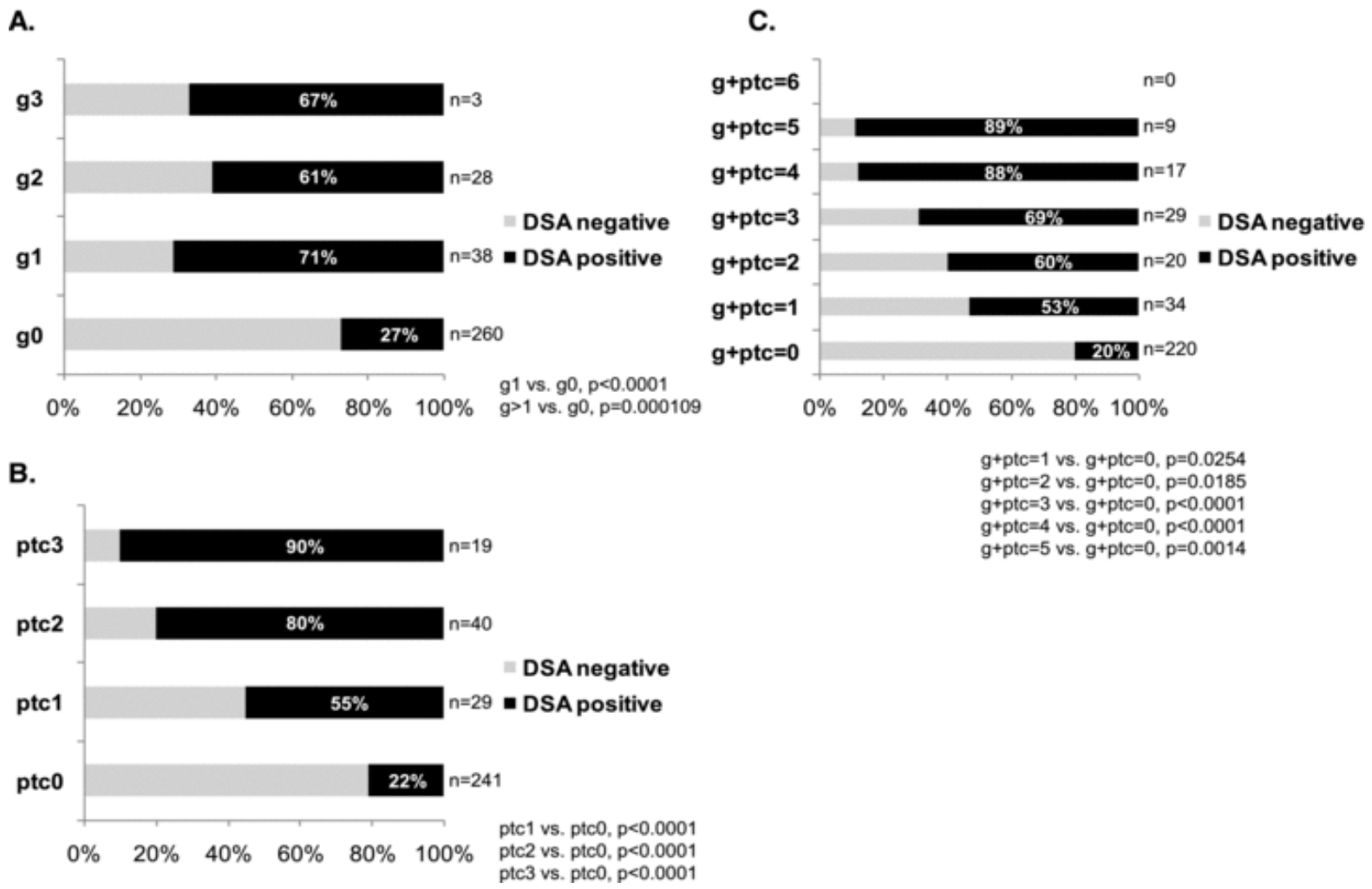
2. What to do with a biopsy showing (g + ptc) ≥2, C4d-, ± TG, and NO DSA?

Microvascular Inflammation (MVI) is NOT Specific for Active ABMR

Examine expression of pathogenesis-based transcript sets (PBTs) previously found to be associated with ABMR in 356 clinically indicated renal allograft biopsies.

209 with MVI = 0 (25% DSA+, 8% C4d+)
67 with MVI = 1 (36% DSA+, 15% C4d+)
80 with MVI $\geq$ 2 (54% DSA+, 50% C4d+)

P values for all PBTs, DSA+ vs. DSA-, within MVI = 1 and MVI $\geq$1 were not significant except for DSASTs
Gupta et al (Albert Einstein), Kidney Int 89: 217-225, 2016



B Sis et al (Edmonton)

Am J Transplant 12: 1168-79, 2012

……..and Neither is Transplant Glomerulopathy (TG) Specific for Chronic ABMR - TG Has Multiple Etiologies

1. Chronic/Persistent Antibody-Mediated Rejection
   (73% of for-cause biopsies with TG at mean of 5.5 yrs post- transplant were C4d+, had concurrent DSA, or both; Sis et al, AJT 7: 1743-1752, 2007)

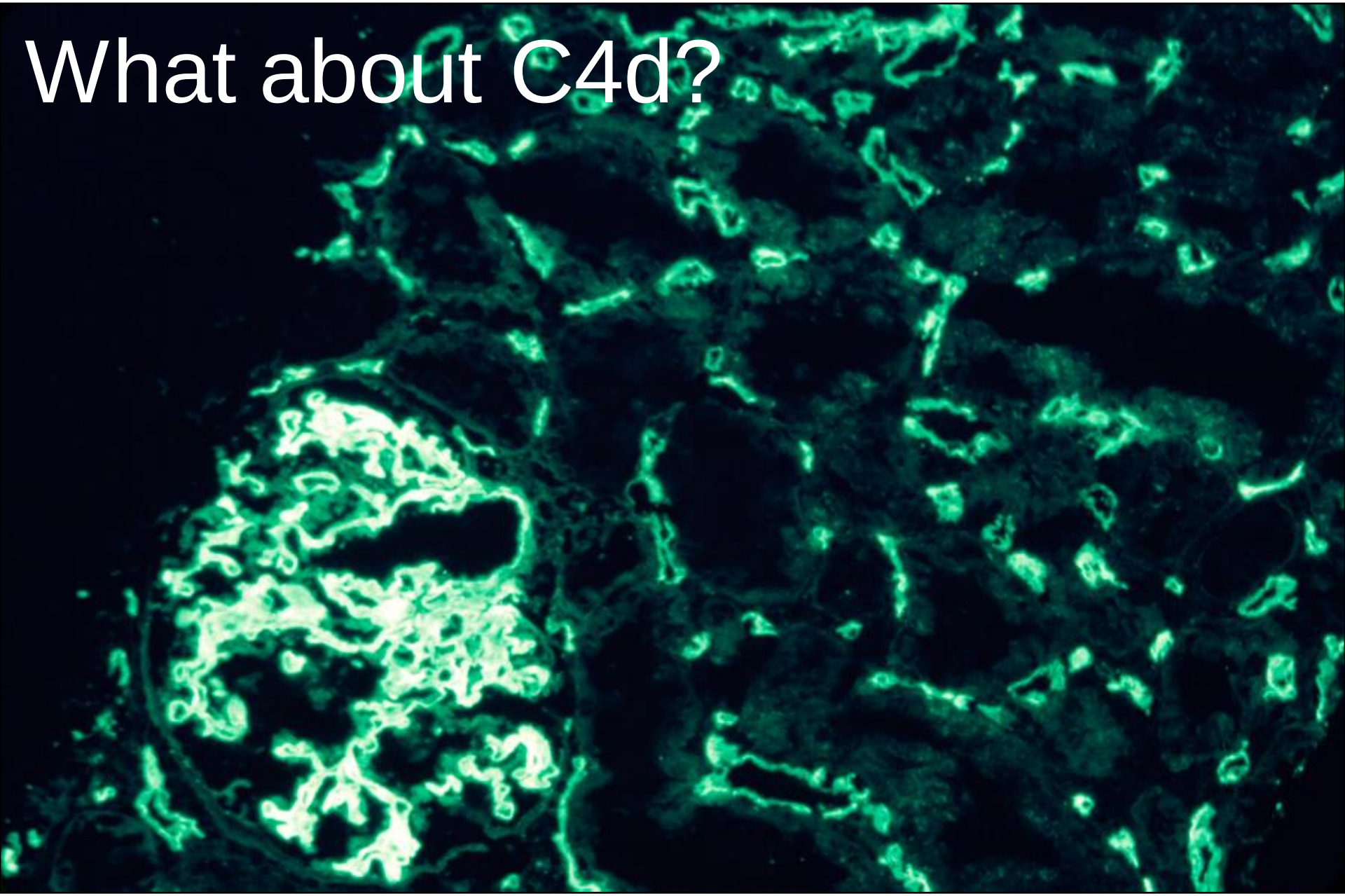
2. Hepatitis C
   - Need to differentiate from recurrent or de novo MPGN, using IF and/or EM
   - Possibly related to TMA associated with anti-cardiolipin antibodies

3. Other forms of TMA

4. Cell-Mediated Rejection (?)

ref: Baid-Agrawal et al, Kidney Int 80: 879-885, 2011

What about C4d?

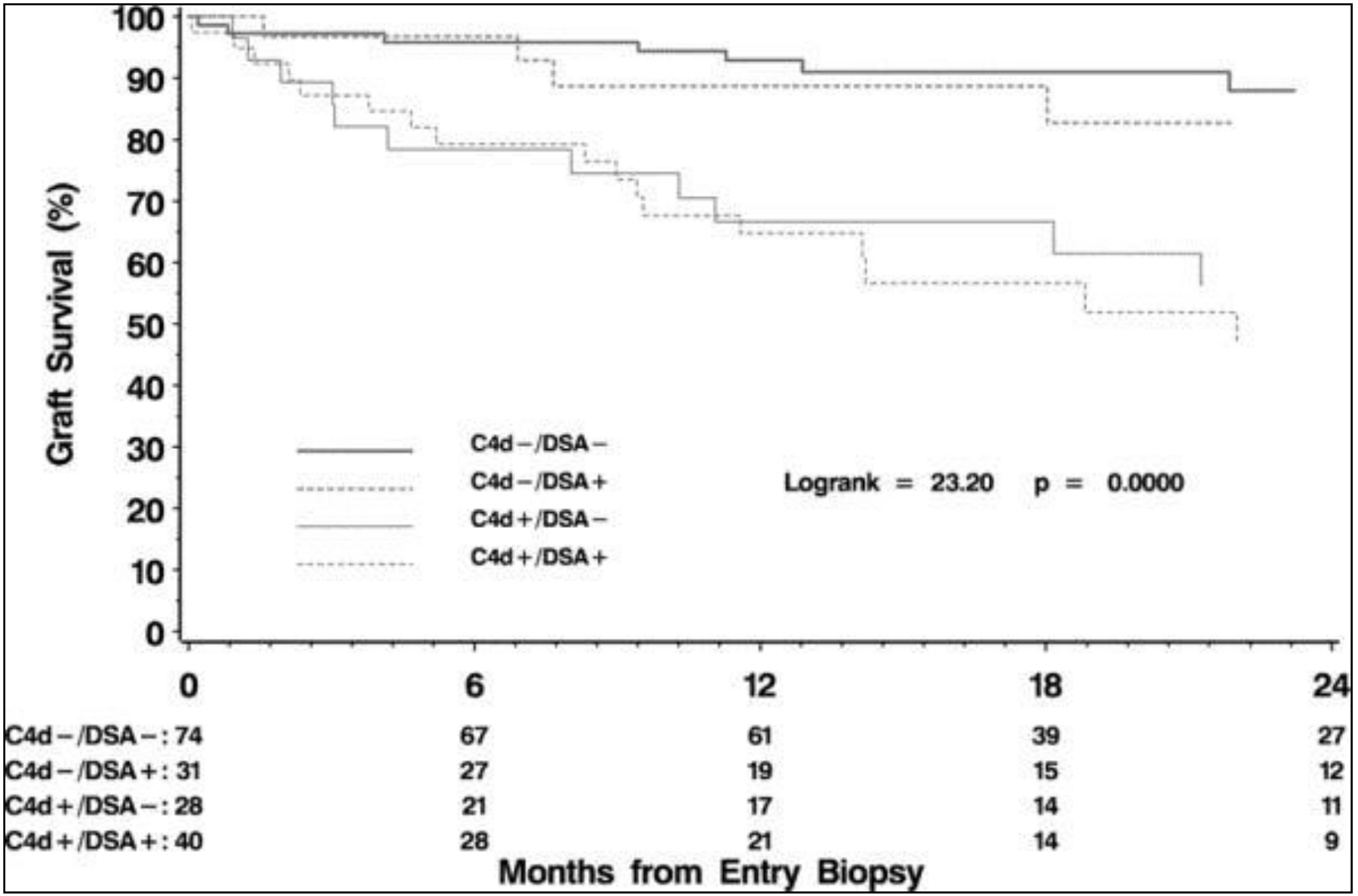


C4d Staining in Renal Allografts: correlation with donor-specific Ab

- Collins et al, JASN 10: 2208-14, 1999
  100% of AR with +DSA were C4d+
  No C4d in DSA- AR, CSA toxicity
- Maueyyedi et al, JASN 13: 779-787, 2002
  30% of early AR C4d+ - 90% had anti-donor antibody
  2 morphologic subtypes of AMR - capillary, arterial
  Arterial (fibrinoid necrosis) had worse outcome
- Bohmig et al, JASN 13: 1091-9, 2002
  21/24 C4d+ cases had DSA by flow cytometric XM
  50% of C4d- biopsies had DSA
  93% specificity, 31% sensitivity (IHC on paraffin sections)

Should DSA be required for ABMR diagnosis in C4d+ biopsies?

Gaston et al (DeKAF Study), Transplantation 90: 68-74, 2010



Influence of DSA and C4d on Outcomes in Chronic, Active ABMR with Transplant Glomerulopathy

Lesage et al (Quebec City), Transplantation 99: 69-76, 2015

61 patients with late indication biopsy (median 79 mo), TG and MVI

- 45 C4d- and DSA- (‘isolated TG”)
- 14 C4d+ and DSA- (6) or C4d- and DSA+ (8)
- 12 C4d+ and DSA+

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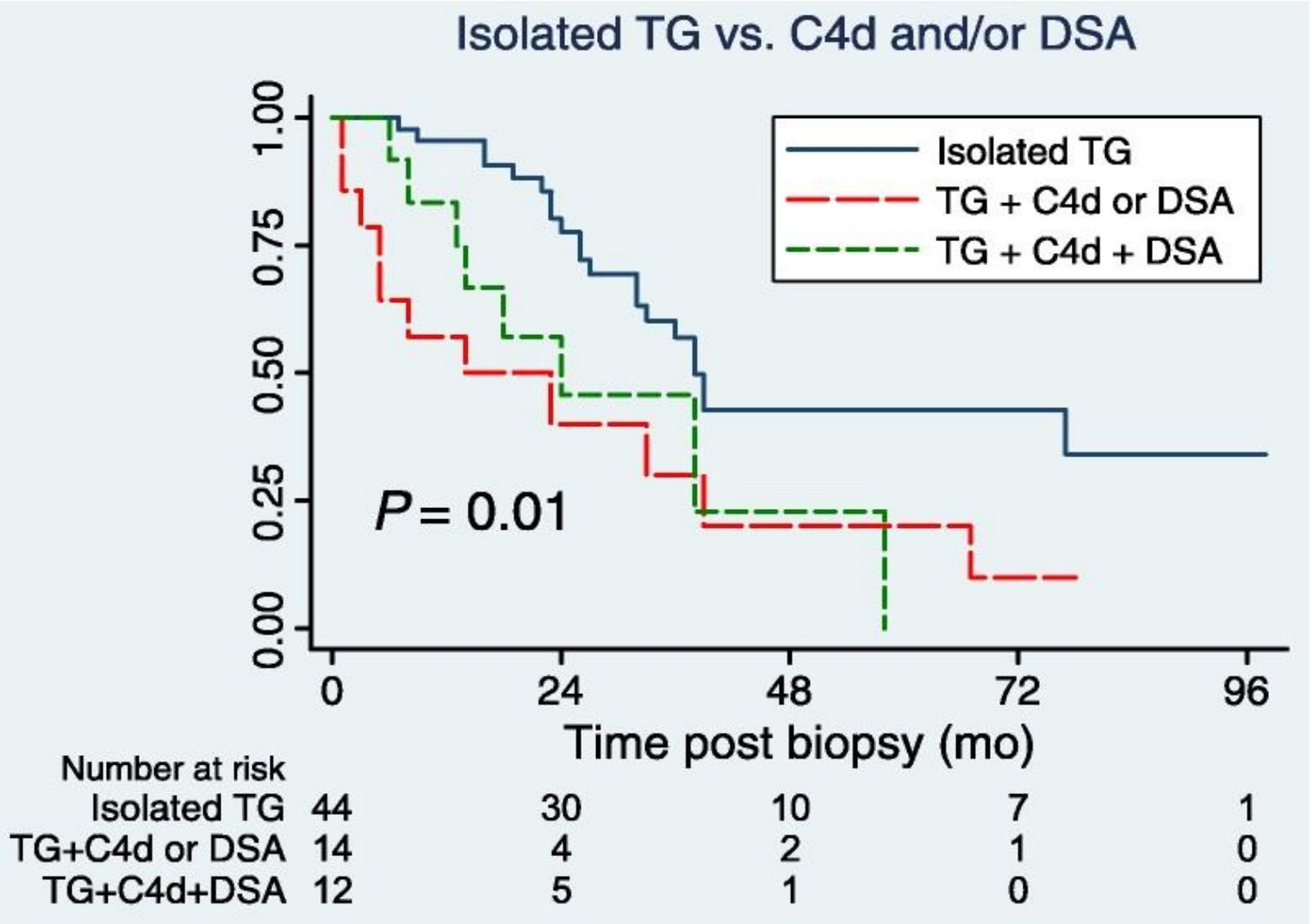
Lesage et al (Quebec City), Transplantation 99: 69-76, 2015



FOR YOUR CONSIDERATION:

Given the high specificity of C4d for DSA and these outcomes data, can DSA requirement for ABMR diagnosis be waived in biopsies of ABO-compatible kidneys with MVI and C4d?

What to do with a biopsy showing (g + ptc) ≥2, C4d-, ± TG, and NO DSA?

Test for non-HLA DSA

-Not all labs do such testing for all relevant non-HLA Abs

-In most labs, routine DSA testing does not include HLA-C and HLA-DP

Consider molecular testing

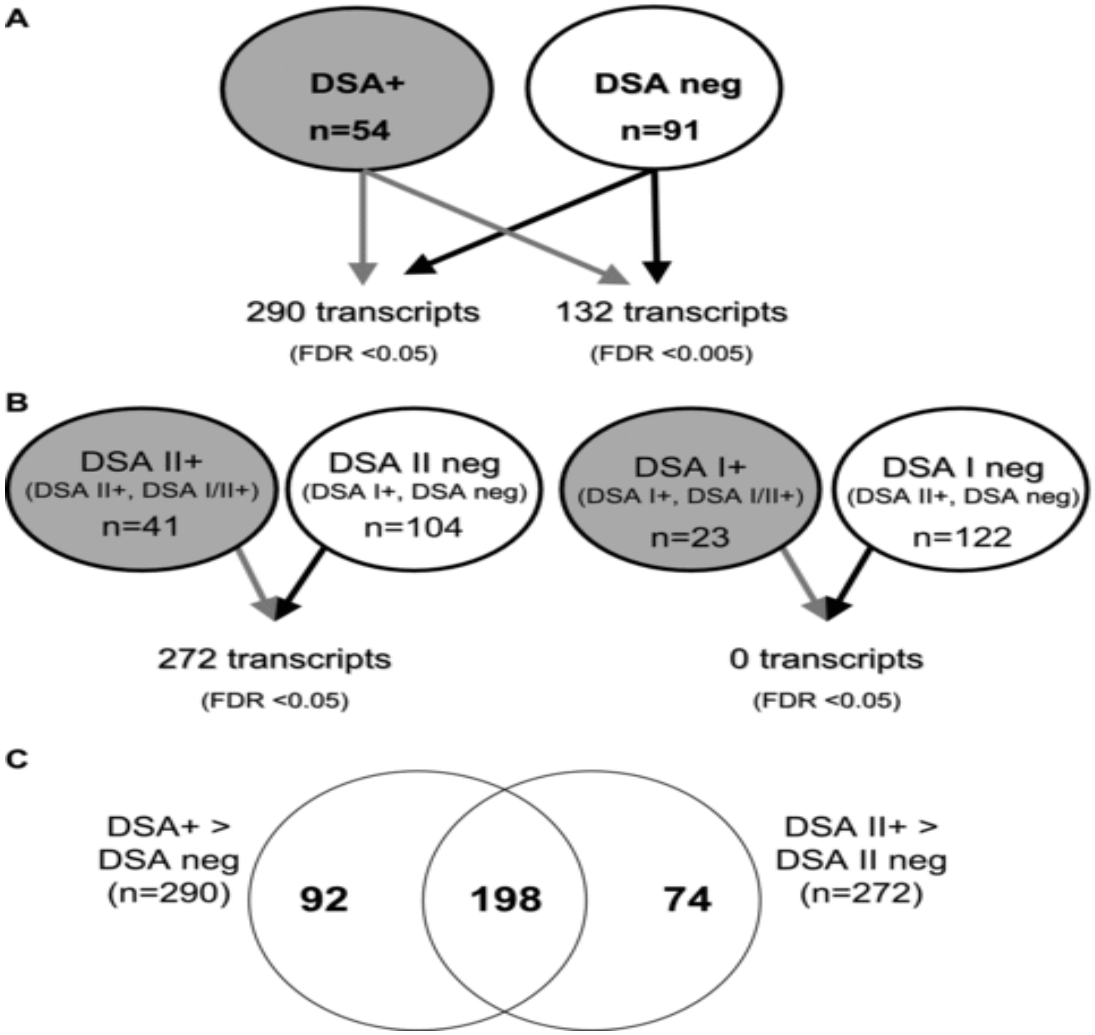
-DSAST transcript set highly correlated with anti-HLA DSA in two independent labs (U. Alberta, Albert Einstein)

-Not known if expression increased with non-HLA DSA

-Doesn't distinguish between IgG subclasses, C1q-binding vs. non-binding, 1 vs. >1 DSA, high vs. low MFI

Defining a Transcript Set Associated with DSA (DSASTs)

Hidalgo et al (Edmonton), Am J Transplant 8: 1812-22, 2010



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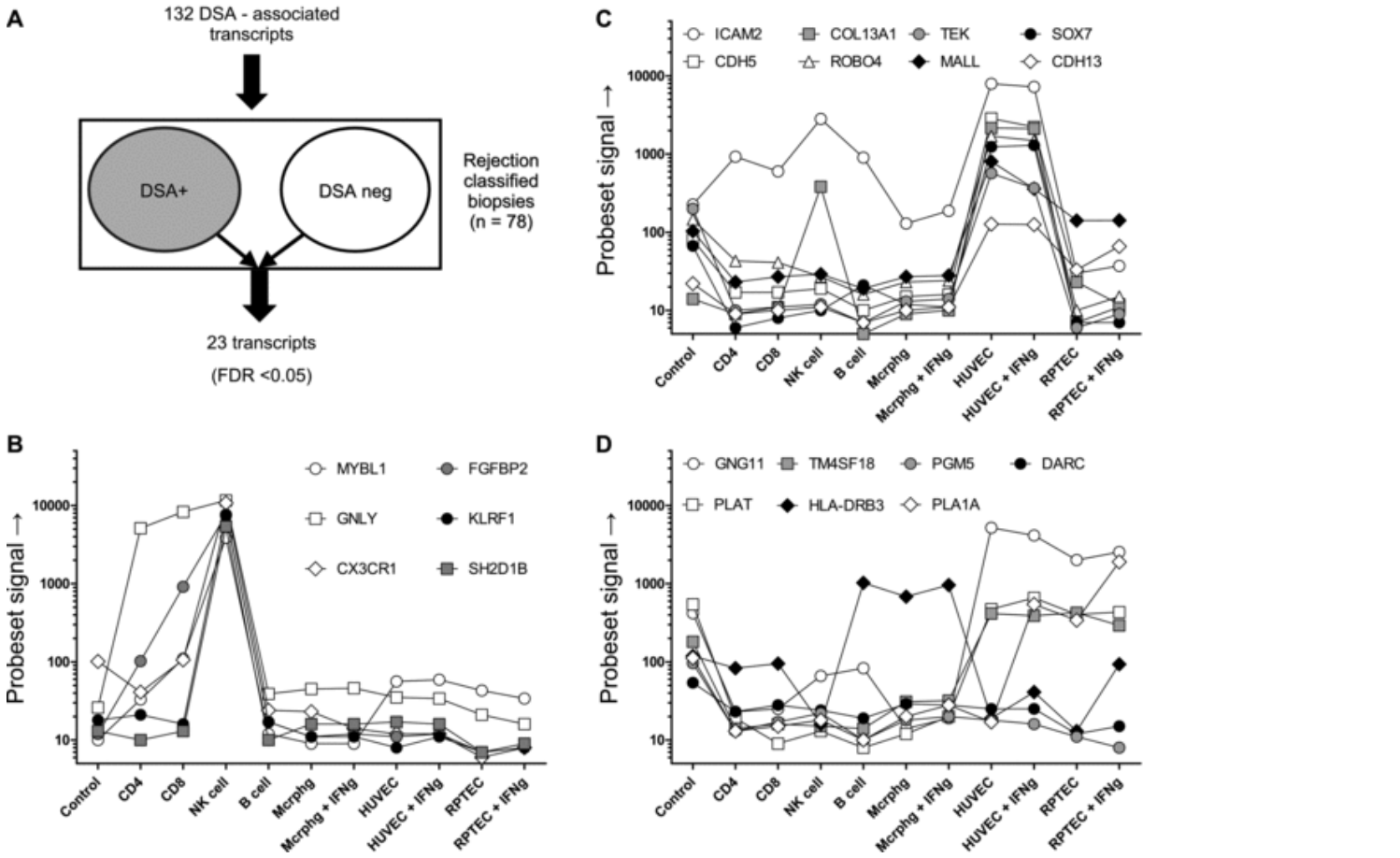

B - Transcripts preferentially expressed in NK cells

C - Transcripts preferentially expressed in endothelial cells

D - Transcripts expressed in endothelial and other cell types

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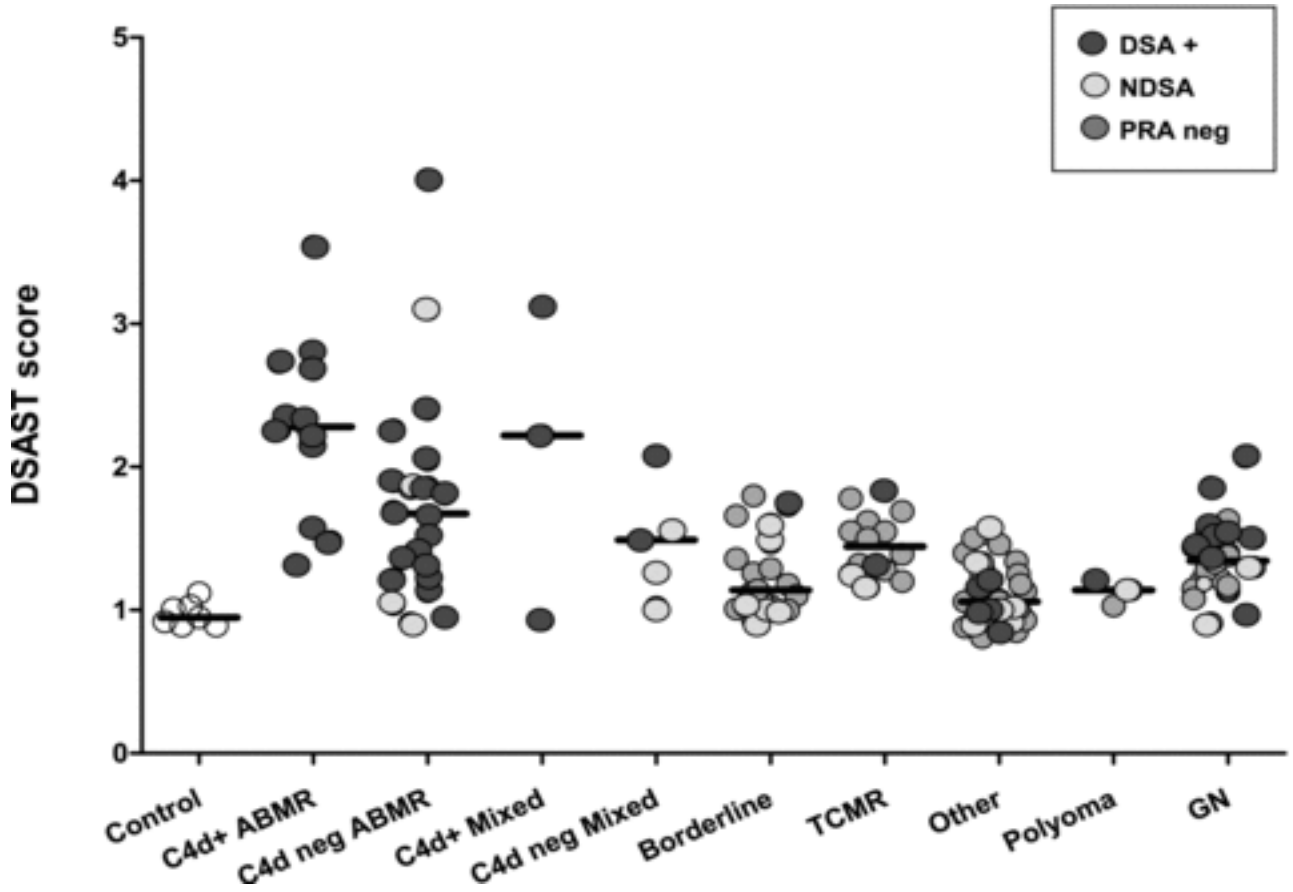
Hidalgo et al (Edmonton), Am J Transplant 8: 1812-22, 2010



Molecular ABMR Classifier Score

J. Sellares et al (Edmonton), AJT 13: 971-83, 2013

Based on 30 non-redundant probes, selected from comparisons between biopsies + or - histologic ABMR (DSA+, C4d+ or C4d-)

Cell types of highest expression, based on literature and/or expression in cell cultures:

Endothelial cells – 17

NK cells – 5

Tubular epithelial cells – 4

T cells – 3

Macrophages – 2

IFN Gamma-induced - 2

Unknown cell type - 5

Association of molecular ABMR score with histologic diagnosis (mixed rejections excluded)

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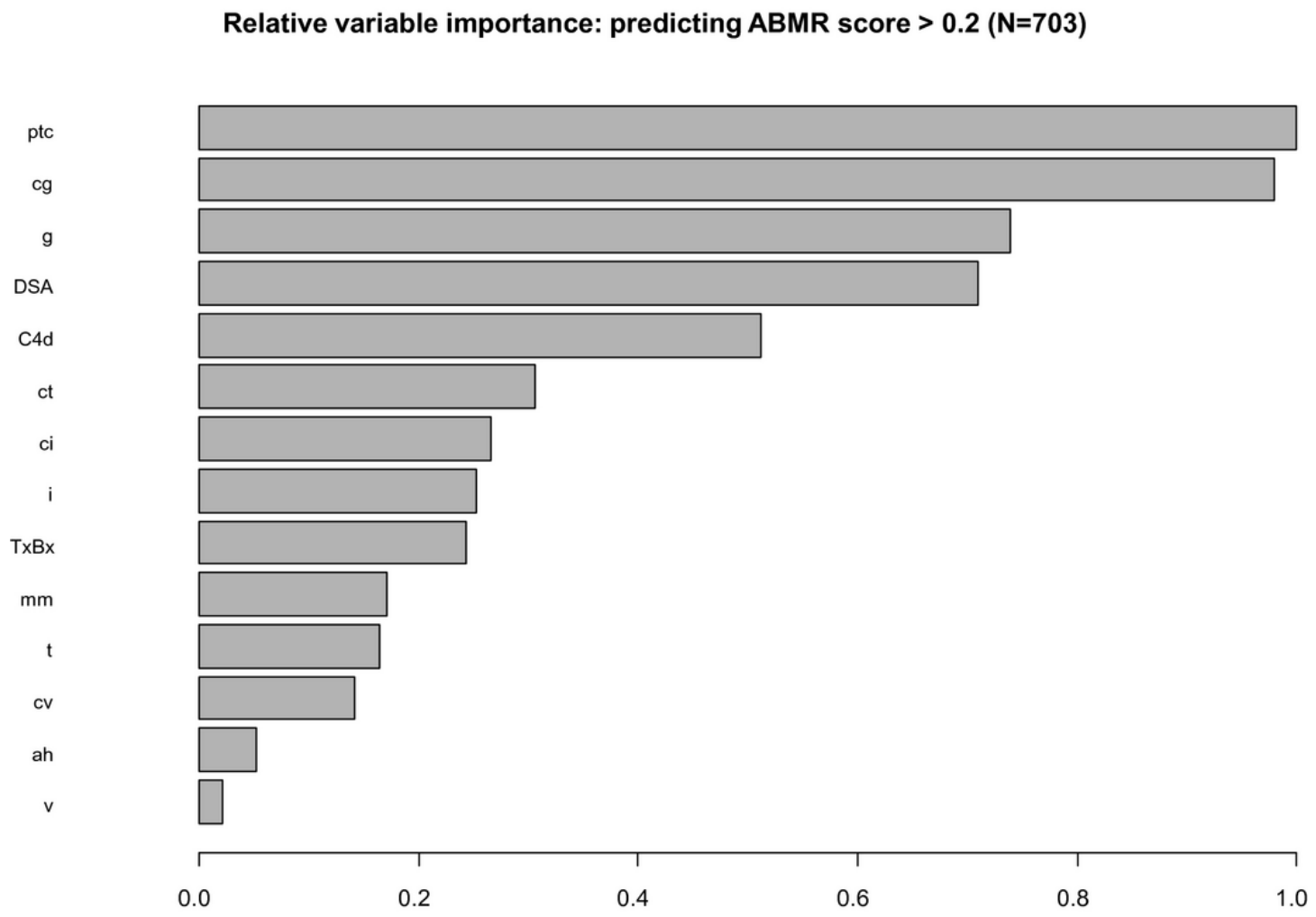
ABMR Score	ABMR	No ABMR	Total
>0.2	64	66	130; PPV=0.49
≤0.2	46	499	545; NPV=0.92
Total	110; sensitivity=0.58	565; specificity=0.87	675; accuracy=0.83

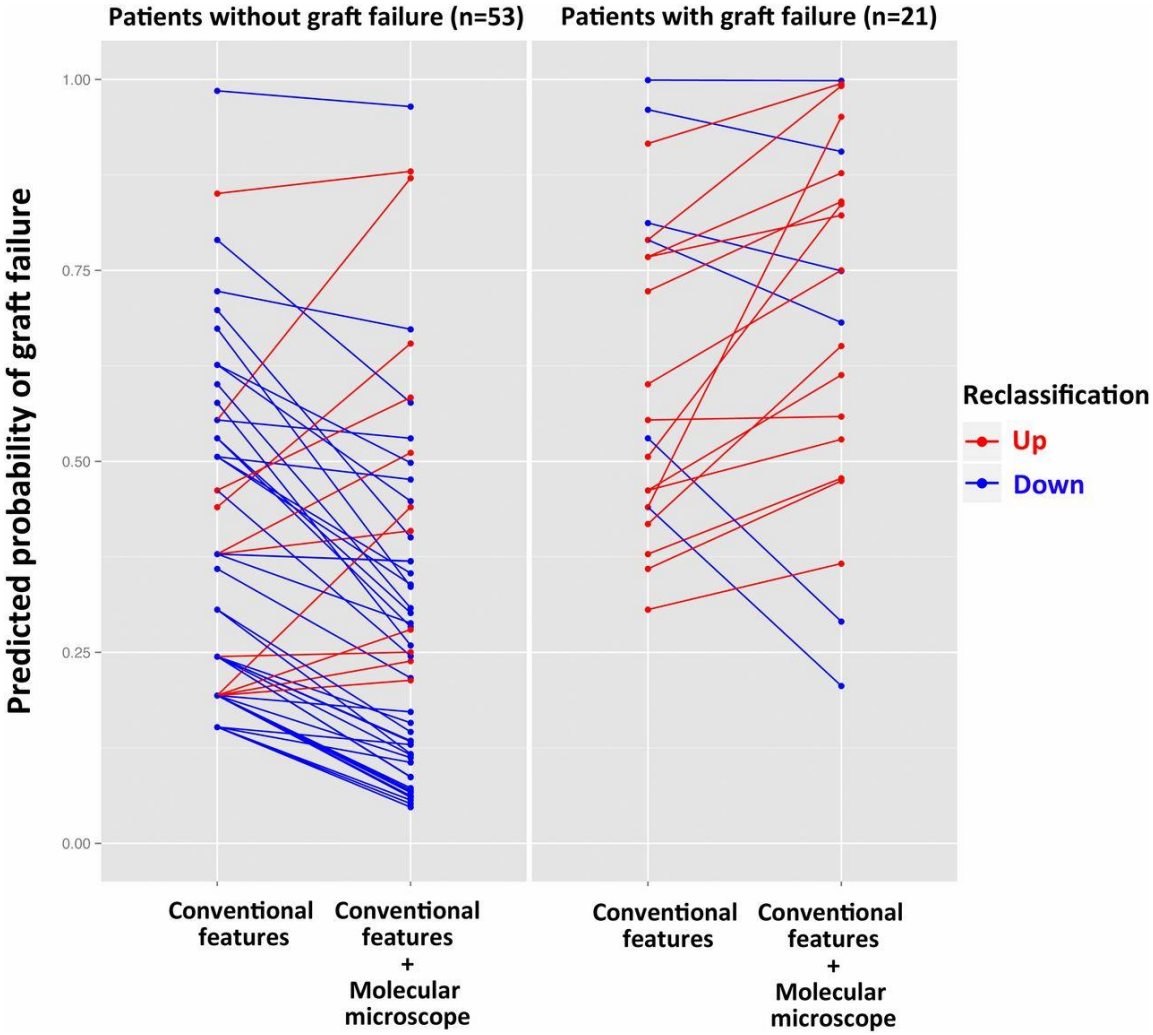
P. Halloran et al, JASN 26: 1711-1720, 2015

A Probabilistic Approach to Histologic Diagnosis of Antibody-Mediated Rejection in Kidney Transplant Biopsies

P Halloran et al, AJT 17: 129-139, 2017



Additive value of the ABMR Molecular Score for reclassification of risk of allograft failure (continuous net reclassification improvement)

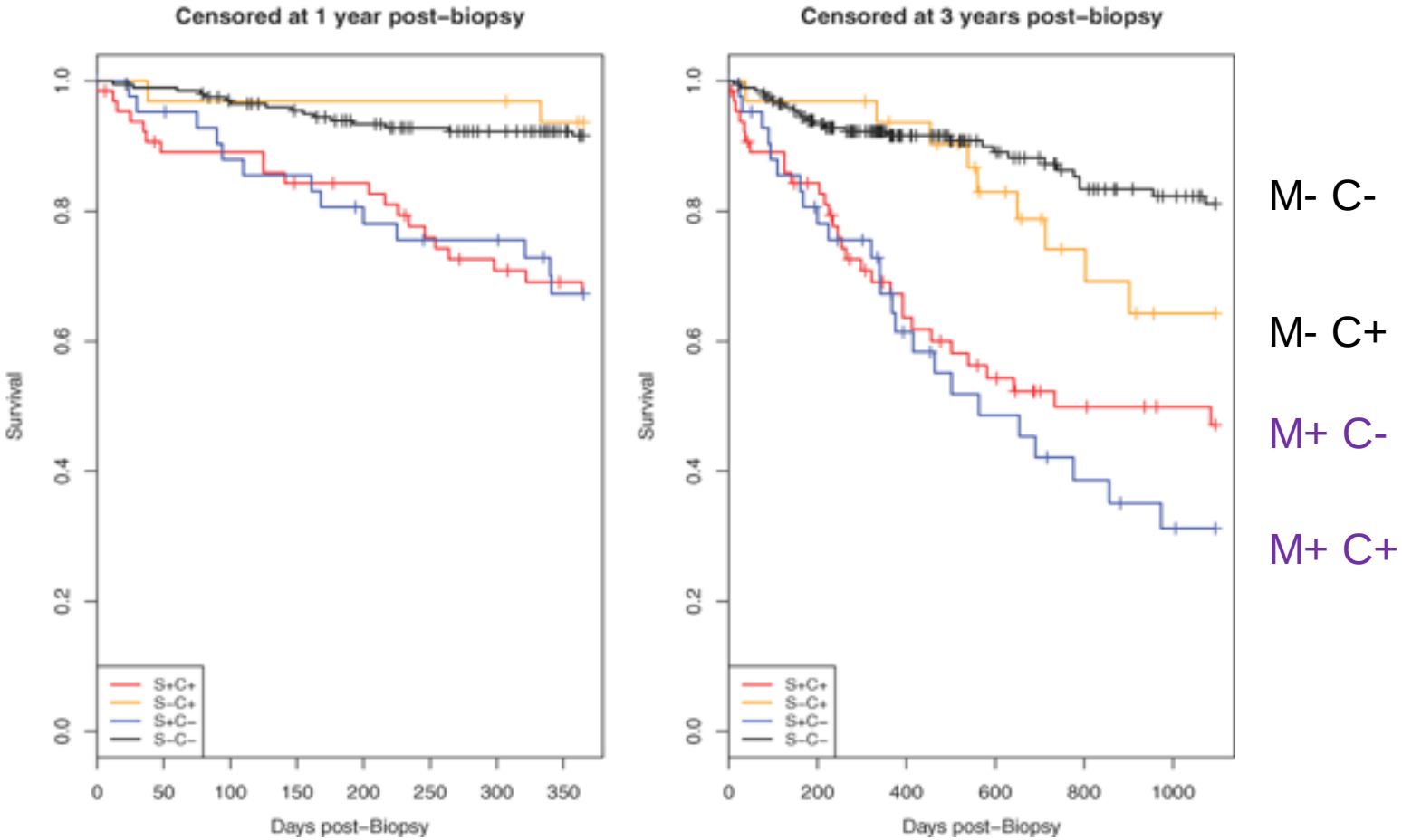


Alexandre Loupy et al. JASN 2014;25:2267-2277

JASN

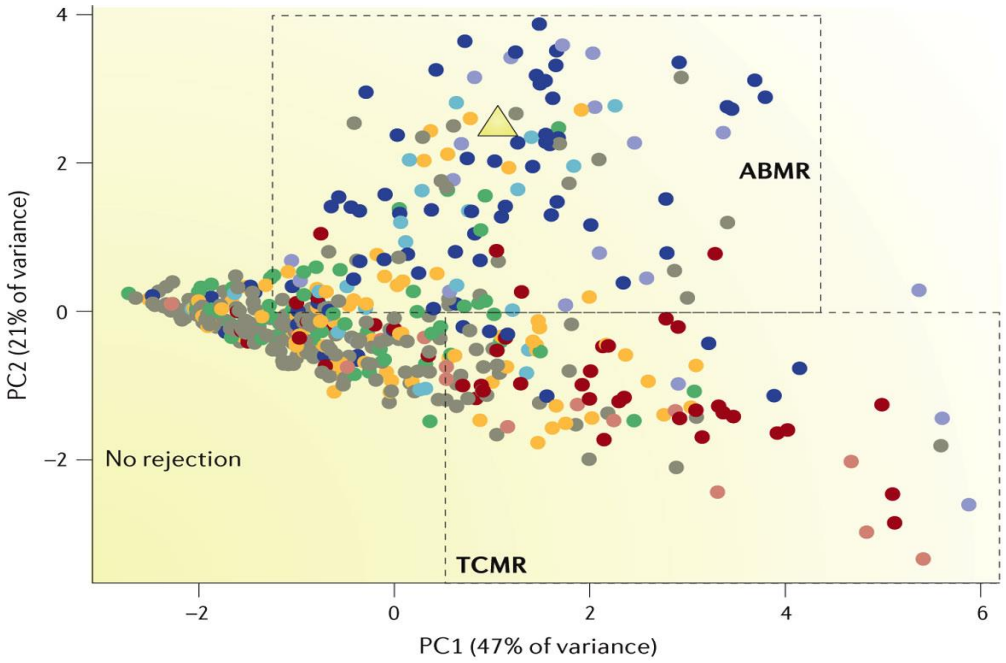
One and Three Year Post-Biopsy Graft Survival As a Function of Microarray and Histologic Diagnosis of ABMR/Mixed Rejection

P Halloran et al, Am J Transplant 13: 2865-74, 2013



M+ = ABMR score >0.2; C+ = diagnostic **or suspected** ABMR C4d+ or C4d-

Schematic of an analysis of a new biopsy sample in relation to a reference set of samples from indication biopsies



Histology diagnosis of the 50 nearest neighbours
C4d⁻ ABMR: 32%
C4d⁺ ABMR: 16%
Borderline: 12%
TG: 11%
Mixed: 11%
GN: 6%
TCMR: 3%
Other: 3%
TA/IF: 3%
No major abnormalities: 2%
AKI: 1%

Proportion of the 50 nearest neighbours surviving
1 year: 0.74
3 years: 0.43

- New sample
- TCMR
- ABMR
- TG
- Mixed transcripts
- Borderline
- BK
- GN
- Others

Nature Reviews | Nephrology

Halloran, P. F. *et al.* Molecular assessment of disease states in kidney transplant biopsy samples *Nat. Rev. Nephrol.* 12: 534-48, 2016

What to do with a biopsy showing (g + ptc) ≥2, C4d-, ± TG, and NO anti-HLA DSA or non-HLA antibodies against the graft?

These are cases where molecular diagnostics have great potential for clinical usefulness, and should now be a specific focus for investigation.

Thank you for your attention. Any questions?