Supplementary Digital Content

C1q single antigen bead assay

Detection of complement-binding antibodies was performed using SAB and C1q screen kits (One Lambda) according to manufacturer's instructions. Samples were analyzed on a Luminex platform (Labscan 100) and data were analyzed with the Fusion software (One Lambda). Sera were inactivated by heating for 30 min at 56 °C, spiked with the complement component C1q, and incubated with 5µL of antigen-coated beads for 20 min at room temperature. Samples were subsequently incubated with 5 µL of phycoerythrin-labeled anti-C1q antibody for 20 min at room temperature, washed twice with 80 µL of wash buffer, and measured using the Luminex assay.

C3d single antigen bead assay

An aliquot (40 µL) of beads bound to HLA antigens was incubated with a small volume of the test serum sample (10 µL). After this initial step, the negative serum reagent (30 µL) was added as a source of complement for an additional incubation. The sensitized beads were washed to remove the unbound antibody. An anti-human C3d antibody conjugated to phycoerythrin was subsequently added (50 µL). After another incubation step, the test sample was washed, diluted, and analyzed using the Luminex instrument. For the test sample, the signal intensity from each bead preparation was compared to the signal intensity of negative control sera to determine if the sample was positive or negative for C3d bound to the antibody/antigen complex.

Detection of intragraft DSA

Elution of the anti-HLA antibody was performed using nonfixed frozen material from biopsy samples using the Gamma ELU-KIT ™ II (lots 426 and 450; Immucor Inc., Norcross, GA, USA). The following steps were performed: (i) defrosting, crushing and grinding the biopsy sample for maximal exposure, (ii) washing with 1 mL of phosphate buffered saline (PBS) and centrifugation at 10 000 rpm for 2 min. The washing procedure was performed 3 times to remove the recipient's biological fluids. The fourth and last wash was performed using 0.1 mL of PBS followed by a centrifugation step. The supernatant was collected and screened for anti-HLA antibodies to remove any contamination from the recipient's blood or extracellular fluid. Washes were continued until anti-HLA antibody screening turned negative. When the test yielded negative results, the pellet was recovered and resuspended in 0.1 mL of acid elution buffer (glycine solution at pH 2.1), incubated at room temperature for 15 min, and then centrifuged at 10 000 rpm for 2 min. The eluate was collected and neutralized with a buffer

solution (tris[hydroxymethyl]aminomethane solution at pH 8.5) until a blue solution of pH 6.8 was obtained. The sample was stored at −80 °C until analysis. All experiments were performed by the same person. Several steps in the elution process might have led to variations in the results, including: (i) the amount of histological material, (ii) pH, (iii) and the examination of the last supernatant. To assess the effect of material amount on results, we studied 12 negative and 12 positive controls, varying the size and the number of glomeruli in the assessed biopsies (data not shown). The results demonstrated an increased MFI value for positive controls when the size and/or number of glomeruli were increased, whereas MFI values of negative controls did not change. We also demonstrated that a small-sized biopsy specimen (<1 mm) could lead to false-negative results. A sample size < 1 mm was considered insufficient for our study. Further, acidity can alter the conformation of the antigen (Ag) fixed on the bead and reveal cryptic Ags that are not accessible in vivo, which may be a source of SAB false-positive results (data not shown). We confirmed that a mixture of acid buffer and neutralizing solution does not result in the denaturation of HLA Ag on SAB, similar to previous data (13) showing that acid treatment with subsequent neutralization does not lead to the denaturation of anti-HLA Abs. To limit the effect of pH on the results, it was adjusted to 6.8 ± 0.3 (as determined by the eluates of positive controls). To mitigate contamination of the eluate due to insufficient washing, we systematically screened the last water wash of all biopsies. If negative, contamination was deemed absent.

Table S1: Comparison of the characteristics of patients according to delay of occurrence of DSA: de novo vs. persistent preexisting DSA

	Patients with de novo DSA N = 59	Patients with persistent preexisting DSA N = 27	p
Age at transplantation (years)	41± 15.8	43 ±11.3	0.614
Sex (F/M)	28/31	11/16	0.705
Sensitization before transplantation			
Pregnancies (among F)	19 (67.8%)	7 (63.6%)	0.517
Blood transfusions	17 (28.8%)	14 (51.8%)	0.041
Previous transplantation	1 (1.7%)	11 (40.7%)	<0.001
Donor: deceased/ living	56 (94.9%)/3	26 (96.2%) /1	0.773
Blood transfusions after transplantation	23 (38.9%)	12 (44.4%)	0.633
Immunosuppression			
ATG (vs. anti-IL2R)	45 (76.2%)	26 (96.2%)	0.011
Tacrolimus (vs. cyclosporine)	9 (15.2%)	12 (44.4%)	<0.001
MPA (vs. azathioprine)	53 (89.8%)	25 (92.5%)	0.932
DSA characteristics			
hsDSA class II (vs. class I)	51 (86.4%)	21 (77.7%)	0.530
hsDSA MFI	10378 ± 1844	6291 ± 2737	0.428
hsDSA > 5000 (vs. < 5000)	45 (76.2%)	16 (59%)	0.276
hsDSA C1q+*	32(55.2%)	13 (48%)	0.546
hsDSA C3d+	30 (50.8%)	12 (44.4%)	0.690
gDSA+	44 (80%)	15 (57.6%)	0.065

**One patient had uninterpretable data for C1q, 5 patients had uninterpretable data for gDSA. ATG, anti-thymocyte globulin; anti-IL2R, interleukin-2 receptor antibodies; MPA, mycophenolic acid; DSA, donor specific antibody; hsDSA $C1q^+$, highest MFI of donor-specific antibody in the serum able to fix C1q; hsDSA $C1q^-$, highest MFI of donor-specific antibody in the serum unable to fix C1q; hsDSA, highest MFI of donor-specific antibody in the serum; MFI: mean fluorescence intensity. Data are presented as means ± SD or N (%) as appropriate*

Table S2: DSA loci repartition among the 86 patients. Analysis by Luminex SAB, One Lambda®

Loci	Total number of hsDSAs and % among class
A	6 (43)
B	5 (36)
C	3 (21)
DR	10 (16)
DQ	**50 (81)**
DP	2(3)

hsDSA, donor-specific antibody with the highest mean fluorescence intensity (MFI) in serum

Table S3: Loci repartition in patients with C1q-positive DSA (N = 45). Analysis by Luminex C1q, One Lambda

Loci	Number of $C1q^+$ hsDSA*
A	2
B	0
C	1
DR	5
DQ	**36**
DP	1

*hsDSA, donor-specific antibody with the highest mean fluorescence intensity (MFI) in serum; *One result was uninterpretable for C1q fixation*

Table S4: Characteristics of patients according to hsDSA C1q positivity

	Patients with C1q⁺ DSA N = 45	Patients with C1q⁻ DSA N = 40	p
Age at transplantation (years)	39.1 ± 14.9	44.5 ± 14.2	0.093
Sex (F/M)	20/25	22/18	0.331
Sensitization before transplantation			
Pregnancies (among F)	12/20 (60%)	17/22 (77%)	0.142
Blood transfusions	17 (37.8%)	14 (35%)	0.790
Previous transplantation	3 (6.7%)	9 (22.5%)	0.034
Donor: deceased/ living	42 (93.3%)/3 (6.7%)	39 (97.5)/1 (2.5)	0.353
Blood transfusions after transplantation	18 (40%)	17 (42.5%)	0.815
Immunosuppression			
ATG (vs. anti-IL2R)	40 (89%)	30 (75%)	0.092
Tacrolimus (vs. cyclosporine)	9 (20%)	12 (31.6%)	0.227
MPA (vs. azathioprine)	34 (75.6%)	33 (86.8%)	0.188
DSA characteristics			
De novo DSA (vs. preformed)	32 (71.1%)	26 (65%)	0.546
hsDSA class II (vs. class I)	42 (93.3%)	29 (64%)	0.008
hsDSA MFI	15 951 ± 4664	3861 ± 2840	< 0.001

**One patient had uninterpretable data for C1q. ATG, anti-thymocyte globulin; anti-IL2R, interleukin 2 receptor antibodies; MPA, mycophenolic acid; DSA, donor specific antibody; hsDSA C1q⁺, highest MFI of donor-specific antibody in the serum able to fix C1q; hsDSA C1q⁻, highest MFI of donor-specific antibody in the serum unable to fix C1q; hsDSA, highest MFI of donor-specific antibody in the serum; MFI: mean fluorescence intensity. Data are presented as means ± SD or N (%) as appropriate*

Table S5: Concordance between C1q* and C3d fixation ability for the highest mean fluorescence of donor-specific antibody in the serum.

	C3d+	C3d-	**Total**
C1q⁺	40	5	45
C1q⁻	1	39	40
Total	41	44	85*

**One result was uninterpretable for C1q fixation*

Table S6: Characteristics of patients and DSA according to the presence of AMR in patients with stable graft function at time of the biopsy (subclinical AMR), n = 28.

	Patients with AMR N = 18	Patients without AMR N = 10	p
Age at transplantation (years)	36.7 ± 14.5	45.2 ± 13	0.139
Sex (F/M)	7/11	7/3	0.111
De novo DSA (vs. preformed)	17 (94.4)	6 (60)	0.024
Class II hsDSA (vs. class I)	17 (94.4)	5 (50)	0.018
hsDSA MFI	9502 ± 6608	5632 ± 6064	0.139
hsDSA > 5000 (vs. < 5000)	13 (72.2)	3 (30)	0.029
hsDSA C1q+*	8 (44)	2 (20)	0.185
hsDSA C3d+	9 (50)	2 (20)	0.110
gDSA+	13 (72.2)	4 (40)	0.105

*AMR, antibody-mediated rejection; DSA, donor-specific antibody; hsDSA, highest MFI of donor-specific antibody in the serum; hsDSA C1q$^+$, highest MFI of donor-specific antibody in the serum able to fix C1q; MFI, mean fluorescence intensity; hsDSA C3d$^+$, highest MFI donor-specific antibody in the serum able to fix C3d; gDSA: intragraft DSA. Data are presented as means ± SD or N (%) as appropriate. *One result of C1q fixation was uninterpretable.*

Table S7: Characteristics of patients and DSA according to the presence of AMR in presence of graft dysfunction at time of the biopsy (N = 58).

	Patients with AMR N = 45	Patients without AMR N = 13	p
Age at transplantation (years)	40.6 ± 16	45.5 ± 11.3	0.310
Sex (F/M)	20/25	8/5	0.276
De novo DSA (vs. preformed)	31 (86.1)	5 (13.9)	0.049
Class II hsDSA (vs. class I)	38 (84.4)	12 (92.3)	0.591
hsDSA MFI	12 518 ± 7216	6609 ± 5843	0.009
hsDSA > 5000 (vs. < 5000)	33 (73.3)	6 (46.1)	0.072
hsDSA C1q+*	31 (68.9)	4 (30.8)	0.010
hsDSA C3d+	28 (62.2)	3 (23.1)	0.020
gDSA+	35 (77.7)	7 (53.8)	0.080

*AMR, antibody-mediated rejection; DSA, donor-specific antibody; hsDSA, highest MFI of donor-specific antibody in the serum; hsDSA C1q$^+$, highest MFI of donor-specific antibody in the serum able to fix C1q; MFI, mean fluorescence intensity; hsDSA C3d$^+$, highest MFI donor-specific antibody in the serum able to fix C3d; gDSA: intragraft DSA. Data are presented as means ± SD or N (%) as appropriate. *One result of C1q fixation was uninterpretable.*

Table S8: Histological lesions of AMR according to DSA MFI, C1q and C3d fixation, n = 86

Banff score	hsDSA MFI < 5000	hsDSA MFI > 5000	*p*	DSA C1q$^+$	DSA C1q$^-$	*p*	DSA C3d$^+$	DSA C3d$^-$	p
t	0.55 ± 0.74	0.39 ± 0.67	0.327	0.58 ± 0.69	0.4 ± 0.74	*0.257*	0.50 ± 0.67	0.44 ± 0.73	0.704
i	0.93 ± 0.88	0.59 ± 0.87	0.097	**1.03 ± 0.81**	**0.59 ± 0.91**	***0.031***	0.97 ± 0.85	0.63 ± 0.89	0.103
ti	1.02 ± 0.99	0.90 ± 0.91	0.596	1.04 ± 0.95	0.92 ± 0.97	*0.569*	0.95 ± 0.94	0.98 ± 0.99	0.907
g	**1.91 ± 1.12**	**1.33 ± 1.24**	**0.040**	1.89 ± 1.17	1.45 ± 1.20	*0.113*	**2.00 ± 1.16**	**1.43 ± 1.20**	**0.040**
ah	1.70 ± 1.26	1.48 ± 1.32	0.488	1.83 ± 1.21	1.41 ± 1.33	*0.155*	1.94 ± 1.17	1.39 ± 1.32	0.066
aah	1.44 ± 1.36	1.42 ± 1.36	0.956	1.49 ± 1.36	1.40 ± 1.36	*0.764*	1.57 ± 1.38	1.33 ± 1.32	0.404
v	0.04 ± 0.19	0.00 ± 0.00	0.288	0.02 ± 0.15	0.02 ± 0.16	*0.934*	0.02 ± 0.15	0.02 ± 0.15	0.987
cg	1.15 ± 1.39	0.90 ± 1.19	0.418	1.22 ± 1.43	0.85 ± 1.19	*0.198*	1.36 ± 1.43	0.74 ± 1.16	0.032
ci	1.22 ± 0.99	1.03 ± 0.95	0.400	1.27 ± 0.96	1.05 ± 0.99	*0.309*	0.74 ± 1.27	0.42 ± 0.93	0.499
cv	1.43 ± 0.93	1.03 ± 0.94	0.076	**1.53 ± 0.88**	**1.05 ± 0.97**	***0.029***	**1.21 ± 0.98**	**1.07 ± 0.99**	**0.047**
ct	1.20 ± 1.16	1.00 ± 1.06	0.290	1.18 ± 1.17	1.05 ± 1.04	*0.598*	1.52 ± 0.91	1.07 ± 0.96	0.617
mm	0.91 ± 1.11	0.94 ± 0.99	0.399	0.97 ± 1.16	0.69 ± 1.06	*0.277*	1.14 ± 1.16	1.02 ± 1.04	0.122
ptc	1.68 ± 1.23	1.19 ± 1.28	0.097	**1.81 ± 1.22**	**1.15 ± 1.23**	***0.021***	**1.06 ± 1.17**	**0.66 ± 1.04**	**0.021**
IF/TA	1.18 ± 0.96	1.13 ± 0.85	0.800	1.2 ± 0.94	1.15 ± 0.89	*0.803*	1.86 ± 1.22	1.19 ± 1.25	0.921
g+ptc	**3.57 ± 1.98**	**2.48 ± 2.31**	**0.031**	3.72 ±1.94	2.53 ± 2.22	***0.015***	3.85 ± 1.92	2.57 ± 2.32	**0.010**

AMR, antibody-mediated rejection; DSA, donor-specific antibody; MFI, mean fluorescence intensity; hsDSA, highest MFI donor-specific antibody in the serum; DSA C1q$^+$, donor specific antibody able to fix C1q; DSA C1q$^-$, donor-specific antibody unable to fix C1q; DSA C3d$^+$, donor-specific antibody able to fix C3d; DSA C3d$^-$, donor-specific antibody unable to fix C3d; t, tubulitis; i, interstitial infiltration; ti, total interstitial inflammation; g, glomerulitis; ah, arterial hyalinosis; aah, hyaline arteriolar thickening; v, vasculitis; cg, chronic glomerulitis; ci, chronic interstitial infiltration; cv ; chronic vasculitis; ct, chronic tubulitis; mm, mesangial matrix increase; ptc, peritubular capillaritis; IF/TA, interstitial fibrosis/tubular atrophy.

Table S9: Banff scoring according to DSA MFI, C1q and C3d fixation, and gDSA among patients with AMR (N = 63)

Banff score	hsDSA MFI < 5000	hsDSA MFI > 5000	*p*	DSA $C1q^+$	DSA $C1q^-$	*p*	DSA $C3d^+$	DSA $C3d^-$	*p*	gDSA $^+$	gDSA $^-$	p
t	0.35 ± 0.61	0.57 ± 0.72	*0.284*	0.56 ± 0.64	0.43 ± 0.79	*0.484*	0.51 ± 0.65	0.50 ± 0.76	*0.940*	0.52 ± 0.65	0.45 ± 0.93	0.781
i	0.75 ± 1.0	1.03 ± 0.85	*0.307*	1.06 ± 0.77	0.78 ± 1.04	*0.258*	1.03 ± 0.82	0.84 ± 0.99	*0.433*	0.92 ± 0.83	0.82 ± 0.98	0.717
ti	1.06 ± 1.03	1.11 ± 0.99	*0.862*	1.08 ± 0.96	1.17 ± 1.07	*0.714*	1.00 ± 0.94	0.98 ± 0.99	*0.369*	1.06 ± 0.95	1.09 ± 0.94	0.929
g	2.18 ± 0.95	2.30 ± 0.81	*0.632*	2.19 ± 0.95	2.32 ± 0.72	*0.605*	2.27 ± 0.94	1.23 ± 1.07	*0.944*	2.22 ± 0.86	2.45 ± 0.82	0.433
ah	1.94 ± 1.18	1.89 ± 1.16	*0.902*	1.94 ± 1.12	1.87 ± 1.22	*0.838*	2.00 ± 1.10	1.80 ± 1.22	*0.530*	1.80 ± 1.16	2.27 ± 1.01	0.225
aah	1.94 ± 1.27	1.59 ± 1.32	*0.431*	1.56 ± 1.33	1.91 ± 1.24	*0.311*	1.62 ± 1.36	1.73 ± 1.25	*0.747*	**1.50 ± 1.29**	**2.45 ± 1.04**	**0.026**
v	0.00 ± 0.00	0.04 ± 0.21	*0.390*	0.03 ± 0.16	0.04 ± 0.21	*0.707*	0.03 ± 0.16	0.04 ± 0.19	*0.803*	0.02 ± 0.14	0.09 ± 0.30	0.254
cg	1.41 ± 1.28	1.37 ± 1.42	*0.915*	1.41 ± 1.45	1.30 ± 1.30	*0.773*	0.84 ± 1.32	1.15 ± 1.29	*0.275*	1.33 ± 1.39	1.55 ± 1.44	0.652
ci	1.24 ± 0.90	1.39 ± 0.95	*0.561*	1.38 ± 0.94	1.35 ± 0.94	*0.882*	1.30 ± 0.97	1.42 ± 0.90	*0.604*	1.35 ± 0.96	1.36 ± 0.67	0.975
cv	1.13 ± 0.96	1.61 ± 0.82	*0.068*	1.61 ± 0.80	1.26 ± 0.97	*0.150*	1.55 ± 0.87	1.36 ± 0.91	*0.432*	1.50 ± 0.88	1.27 ± 0.79	0.441
ct	1.00 ± 1.06	1.26 ± 1.16	*0.422*	1.23 ± 1.16	1.17 ± 1.11	*0.850*	1.24 ± 1.16	1.12 ± 1.11	*0.663*	1.23 ± 1.17	1.18 ± 1.08	0.903
mm	1.00 ± 1.21	1.08 ± 1.15	*0.821*	1.13 ± 1.18	0.96± 1.15	*0.592*	1.21 ± 1.18	0.88 ± 1.13	*0.305*	0.93 ± 1.10	1.27 ± 1.19	0.364
ptc	2.06 ± 1.08	2.00 ± 1.10	*0.854*	2.06 ± 1.11	1.91 ± 1.08	*0.620*	2.06 ± 1.12	1.96 ± 1.06	*0.724*	2.07 ± 1.07	1.82 ± 1.25	0.502
IF/TA	1.24 ± 0.83	1.33 ± 0.94	*0.728*	1.28 ± 0.94	1.39 ± 0.84	*0.649*	1.19 ± 0.97	1.46 ± 0.81	*0.245*	1.29 ± 0.92	1.36 ± 0.67	0.808
g+ptc	4.24 ± 1.56	4.26 ± 1.37	*0.947*	4.29 ± 1.34	4.13 ± 1.46	*0.684*	4.33 ± 1.47	4.16 ± 1.38	*0.656*	4.27 ± 1.40	4.27 ± 1.55	0.993

AMR ; antibody-mediated rejection; DSA, donor-specific antibody; MFI, mean fluorescence intensity; hsDSA. highest MFI donor-specific antibody in the serum; DSA $C1q^+$, donor-specific antibody able to fix C1q; DSA $C1q^-$, donor-specific antibody unable to fix C1q; DSA $C3d^+$, donor-specific antibody able to fix C3d; DSA $C3d^-$, donor-specific antibody unable to fix C3d; $gDSA^+$, presence of intragraft DSA; $gDSA^-$, absence of intragraft DSA; tubulitis; i, interstitial infiltration; ti, total interstitial inflammation; g, glomerulitis; ah, arterial hyalinosis; aah, hyaline arteriolar thickening; v, vascularitis; cg, chronic glomerulitis; ci, chronic interstitial infiltration; cv, chronic vacularitis; ct, chronic tubulitis; mm, mesangial matrix increase; ptc, peritubular capillaritis; IF/TA, interstitial fibrosis/tubular atrophy.

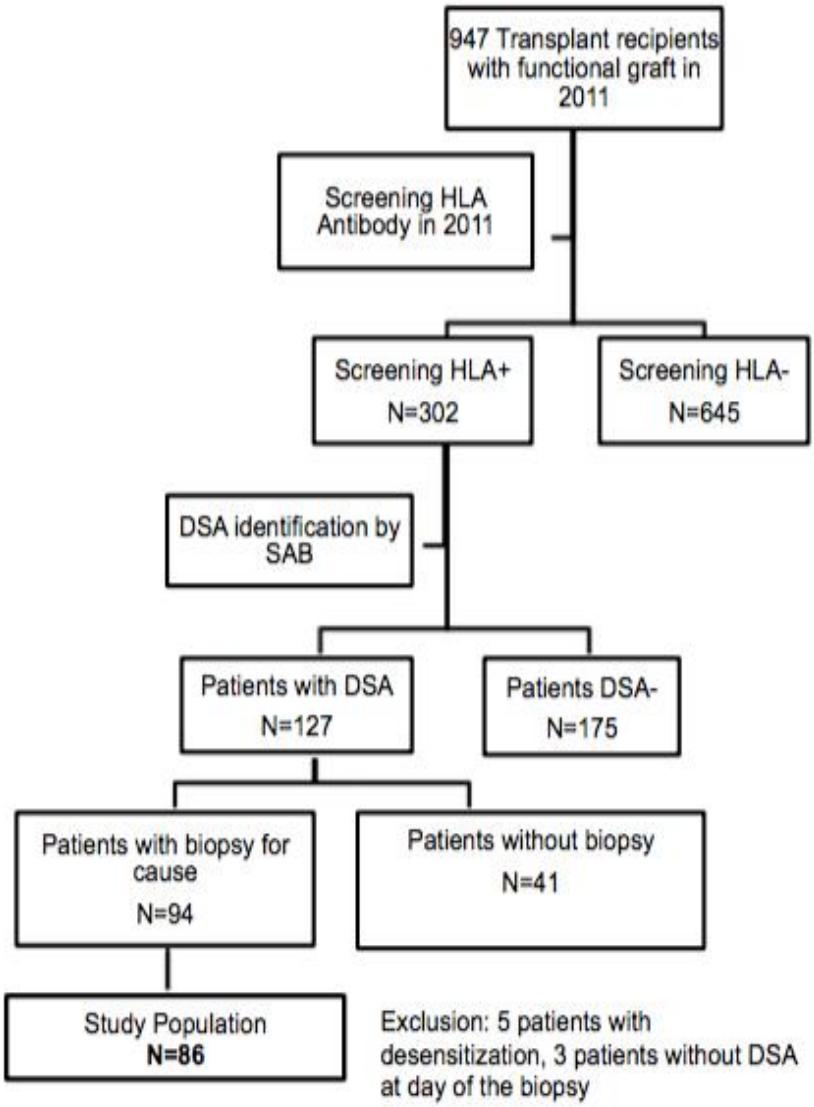

Figure S1: Study population flow chart DSA; donor specific antibody

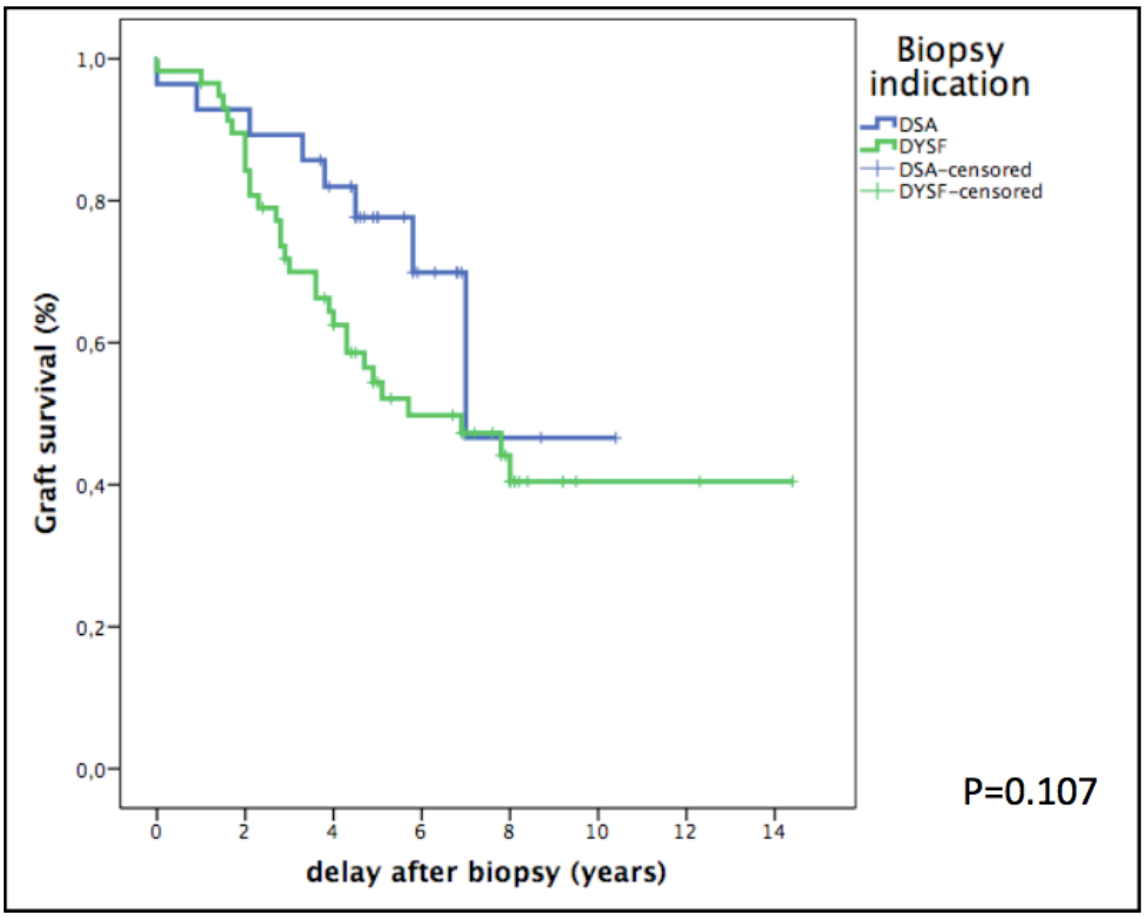

Figure S2: Kaplan-Meier plots of graft survival in 86 patients according to the clinical status at the time of allograft biopsy. Graft dysfunction (DYSF, green curve) versus stable graft function (DSA, blue curve; p = 0.107).