Supplemental material

Table of contents

Supplemental Table S1. Graft function after transplant according to C3d-fixing DSA status. Shown are the proteinuria (g/l) and serum creatinine (µmol/l) at month 3, year 1, year 3, year 5 and year 10 after transplant. Number of values indicates the number of transplants used in the calculation, as some transplants are lost due to graft failure, patient death or lost to followup.

	non-C3d-fixing DSA (N=470)			C3d-fixing DSA (N=97)			
	Mean	SD	Number of values	Mean	SD	Number of values	P-value*
Proteinuria (g/l), Month 3	0.53	1.75	401	0.85	3.92	86	0.473
Proteinuria (g/l), Year 1	0.58	3.76	375	0.33	0.58	81	0.414
Proteinuria (g/l), Year 3	0.37	0.88	327	0.41	0.72	71	0.077
Proteinuria (g/l), Year 5	0.40	0.83	289	0.49	0.69	67	0.152
Proteinuria (g/l), Year 10	0.46	0.89	173	0.44	0.90	32	0.300
Serum Creatinine (µmol/l), Month 3	191	176	433	199	190	89	0.563
Serum Creatinine (µmol/l), Year 1	153	99	397	161	100	86	0.649
Serum Creatinine (µmol/l), Year 3	160	136	351	168	117	76	0.704
Serum Creatinine (µmol/l), Year 5	159	112	324	183	129	71	0.049
Serum Creatinine (µmol/l), Year 10	153	80	207	168	138	41	0.983

**Mann-Whitney U test for continuous variables*

Supplemental Table S2. Comparison of our study to all published studies that used the Lifecodes C3d assay (Immucor) to determine C3d-fixing DSA in kidney transplant patients.

Reference	Patient population	Patient sera	IgG-DSA positivity cut-off	C3d-DSA positivity cut-off	Patients with C3d-fixing DSA
This study	567 transplants with pretransplant DSA	Pretransplant	*Immucor LSA*: According to manufacturer (if two of the three values are above a lot-specific cut-off (LSA1: BCM>1500, BCR>3 and/or AD-BCR>4 and LSA2: BCM>1500, BCR>4 and/or AD-BCR>5).	According to manufacturer	97/567=17%
(Comoli et al. 2016)	39 unsensitized pediatric patients who developed de novo DSA	Posttransplant	*One Lambda LSA*: MFI>1000	According to manufacturer	9/39=23%
(Kim et al. 2017)	65 pediatric transplants with de novo DSA	Posttransplant screening; first DSA-positive serum was further tested with C3d assay	*One Lambda LSA*: No threshold MFI for positive DSA was set as an a priori criteria *Immucor LSA*: unknown	According to manufacturer	23/65=35%
(Sicard et al. 2015)	69 transplants with AMR	Time of biopsy with AMR	*Immucor LSA*: MFI>500 and AD-BCR>5	According to manufacturer	40/69=58%
(Pelletier et al. 2017)	265 recipeints with posttransplant DSA	Posttransplant screening	*Immucor LSA: MFI>2000*	According to manufacturer	179/265=67.5%

Supplemental methods: LIFECODES C3d detection

567 serum samples with known DSA were analyzed for the presence of C3d-fixing DSA with the modified SAB assays from Immucor Lifecodes Transplant Diagnostics, Herentals, Belgium. We reduced the reagents to 50% of what the manufacturer recommends. Briefly, 20 µl of the SAB beads (LSA class I or II, Immucor) were incubated with 5 µl of patient serum or control serum in the dark for 30 minutes on a shaking platform in an incubator at 21°C. After 30 minutes 15 µl of complement serum was added. After the 30 minute incubation 100 µl washbuffer was added and plate was aspirated. Next, plates were washed four times using 250 µl wash buffer to remove unbound antibody and 25 µl PE-conjugated anti-human C3d antibody was added. After 30 minute incubation on a shaking platform in an incubator at 21°C, 100 µl of wash buffer was added and plate was aspirated. Samples were washed one more time with 250 µl wash buffer. Finally, each sample was resuspended in 100 µl wash buffer and analyzed on the Luminex platform.

We defined beads as positive according to manufacturers' recommendations. Briefly, a bead is considered positive if two or more values are above the cutoff values. For the lot that we used (03146A) these were for the BG Adjusted MFI, BCR-Neg and R-strength 1500, 4 and 4 respectively. These values are calculated as followed:

A. Subtract the MFI values of the Negative Control serum (MFI of NC serum) from the raw MFI for each individual bead to calculate the Background Adjusted MFI (BG Adjusted MFI):

$$(1)\ BG\ Adjusted\ MFI = Raw\ MFI\ of\ bead - MFI\ of\ NC\ serum$$

B. Then, divide the BG Adjusted MFI by the MFI of the Calculated Control (CalcCON) of its respective locus to generate the background corrected ratio (BCR-Neg). The CalcCON for each locus is the raw MFI value of the lowest ranked antigen bead for that locus.

$$(2)\ BCR - Neg = \frac{BG\ Adjusted\ MFI}{\text{raw MFI value of the lowest ranked antigen}}$$

C. Last, divide the BG Adjusted MFI of antigen by the corresponding MFI value of the antigen for the LSA NC serum to generate the relative strength (R-strength).

$$(3)\ R - strength = \frac{BG\ Adjusted\ MFI}{raw\ MFI\ of\ antigen}$$