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Does gene transcript analysis in renal transplant biopsies assist in the distinction of antibody-mediated rejection from glomerulonephritis?

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Glomerulonephritis (GN) and Antibody-mediated rejection (AMR)

- Both immune complex GN and AMR cause inflammation of the glomerulus
- Both GN and AMR are important causes of late allograft loss
- Establishing the distinction between these two diagnoses is important for patient management

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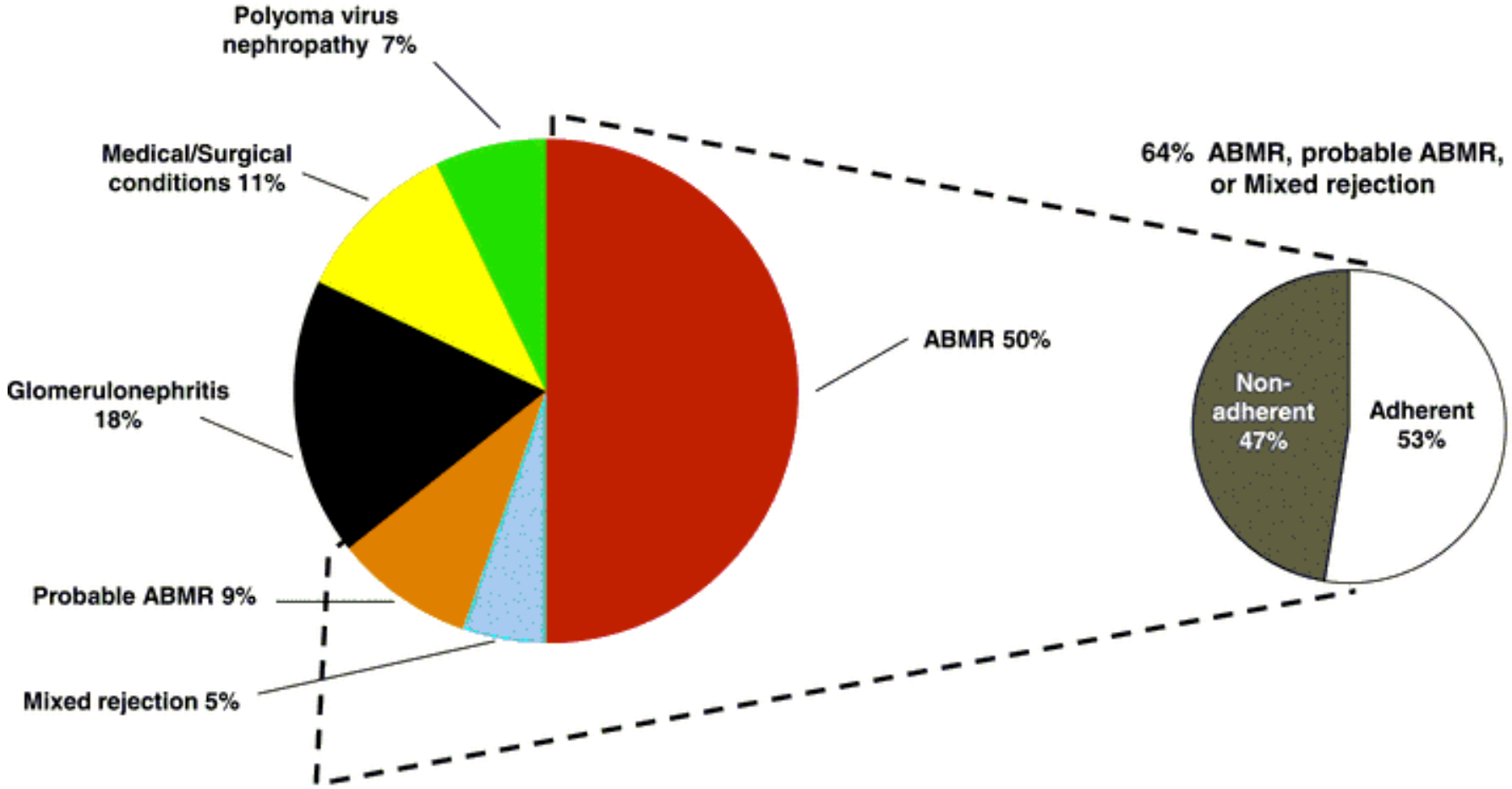
Distribution of causes of transplant renal failures



References: Sellarés, J., de Freitas, D. G., Mengel, M., Reeve, J., Einecke, G., Sis, B., Hidalgo, L. G., Famulski, K., Matas, A. and Halloran, P. F. (2012), Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence. American Journal of Transplantation, 12: 388–399. doi:10.1111/j.1600-6143.2011.03840.x

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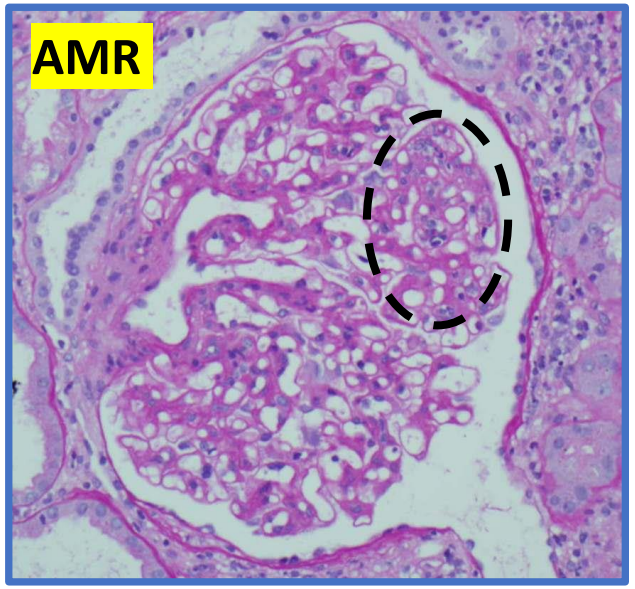
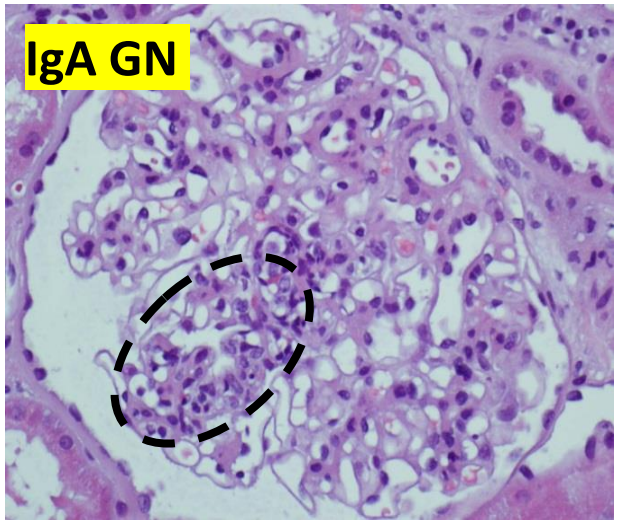
Conventional histological diagnosis

	AMR	GN
Light microscopy	Glomerulitis Capillary wall double contours Peritubular capillaritis	Glomerulitis Capillary wall double contours
Immunofluorescence	Glomeruli negative for immunoglobulins and complement	Variable positivity for immunoglobulins and complement
Electron microscopy	No or few electron dense deposits in glomeruli Peritubular capillary basement membrane multilayering	Electron dense deposits in glomeruli





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Transcript analysis of renal transplant biopsies

- Endothelial associated gene transcripts and natural killer cell–associated transcripts are elevated in AMR (Halloran et al., (2015). *Current Opinion in Organ Transplantation*, 20(3), pp.359-367.)
- Increased AMR associated transcripts are now part of Banff 2017 definition of AMR
- **Use of Quantitative Real Time Polymerase Chain Reaction to Assess Gene Transcripts Associated With Antibody-Mediated Rejection of Kidney Transplants**

Dominy, Katherine M.[1]; Roufosse, Candice[1,2]; de Kort, Hanneke[1]; Willicombe, Michelle[3]; Brookes, Paul[4]; Behmoaras, Jacques V.[1]; Petretto, Enrico G.[5]; Galliford, Jack[3]; Choi, Peter[3]; Taube, David[3]; Cook, H. Terence[1,2]; Mclean, Adam G.[3]

Transplantation: September 2015 - Volume 99 - Issue 9 - p 1981–1988

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Hypothesis

- In biopsies with glomerulitis, the AMR related gene signature identifies cases with an AMR component

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Methods

- 241 biopsies, graded using Banff 2015 classification
  - Control (n=135): patients without rejection or GN
  - AMR (n=76): active AMR (n=54)+chronic active AMR (n=22)
  - GN (n=20): IgA nephropathy (n=16), IgM nephropathy (n=1), membranous GN (n=1), ANCA vasculitis (n=1), Hepatitis C GN (n=1)
  - AMR+GN (n=8): IgA nephropathy (n=4), C3 GN (n=2), membranous GN (n=1), post-infectious GN (n=1)

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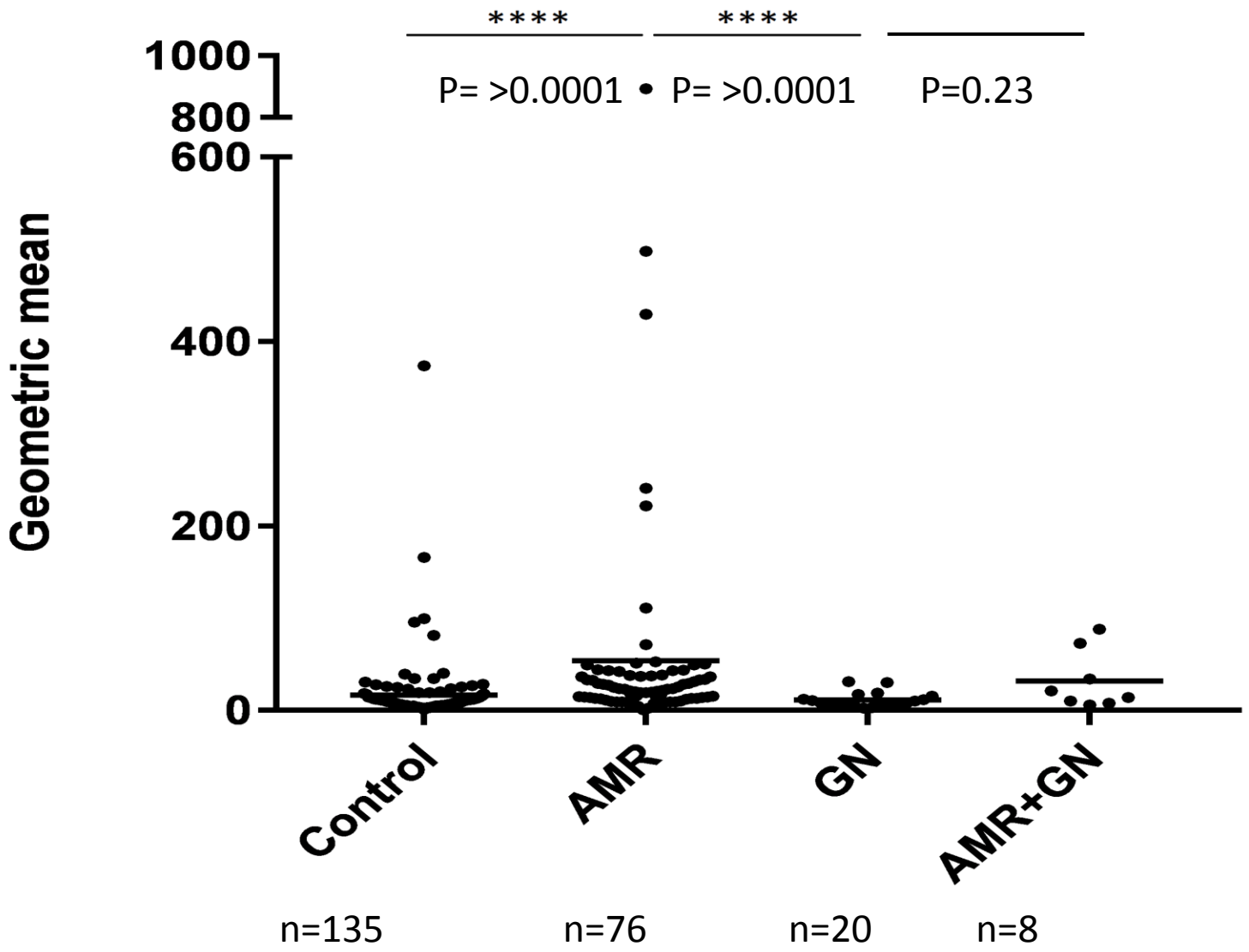
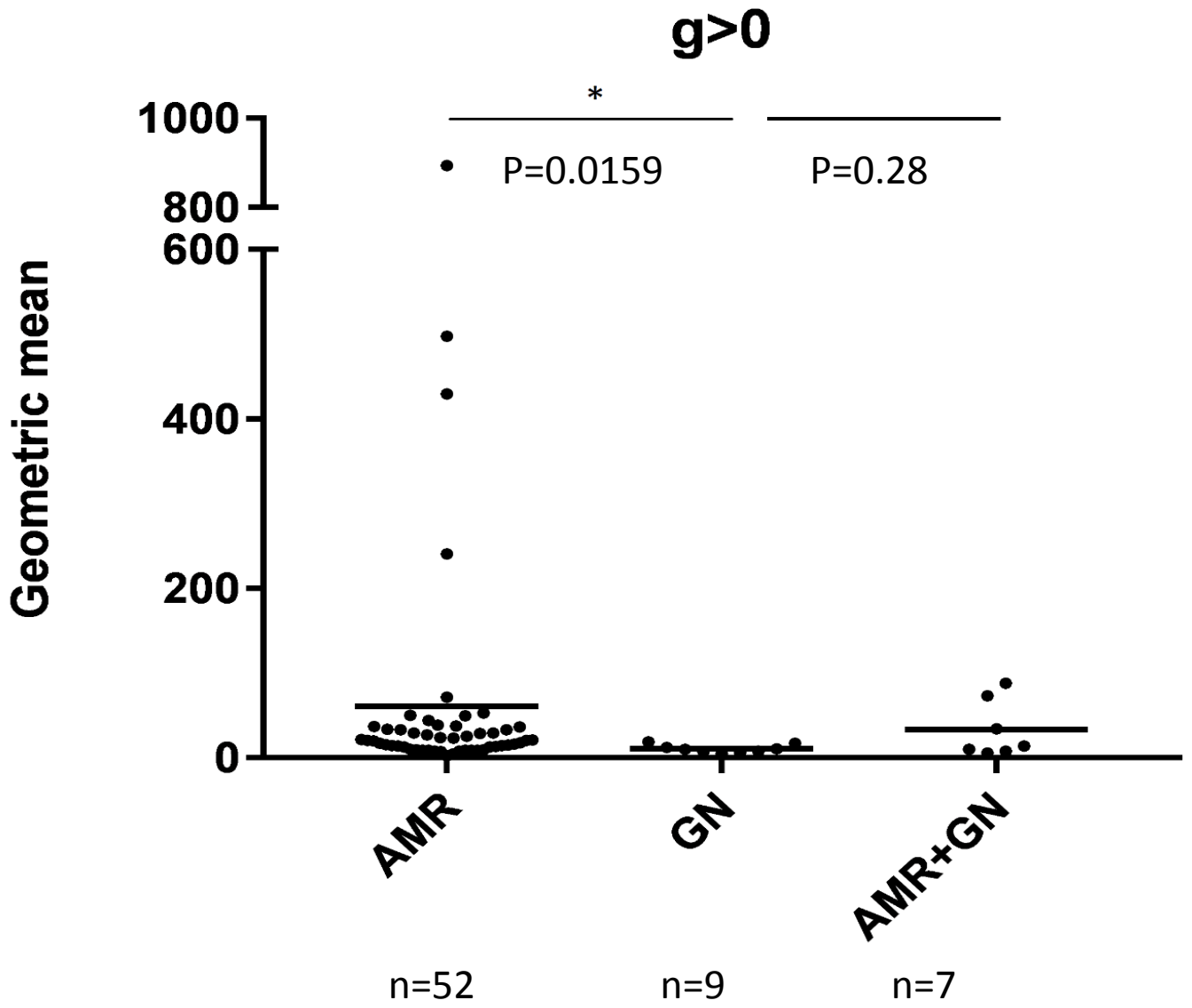
Gene selection

- 6 AMR-related gene transcripts, previously shown to be strongly statistically different between control and AMR groups
  - *DARC, PECAM1->endothelial*
  - *KLRF1, MYBL1, FGFBP2, SH2D1b->NK*
- ΔΔCT method
- Calculation of geometric mean
- Kruskal-Wallis test

Results


****
****
P= >0.0001
P= >0.0001
P=0.23
1000
800
600
400
200
0
Geometric mean
Control
AMR
GN
AMR+GN
n=135
n=76
n=20
n=8

Results


g>0
*
P=0.0159
P=0.28
Geometric mean
1000
800
600
400
200
0
AMR
GN
AMR+GN
n=52
n=9
n=7

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Conclusion

- Confirms qRT-PCR of AMR transcripts as a potential diagnostic tool in the diagnosis of AMR
- AMR-related transcripts are not increased in GN
- Glomerular inflammation is likely mediated by different mechanisms comparing AMR and GN
- Increased AMR-related transcripts in cases with GN may complement histology to help identify cases where both events are occurring

Acknowledgments

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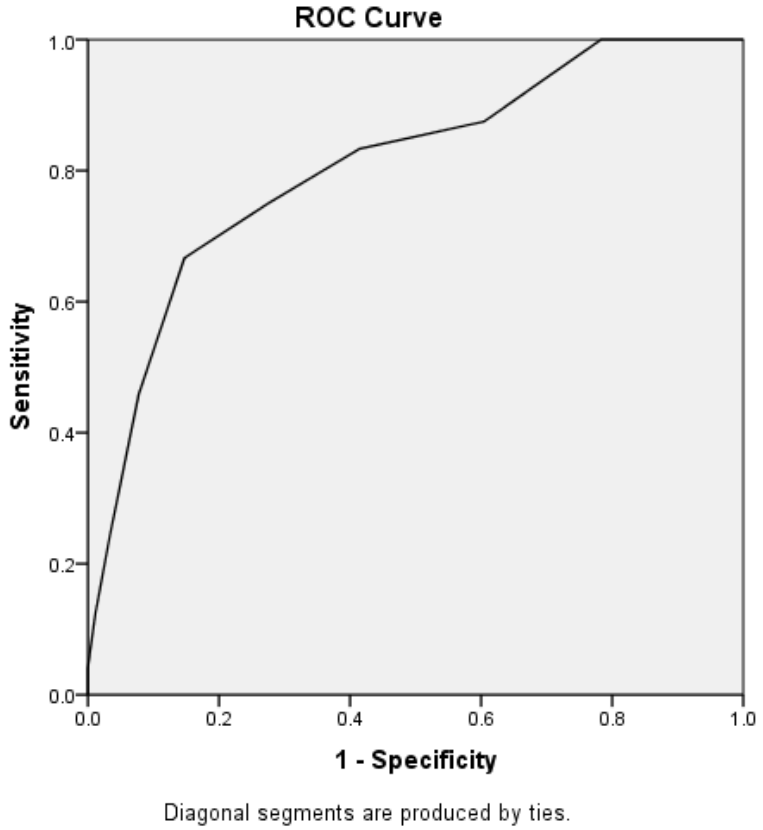
AMR+GN

Patient no	GN	DSA	g	Ptc	MI	C4d	Geometric mean
1	IgA	O	3	0	3	1	5.912
2	C3 GN	0	2	0	2	2	7.847
3	C3 GN	0	3	0	3	3	10.396
4	IgA	Yes	2	1	3	1	73.044
5	IgA	Yes	2	1	3	0	88.249
6	Post inf	0	3	2	5	0	13.945
7	IgA	Yes	3	3	6	0	34.323
8 (no g)	Mb	Yes	0	3	3	3	21.47

N= 282 transplant biopsies, wide range of diagnoses

Sum risk from 8 transcripts
ENDAT = vWF, DARC, PECAM1, CDH5
NK = GNLY, MYBL, SH2D1B, KLRF

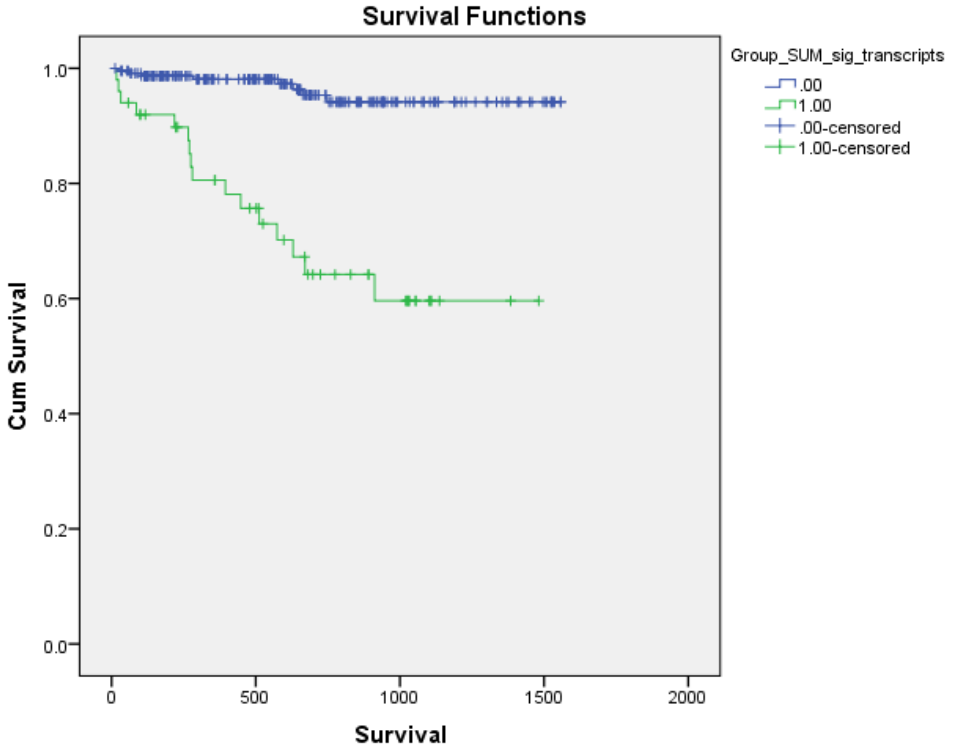
Outcome = graft loss



>4 risk transcripts = high risk group

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	38.886	1	.000



Genes

- MYBL1- Strong transcriptional activator, could have a role in the proliferation and/or differentiation of neurogenic, spermatogenic and B-lymphoid cells
- DARC- duffy antigen/chemokine receptor- the Duffy antigen has been found to act as a multispecific receptor for chemokines of both the C-C and C-X-C families
- KLRF1- killer cell lectin like receptor F1- codes for several lectin-like receptor genes expressed by NK cells as well as by other hematopoietic cells
- SH2D1b-EAT2- regulates signal transduction through receptors expressed on the surface of antigen-presenting cells
- PECAM1- Platelet endothelial cell adhesion molecule-key role in removing aged neutrophils from the body
- FGFBP2- fibroblast growth factor binding protein 2-serum protein that is selectively secreted by cytotoxic lymphocytes