

Donor-specific antibody is associated with increased expression of rejection transcripts in renal transplant biopsies classified as no rejection

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Supplementary Material

Table of Contents

Supplementary Table 1. Participating Centers 3

Supplementary Table 2. List of abbreviations and their definitions used in the manuscript..... 4

Supplementary Table 3. Demographics and clinical features of the 1679 biopsy cohort and 1012 No Rejection only biopsy cohort 5

Supplementary Table 4. Histologic diagnoses and DSA status in the kidney 1679 cohort, and in the 1012 no rejection cohort (N, % of total) 6

Supplementary Table 5. DSA detail in full population (1394 tested of 1679 total) and in NR biopsies (835 tested of 1012 total). 7

Supplementary Table 6. Mean PBT scores in DSA+ vs DSA- biopsies within archetypally-assigned NR, ABMR, and TCMR groups. 8

Supplementary Figure 1. Venn diagram showing relationships between A) histologically and B) molecularly defined NR (blue) and ABMR (purple), the DSAprob score >0.47 (orange), and the ABMRpm score >0.2 (green)..... 9

Supplementary Table 1. Participating Centers

Location	Principal investigators	Samples contributed
Birmingham, AL, USA	Roslyn Mannon	28
Barcelona, Spain	Daniel Serón and Joana Sellarés	71
Bronx, NY, USA	Enver Akalin	33
Manchester, UK	Declan de Freitas and Michael Picton	39
Baltimore, MD, USA	Jonathan Bromberg and Matt Weir	72
Berlin, Germany	Klemens Budde and Timm Heinbokel	12
Hannover, Germany	Gunilla Einecke	67
Harrisburg, PA, USA	Harold Yang and Seth Narins	12
Detroit, MI, USA	Milagros Samaniego-Picota	1
Paris, France	Carmen Lefaucheur, Alexandre Loupy	212
Poland	Marek Myslak and Agnieszka Perkowska-Ptasinska	2
San Antonio, TX, USA	Adam Bingaman	83
St Louis, MO, USA	Daniel Brennan and Andrew Malone	20
Minneapolis, MN, USA	Bertram Kasiske	6
Edmonton, AB, CA	Philip F Halloran	487
Minneapolis, MN, USA	Arthur Matas	76
Madison, WI, USA	Arjang Djamali	10
Vienna, Austria	Georg Böhmig and Farsad Eskandary	198
Richmond, VA, USA	Gaurav Gupta	250
TOTAL		1679

Supplementary Table 2. List of abbreviations and their definitions used in the manuscript	
Abbreviation	Definition
ABMR	antibody-mediated rejection
ABMRpm	ABMR classifier (ABMR, [C4d-negative and C4d-positive] vs. everything else)
AUC	area under the curve
DSA	donor-specific HLA antibody
DSA _{Prob}	DSA probability
eGFR	estimated glomerular filtration rate
INTERCOMEX	Diagnostic and Therapeutic Applications of Microarrays in Organ Transplantation (ClinicalTrials.gov Identifier: NCT01299168)
EABMR	early-stage ABMR
FABMR	fully-developed ABMR
LABMR	late-stage ABMR
MMDx	Molecular Microscope [®] Diagnostic System
NR	no rejection
PCA	principal component analysis
PC1	principal component 1
PC2	principal component 2
PC3	principal component 3
SOC	standard-of-care
TCMR	T cell-mediated rejection
UMAP	uniform manifold approximation and projection

Supplementary Table 3. Demographics and clinical features of the 1679 biopsy cohort and 1012 No Rejection only biopsy cohort

Patient Demographics		All patients (N=1381)	NR patients (N=894)
Mean recipient age (range)		51 (8 – 91)	52 (19 – 91)
Recipient Gender Male (% male)		702 (51%)	524 (64%)
Ethnicity	Caucasian	607	423
	Black	171	120
	Other	147	101
	Not available ^a	456	250
Primary Disease	Diabetic nephropathy (DN)	211	153
	Hypertension / large vessel disease	94	77
	Glomerulonephritis / vasculitis (GN)	47	258
	Interstitial nephritis / pyelonephritis	26	62
	Polycystic kidney disease	125	99
	Others	767	78
	Unknown etiology	111	167
Mean donor age (range)		44 (0.03 – 85)	45 (3 – 85)
Donor gender (% male)		386 (28%)	297 (46%)
Donor type (% deceased donor transplants)		889 (64%)	608 (70%)
Latest kidney status (% of total)	Functioning graft	964 (70%)	684 (77%)
	Graft failure/return to dialysis	208 (15%)	116 (13%)
	Patient death with functioning graft	26 (6%)	16 (2%)
	Mean (median) follow-up (functioning grafts) in days	651 (285)	805 (493)
Biopsy data		All biopsies (N=1679)	NR biopsies (N=1012)
Median time of biopsy post-transplant in days (range)		650 (1 – 12371)	375 (1 – 11453)
Early biopsies (< 1 year) (% total)		709 (42%) ^b	496 (49%)
Late biopsies (≥ 1 year) (% total)		966 (57%) ^b	516 (31%)
Indication for biopsy (% of total)	Primary non-function	10 (1%)	10 (1%)
	Rapid deterioration of graft function	292 (17%)	145 (14%)
	Slow deterioration of graft function	307 (18%)	189 (19%)
	Stable impaired graft function	92 (5%)	72 (7%)
	Investigate proteinuria/rejection/BK/creatinine	247 (15%)	284 (28%)
	Delayed graft function	74 (4%)	57 (6%)
	Others	617 (37%)	233 (23%)
	Indication not specified	40 (2%)	22 (2%)

Abbreviations: NR, no rejection. DN, diabetic nephropathy. GN, glomerulonephritis. BK, polyoma virus.

^aSome centers preferred not to identify ethnicity

^bFour biopsies had no provided date of transplant

Supplementary Table 4. Histologic diagnoses and DSA status in the kidney 1679 cohort, and in the 1012 no rejection cohort (N, % of total)

			All biopsies		NR biopsies	
			N (% of total=1679)	DSA+ N (% of known DSA per row)	N in NR (% of total = 1012)	DSA+ in NR: N (% of known DSA per row)
Histologic Rejection N = 612 (36% of all diagnoses)	ABMR-related	ABMR	333 (20%)	219 (74%)	78 (8%)	46 (68%)
		Transplant glomerulopathy	51 (3%)	11 (27%)	20 (2%)	3 (18%)
		ABMR suspected	33 (2%)	8 (30%)	14 (1%)	2 (18%)
	Mixed (TCMR plus ABMR)		56 (3%)	28 (60%)	3 (0%)	1 (33%)
	TCMR ^a		139 (8%)	32 (29%)	42 (4%)	8 (21%)
Borderline			128 (8%)	32 (28%)	91 (9%)	22 (27%)
Histologic No Rejection N = 939 (56% of all diagnoses)	AKI		117 (7%)	30 (33%)	108 (11%)	26 (31%)
	BK		52 (3%)	5 (12%)	28 (3%)	2 (9%)
	Diabetic Nephropathy		24 (1%)	7 (54%)	19 (2%)	5 (56%)
	Glomerulonephritis		108 (6%)	27 (34%)	84 (8%)	19 (31%)
	IFTA not otherwise specified		193 (11%)	49 (30%)	160 (16%)	38 (28%)
	No major abnormalities		371 (22%)	113 (35%)	311 (31%)	88 (33%)
	Others ^b		74 (4%)	15 (29%)	54 (5%)	11 (31%)
	All NR excluding Borderline		939 (56%)	246 (32%)	764 (75%)	189 (31%)
Patient HLA antibody status at DSA at time of biopsy (N = 1679)			N (% of N=1394 tested)		N in NR (% of N=835 tested)	
DSA positive			576 (41%)		271 (32%)	
DSA negative, PRA positive			277 (20%)		175 (21%)	
DSA negative, PRA unknown			148 (11%)		92 (11%)	
PRA negative/DSA negative			393 (28%)		297 (36%)	
DSA not tested			264		163	

Abbreviations: ABMR, antibody-mediated rejection. AKI, acute kidney injury. BK, polyoma virus. DSA, donor specific antibody. HLA, human leukocyte antigen. IFTA, interstitial fibrosis and tubular atrophy. NR, no rejection. PRA, panel reactive antibody. TCMR, T cell-mediated rejection.

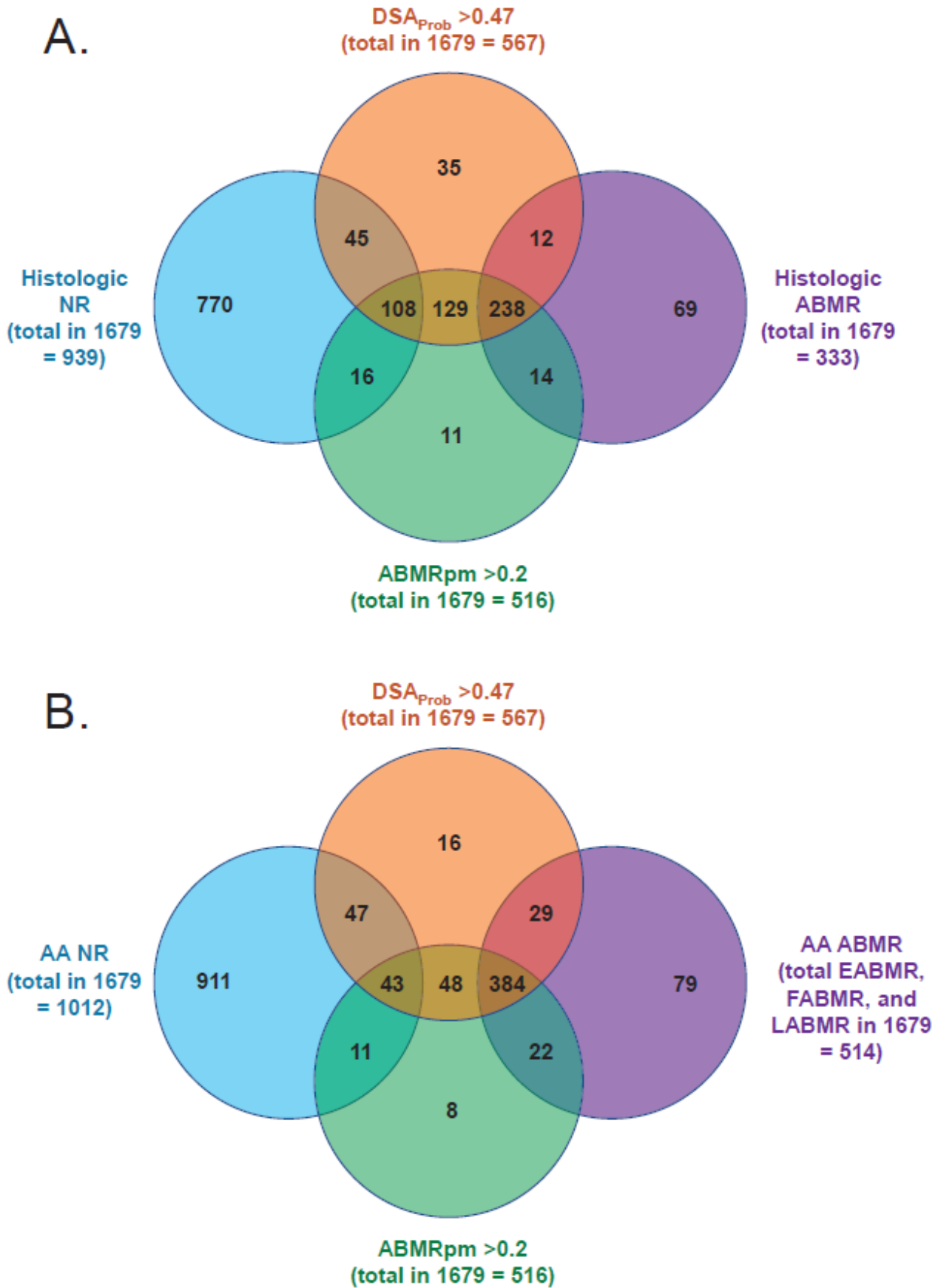
^aThree biopsies had histology diagnoses of both TCMR and BK virus - we have categorized these as TCMR in this table and throughout the paper.

^bOthers includes calcineurin inhibitor toxicity, C4d deposition without morphologic evidence for active rejection, donor origin vascular disease, pyelonephritis, systemic infection/diarrhea, and bacterial infection.

Supplementary Table 5. DSA detail in full population (1394 tested of 1679 total) and in NR biopsies (835 tested of 1012 total).

	All biopsies N (% of N=1394 tested)	NR biopsies N (% of N=835 tested)
DSA Class I	74 (5%)	35 (4%)
DSA Class I/II	113 (8%)	44 (5%)
DSA Class II	191 (14%)	97 (12%)
DSA Negative	818 (59%)	564 (68%)
DSA Positive	198 (14%)	95 (11%)
Not done/Not available	285	163

Supplementary Table 6. Mean PBT scores in DSA+ vs DSA- biopsies within archetypally-assigned NR, ABMR, and TCMR groups.							
		NR (N=1012, 835 with DSA data)		ABMR (N=514, 436 with DSA data)		TCMR (N=116, 94 with DSA data)	
		DSA+ (N=271)	DSA- (N=564)	DSA+ (N=262)	DSA- (N=174)	DSA+ (N=26)	DSA- (N=68)
TCMR	QCAT	1.54	1.51	2.43	2.45	4.81	4.82
	TCB	1.90	1.85	3.07	3.14	8.12	7.99
	TCMR-RAT	1.47	1.44	2.18	2.19	5.25	4.98
ABMR	DSAST	1.06 ^a	1.03	1.58 ^b	1.44	1.19 ^a	1.12
	NKB	1.30 ^a	1.26	2.32 ^a	2.09	1.78	1.80
	ABMR-RAT	1.40 ^b	1.30	2.64 ^a	2.42	3.10	2.96
Rejection	GRIT1	1.37 ^a	1.31	1.96	1.90	2.62	2.66
	Rej-RAT	1.65 ^a	1.51	3.37 ^a	3.13	5.46	5.24
Macrophages	AMAT1	1.32	1.30	1.74	1.75	2.62	2.53
	QCMAT	1.24	1.23	1.55	1.54	2.44	2.36
Recent injury	IRITD3	1.01	1.02	1.06	1.07	1.15	1.15
	IRITD5	1.23	1.25	1.32 ^a	1.36	1.55	1.54
	IRRAT30	1.17	1.17	1.32	1.37	2.03	2.21
Atrophy-fibrosis	IGT	1.60	1.48	2.42	2.24	5.62	3.56
^a t-test <i>P</i> value of DSA+ vs. DSA- <0.05 ^b t-test <i>P</i> value of DSA+ vs. DSA- <0.001							



Supplementary Figure 1. Venn diagram showing relationships between A) histologically and B) molecularly defined NR (blue) and ABMR (purple), the DSA_{prob} score >0.47 (orange), and the ABMR_{pm} score >0.2 (green). Cutoffs are the same as those used in Figure 3. Overlaps are labeled with the number of biopsies that fit those criteria.