

CLINICAL SCIENCE

The impact of pretransplant donor-specific antibodies on graft outcome in renal transplantation: a six-year follow-up study

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OBJECTIVE: The significance of pretransplant, donor-specific antibodies on long-term patient outcomes is a subject of debate. This study evaluated the impact and the presence or absence of donor-specific antibodies after kidney transplantation on short- and long-term graft outcomes.

METHODS: We analyzed the frequency and dynamics of pretransplant donor-specific antibodies following renal transplantation from a randomized trial that was conducted from 2002 to 2004 and correlated these findings with patient outcomes through 2009. Transplants were performed against a complement-dependent T- and B-negative crossmatch. Pre- and posttransplant sera were available from 94 of the 118 patients (80%). Antibodies were detected using a solid-phase (Luminex[®]), single-bead assay, and all tests were performed simultaneously.

RESULTS: Sixteen patients exhibited pretransplant donor-specific antibodies, but only 3 of these patients (19%) developed antibody-mediated rejection and 2 of them experienced early graft losses. Excluding these 2 losses, 6 of 14 patients exhibited donor-specific antibodies at the final follow-up exam, whereas 8 of these patients (57%) exhibited complete clearance of the donor-specific antibodies. Five other patients developed "de novo" posttransplant donor-specific antibodies. Death-censored graft survival was similar in patients with pretransplant donor-specific and non-donor-specific antibodies after a mean follow-up period of 70 months.

CONCLUSION: Pretransplant donor-specific antibodies with a negative complement-dependent cytotoxicity crossmatch are associated with a risk for the development of antibody-mediated rejection, although survival rates are similar when patients transpose the first months after receiving the graft. Our data also suggest that early posttransplant donor-specific antibody monitoring should increase knowledge of antibody dynamics and their impact on long-term graft outcome.

KEYWORDS: Renal Transplantation; Donor-Specific Antibodies; Solid-Phase Assay; Luminex; DSA.

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INTRODUCTION

The introduction of solid-phase assays for the detection of anti-HLA antibodies (ABs) as well as C4d staining for the evaluation of allograft biopsies has revolutionized the current era of assessing acute and chronic donor-specific antibody-mediated rejection in clinical practice.

Preformed, donor-specific HLA ABs (DSA) are responsible for some renal allograft rejections. The detection of these ABs prior to transplantation is an important step in the assessment of a patient's immunological risk and the exclusion of incompatible donors.

However, these new methods used for antibody recognition are performed in special in vitro conditions that may not accurately reflect conditions in vivo. Pre-Tx HLA-DSA are not necessarily harmful to the transplanted kidney, and these ABs may also preclude the implant of a transplantable organ due to a new technological barrier.

The relevance of pre-TX DSA in patients with a negative crossmatch remains controversial (1-5). These discrepant results may be due to the dynamics of DSA generation.

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No potential conflict of interest was reported.

Following transplantation, the blood levels of DSA may increase, decrease or be completely cleared from the recipient's blood post-transplantation, and these changes may therefore impact graft outcomes.

A prospective trial to assess the impact of DSA dynamics on allograft outcome is a high-risk and potentially unethical study.

Therefore, the current study examined previously frozen and stored sera from a retrospective population of renal patients who had received transplants with a negative complement-dependent cytotoxicity crossmatch (CDC-XM). As the currently used assays were unavailable at the time of serum collection, the current study examined these retrospective samples using modern solid-phase assays.

This cross-sectional study analyzed the frequency of pre-Tx DSA and the presence/absence of these ABs following Tx using single antigen beads to assess the short- and long-term outcomes of MoDIFY trial participants who received transplants between 2002 and 2004.

METHODS

Patients

Male and female patients between the ages of 18 and 65 years who had received a non-identical twin kidney allograft and who presented an ELISA panel-reactive antibody (PRA) level <50% during a prospective, randomized and controlled trial of tacrolimus (TCL) (Prograf[®], Astellas, Deerfield, IL, USA) minimization (the MoDIFY study - Modification of Doses to Improve Function through the Years) (6) were eligible for the current trial. Subjects were excluded if they had received a nonrenal organ or induction with antilymphocyte preparations.

Subjects who fulfilled the inclusion criteria were randomized (2:1) to receive TCL at either an initial dose of 0.15 - 0.25 mg/kg/day or a cyclosporine microemulsion (Neoral[®], Novartis Pharma, USA) at an initial dose of 10 mg/kg/day. Drug doses were subsequently adjusted according to the whole blood levels. For patients in the TCL groups, target pre-dose (C0) levels were 10-15 ng/mL during the first month of treatment but progressively decreased to 3-5 ng/mL at 6 months. In the CyA group, the dose was adjusted according to the CyA concentration during the second hour (C2) following the oral dose, and the target concentrations were between 1,400 and 1,700 ng/mL during the first month and between 800 and 1,000 ng/mL after 6 months. All patients received 1.5 to 2 g/day mycophenolate mofetil (MMF) (Cellcept[®], Hoffman La Roche). All groups received an initial dose of 0.5 mg/kg/day prednisone, which was tapered to 0.1 mg/kg/day prior to the third month of treatment. TCL, CyA and MMF were administered in two 12 h interval oral doses, typically at 8 a.m. and 8 p.m. All patients received induction with an anti-IL2R antibody, either basiliximab (Simulect[®], Novartis Pharma) or daclizumab (Zenapax[®], Hoffman La Roche). The Institutional Ethics Committee approved this study, which was also conducted in full compliance with the Guidelines for Good Clinical Practices.

Renal biopsies

Available biopsies from patients with acute allograft dysfunction were reviewed according to the Banff 2007 classification (7). C4d staining was performed using IHC (immunohistochemistry) from paraffin-embedded biopsies.

Staining of more than 10% of the PTC (peritubular capillaries) was considered positive, as this has been shown to correlate with graft loss and the presence of DSA (8,9).

HLA typing

All donors and recipients were typed for HLA-A, -B and -DR using low-resolution polymerase chain reaction single-strand polymorphisms (PCR-SSPs) (One Lambda, Canoga Park, CA). HLA-DP, -DQ and -Cw specificities were not examined in this study because these antigens were not routinely typed in our region at that time. Donors and recipients were also not typed for these loci because it was not possible to re-type all of the donors for these antigens.

Pretransplant crossmatch

All patients exhibited negative pretransplant CDC-XM with antihuman globulin CDC (CDC-AHG-XM) for T cells and long-incubation CDC for B cells at the time of transplantation. The presence of IgM antibodies was excluded by testing for CDC-XM in the presence of dithiothreitol (DTT).

Pre- and post-Tx serum collection

Two serum samples from each patient were analyzed, one pre-Tx and the other either after the last follow-up exam or prior to death or graft loss. Serum was collected from all MoDIFY study participants prior to Tx and was stored frozen (-70° C). The second serum samples (post-Tx) were collected under the following conditions: 1 - posttransplant serum was collected from active patients who agreed to participate at the time of providing informed consent; 2 - patients who returned for dialysis were invited to participate; and 3 - the hospital laboratories were searched for sera samples that had been collected and frozen from patients who had died with a functioning graft.

Panel-reactive antibodies (PRAs)

PRA levels (% PRA) were measured using flow cytometry for the detection of anti-HLA class I and II antibodies, as recommended by the manufacturers.

Donor-specific antibodies (DSAs)

All patients were tested for the presence of DSA in pre- and posttransplant sera using a solid-phase assay consisting of a single HLA antigen-coated microspheres (Luminex[®] - One Lambda, Canoga Park, CA). Briefly, 5 µl of class I and class II beads were mixed with 20 µl of the serum sample, and the mixture was incubated in the dark for 30 minutes at room temperature. After 3 washes, 100 µl of 1:100 FITC goat antihuman IgG was added, and the samples were incubated at the same conditions for 30 minutes. The microbeads were washed twice and resuspended in 80 µl of fixing solution. Class I and II antibodies were distinguished using a single test, and the tests were read on the LABScreen[™]100 Luminex machine (One Lambda, Inc.).

The immunodominant DSA (iDSA), i.e., the antidonor HLA antibody with the highest median fluorescent intensity (MFI), was used for the statistical analysis when more than one antibody was detected. The charts display each of the recipients' DSA-MFIs in addition to the iMFIs.

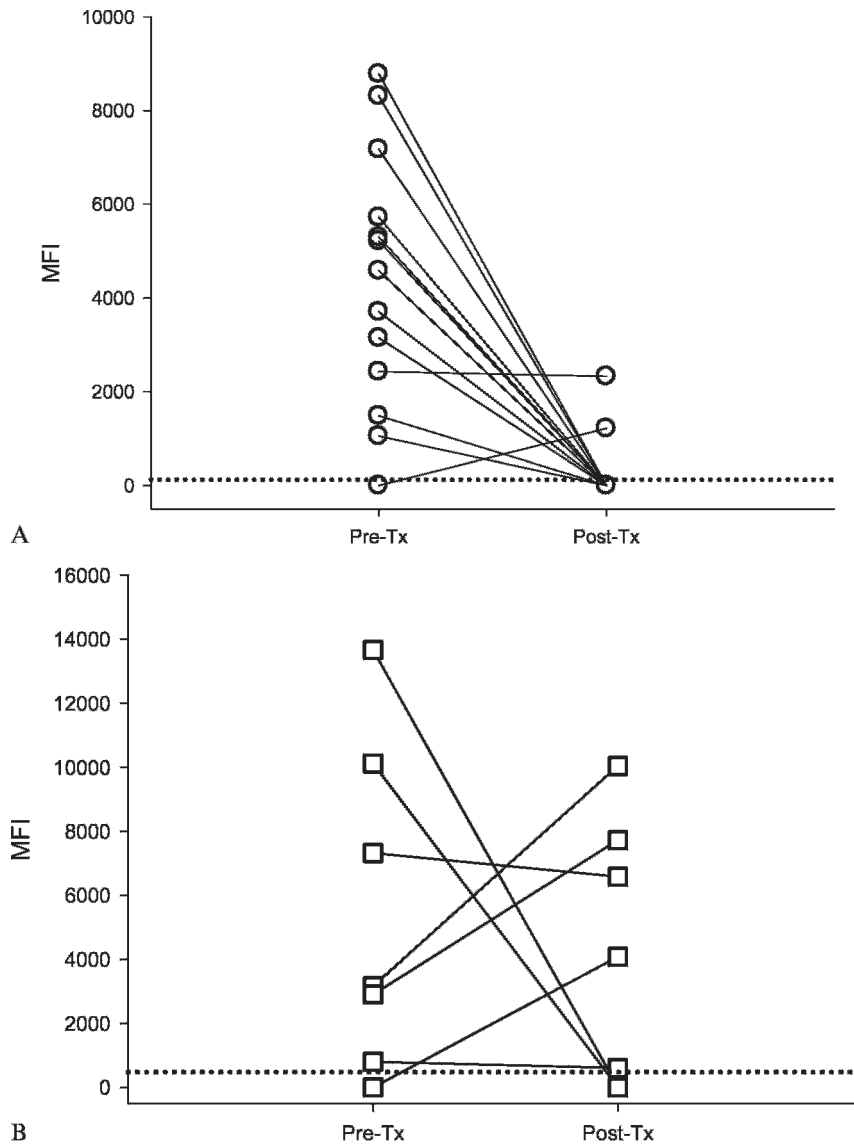


Figure 1 - Pre- and posttransplant MFIs of each of the detected DSAs in 11 patients who remained active until the last follow-up visit (figure 1a) and in 5 patients who lost their allografts or died (figure 1b). The dotted line represents the standard cut-off MFI value for this method.

DATA ANALYSIS

Delayed graft function was defined as the need for dialysis during the first week after transplantation. Any cases with the detection of Cytomegalovirus disease (syndrome or invasive) were classified as CMV-positive. Rejection was defined as any treated or biopsy-proven acute rejection episode. Additional variables that were used for the statistical analysis included the following: recipient and donor age, gender, race, number of HLA-A, -B and -DR incompatibilities, deceased/live donor, type of IL-2R AB administered, doses and blood levels of IS drugs (TCL, CyA and MPA) at 1, 3, 6 and 12 months and 2, 3, 4 and 5 years after Tx, serum creatinine level, eGFR (estimated glomerular filtration rate) according to the MDRD equation and the urinary protein/creatinine from the same time-points as when the immunosuppressive drugs levels were measured.

Data are reported as the means \pm SD, and the medians and 95% C.I. are reported for nonparametric data. Data were analyzed during the first 6 months to identify an early

impact of DSA, and the data were re-analyzed to verify the long-term impact of DSA on long-term allograft outcomes for grafts that had survived the first 6 months. Cox proportional analysis evaluated the hazards of each available variable to predict death-censored graft survival. The PASW statistics 18 (SPSS) software was used for the statistical analyses.

RESULTS

Study population

Twenty-four of the 118 patients in the Modify study did not participate in this analysis. Fifteen patients lost their grafts during the first year due to vascular reasons and/or renal rupture (n=5), death (n=8) or acute rejection (n=2). No available post-Tx serum samples were available for these patients. A post-Tx serum sample was also not available for one patient who died after 7 years of follow-up. Eight patients with functioning grafts at the time of

enrollment refused to participate in the study. Therefore, 94 patients (80%) from the MoDIFY study population were analyzed.

Time of pre- and post-Tx sera collection

Pre-Tx sera were collected and frozen at a median of 61 (-134 to 30) days prior to Tx.

Post-Tx sera were collected from all 75 active patients at our outpatient clinic at a mean of 1834 ± 389 days posttransplant. Nine patients died with a functioning graft at a mean of 1080 ± 810 days after Tx, and post-Tx serum samples were collected at a mean of -613 ± 623 days prior to death.

Ten patients lost their grafts and returned to dialysis. Serum samples were collected from two of these patients at 38 and 222 days prior to graft loss. The remaining 8 cases were recruited from dialysis facilities for the collection of sera at a median of 836 [303–1,114] days after graft loss.

Donor-Specific Antibodies (DSA)

Sixteen of the 94 study patients (17%) were transplanted with DSA (pre-Tx DSA), and 78 patients (83%) were transplanted without DSA (no DSA).

Table 1 describes the demographics and transplant characteristics of the study population.

The pre-Tx DSA and no DSA groups were similar at baseline, although more patients in the pre-Tx DSA group exhibited a PRA class I and II level above 0% as well as a higher class I and II PRA percentage. The numbers of biopsy-proven AR (BPAR) and AMR cases were significantly higher in the pre-Tx DSA-positive group.

Patients with pre-Tx antibodies

Table 2 lists the DSA specificities, MFIs and outcomes of patients who received transplants with pre-Tx DSA. Twelve pts (75%) exhibited anti-HLA class I antibodies, three patients (19%) exhibited anti-class II antibodies, and one patient exhibited (6%) both class I and II ABs.

Ten of these 16 (62%) patients did not develop acute rejection episodes (AR), whereas six (38%) patients developed ARs, including one patient with a late AR episode on PO day 797. The biopsies revealed that only three (3%) patients in the pre-Tx DSA group developed the full clinical picture of antibody-mediated rejection (AMR) with C4d-positive staining in peritubular capillaries. No cases of hyperacute rejection were observed. The cases with ARs were classified as AMR (n=3), Banff Ib (n=1), Banff IIa (n=1), or clinically diagnosed but not biopsied (n=1). The three cases of AMR were treated with thymoglobulin, and two of these cases were also treated with intravenous immunoglobulin between 2002 and 2004 due to the diagnosis of “acute transplant vasculopathy” that suggested a “humoral” component of the AR. Two of the three patients with AMR lost their allografts. AMR only occurred in patients with class I DSA, as none of the three patients with only class II DSA developed AMR.

The mean iMFI-DSA did not differ between pre-Tx DSA patients who developed AMR (n=3) and those patients who did not develop AMR (n=13) (6106 ± 6600 vs 5423 ± 2910, p=NS, respectively, p=NS). The same results were observed when comparing patients who developed AR (AMR or not, n=6) to those who did not develop AR (n=10) (6609 ± 4948 vs 4917 ± 2511, respectively, p=NS). A ROC

Table 1 - Demographics and transplant characteristics of the study population.

	No pre-Tx DSA	Pre-Tx DSA	p-value
n	78 (83%)	16 (17%)	
Gender (F/M)	33/45	10/6	NS
Race (white/no white)	55/23	11/5	NS
Age (years)	42 ± 12	40 ± 12	NS
Tx (1 st /pre-Tx)	74/4	13/3	0.093
Donor (D/L)	39/39	13/3	0.028
PRA c-I (+)	11 (15%)	10 (62%)	<0.001
% PRA c-I	2 ± 7	18 ± 23	0.000
PRA c-II (+)	8 (10%)	7 (44%)	0.003
% PRA c-II	3 ± 11	26 ± 35	0.000
TCL/CyA (n=)	53/25	11/5	NS
HLA-A mm (0/1/2)	14/37/27	0/9/7	NS
HLA-B mm (0/1/2)	10/42/26	0/7/9	NS
HLA-DR mm (0/1/2)	23/39/14	6/8/2	NS
AR (clinical)	16 (20%)	6 (38%)	NS
BPAR	8 (10%)	5 (31%)	0.046
AMR	0 (0%)	3 (19%)	0.004
DGF	24 (61%)	8 (50%)	NS
(in deceased donor)			
CMV	17 (22%)	4 (25%)	NS
Follow-up (mo)	69 ± 16	70 ± 15	NS

AR: clinically diagnosed acute rejection episodes; BPAR: biopsy-proven acute rejection episodes; AMR: antibody-mediated rejection; DGF: delayed graft function; CMV: cytomegalovirus disease; TCL: tacrolimus; CyA: Cyclosporin-A.

curve analysis did not identify a cut-off MFI for pre-Tx DSA that could predict the development of AMR.

Two patients, of the 14 pts with pre-Tx DSA who survived the first six months after transplantation, died with a functioning graft (DwFG) at 23 and 72 months posttransplantation, and one patient lost his/her graft due to chronic transplant nephropathy at 49 months posttransplantation.

Profile of pre- and posttransplant DSA frequencies and outcomes

The post-Tx DSA evaluation from the last follow-up visit, excluding the two patients who lost their allografts due to early AMR, revealed that six of the 14 patients demonstrated DSA, whereas eight patients had completely cleared the DSA (Figure 1A and 1B). Seven patients had cleared class I DSA and one patient had cleared class II DSA. Changes in DSA specificities were observed in three cases (patients # 1, 13, and 16 in Table 2).

Patients who had completely cleared the DSA were not significantly different from patients who had not cleared the DSA in regards to all baseline variables, including the pre-Tx class I PRA level (22 ± 29 vs. 8 ± 14%, respectively, p=NS) and class II PRA level (26 ± 37 vs. 21 ± 29%, respectively, p=NS). The pre-Tx immunodominant MFI-DSA (iMFI-DSA) was not different among patients who had cleared the DSA, as compared to patients who had not (6371 ± 2773 vs. 3503 ± 2607, respectively, p=0.07). The oral dose and blood level of the IS drugs were not different at 1, 3, 6, and 12 months or 2, 3, 4, and 5 years post-Tx between these two groups.

Overall graft survival for pre-Tx DSA-positive patients was lower than for patients without pre-Tx DSA, although this difference was not statistically significant (Log Rank=0.21) (Figure 2). Death-censored graft survival was similar between the two groups, and renal function was also

Table 2 - HLA-DSA and outcomes.

Patient #	Pre-Tx					Day of serum harvest prior to death and after loss	Post-Tx				Outcome /cause/ Fup (months)
	cl - I	MFI-Cl -I	cl -II	MFI cl -II	AR(Banff) PO day		cl-I	MIF Cl-I	cl -II	MIF cl -II	
1	A33	3169	DR1	2913	AMR I 13PO	+1151	A33	10047	DR1 DR7	7727 4090	GL/AMR 2
2	B7	13666	--	--	AMR II 28PO	+1710	B7	1472	--	--	GL/AMR 4
3	A30	10122	--	--	AR clinical 13 PO	+600	--	--	--	--	GL/CAN 49
4	A31	800	--	--	No AR	-324	A31	612	--	--	DwFG/ Suicide 23
5	B44	7322	--	--	No AR	-134	B44	6584	--	--	DwFG/ Infection 72
6	A11	4583	--	--	No AR	--	--	--	--	--	Active 62
7	B14	7173	--	--	No AR	--	--	--	--	--	Active 62
8	A68	5215	--	--	No AR	--	--	--	--	--	Active 62
9	A31	1050	--	--	No AR	--	A31	871	--	--	Active 72
10	A23	8312	--	--	No AR	--	--	--	--	--	Active 73
11	B51 A26	1485 1047	--	--	AMR I 27PO	--	--	--	--	--	Active 73
12	--	--	DR103	5297	No AR	--	--	--	--	--	Active 76
13	--	--	DR16 DR1	1048 5721	No AR	--	A31	1215	--	--	Active 77
14	A2 A29	8782 3148	--	--	Ib 797 PO	--	--	--	--	--	Active 79
15	A11	2429	--	--	Ila 6PO	--	A11	2325	--	--	Active 81
16	--	--	DR4	3703	No AR	--	A68	886	DR53	14377	Active 84

class I and class II: Donor-specific anti-HLA antibodies anti-class I and anti-class II; MFI: median fluorescent intensity; AMR: antibody-mediated rejection; GL: graft loss; DwFG: death with a functioning graft; CAN: chronic allograft nephropathy; Fup: follow-up.

similar between patients with and without pre-Tx DSA (64 ± 15 vs 58 ± 22 ml/min, respectively, $p=NS$). Additionally, the urinary P/Cr levels did not differ between non-pre-Tx DSA patients and pre-Tx DSA-positive patients (0.49 ± 0.83 vs. 0.86 ± 1.1 , respectively, $p=NS$).

A Cox univariate analysis revealed that the only variable with a risk for death-censored graft loss was the presence of a positive post-Tx DSA, including patients with "de novo" post-Tx DSA and patients with early graft loss due to AMR (HR: 5.1, 1.4-18.1, $p=0.012$). The presence of pre-Tx DSA for class I and/or II was not associated with a risk of death-censored graft loss ($p=0.297$).

DISCUSSION

This cross-sectional study analyzed the impact of pre-Tx DSA on the long-term outcomes of patients in a low-risk renal transplant population.

The novel aspect of our study was the analysis of post-Tx DSA, which revealed the dynamics of pre-Tx DSA responses after transplantation.

The first finding of our study was that the majority of patients with low levels of pre-Tx DSA did not develop AMR. However, occurrences of AMR were most often

severe and frequently led to early graft loss. The incidence of AMR in our study (19%) was lower than that in previous studies, particularly in patients with strongly positive DSA and a historic positive cross-match (10). One explanation for this result could have been the low levels of DSA in our population, which were evidenced by the negative pre-Tx CDC-XMs of the included patients. The AMR rates vary between studies; in one large retrospective study, the prevalence of AMR was only 2%, and all patients with AMR had at least one strong donor-specific DSA with an MFI value greater than 6,000 (11).

However, the disparity in AMR rates between studies may be due to the absence of protocol biopsies for the diagnosis of subclinical AMR. A cohort study of 54 DSA-positive kidney transplant recipients demonstrated that 31% of patients met the criteria for subclinical antibody-mediated rejection (SAMR) at the time of a three-month protocol biopsy (12). We used the BANFF-2007 classification, which requires positive C4d staining for an AMR diagnosis. We also performed C4d staining retrospectively using IHC. As IF is more sensitive than IHC (13,14), we may have underdiagnosed cases of AMR due to the false-negative C4d staining using IHC and the absence of protocol biopsies.

Table 3 - Causes of graft loss and death with a functioning graft prior to 6 months and at the final follow-up visit post-Tx.

	Non-DSA group	DSA group	p-value
Pts at risk n =	78	16	
Early Losses (≤6 mo)	3 (3.8%)	2 (12.5%)	0.056
due to ARE	1 (1.3%)	2 (12.5%)	0.056
due to DwFG	2 (2.5%)	-- (0%)	
Pts at risk n =	75	14	
Late Losses (> 6 mo)	11 (14.6%)	3 (21.4%)	0.74
CAN	5 (6.6%)	1 (7.1%)	NS
DwFG	5 (6.6%)	2 (14.3%)	NS
Other	1 (1.4%)	-- (0%)	NS

DSA: donor-specific antibody; PTS: patients; ARE: acute rejection episodes; DwFG: death with a functioning graft; CAN: chronic allograft nephropathy.

However, our results also suggest that patients with low levels of pre-Tx DSA rarely develop AMR, which suggests the possibility of an association between a low concentration of pre-Tx DSA and a negative CDC-XM. This rationale is consistent with previous data demonstrating an association between assay results and, graft loss (15). A retrospective analysis of transplanted patients who had a negative CDC-XM but positive results from a solid-phase assay or FCXM revealed that most patients neither developed AMR episodes nor exhibited impaired graft survival (5). However, two of the three patients who developed AMR lost their grafts. Unfortunately, it was not possible to retest the pre-Tx sera against the donor lymphocytes to perform FCXM in our population.

Whether pre-Tx DSA-MFI levels can predict the development of AMR is an additional topic of debate. The prevalence of AMR increases with increasing pre-Tx DSA-MFIs, and a peak serum MFI between 465 and 1500 in the peak serum is associated with a 25-fold increase in the relative risk for the development of AMR (10). However, we could not detect a pre-Tx MFI cutoff value that could predict the occurrence of AMR. The reasons for these discrepant results remain unclear, although the examination of only pre-Tx serum, rather than serum from the peak of the response, may provide one explanation.

Another important finding of our study was related to the discordance between previous studies as to the impact of pre-Tx DSA levels (not detected by CDC-XM) on transplant outcome. Some authors have observed worse outcomes, whereas others have not (5,10,16). A retrospective study was performed on 113 kidney transplant recipients with negative prospective T- and B-cell CDC-XM at the time of transplant, and these patients were screened for the presence of circulating anti-HLA antibodies and DSA using the Luminex assay. One-year allograft survival rates were similar between the Luminex pre-Tx DSA-positive and DSA-negative groups (17). Patients with DSA who did not experience AMR exhibited the same graft survival rates as patients without DSA (10), and the results of the current study are in line with these previous findings.

The major finding of the current study was that differences in long-term graft survival among individuals with pre-Tx DSA may be due to the dynamics of the DSA response. This is

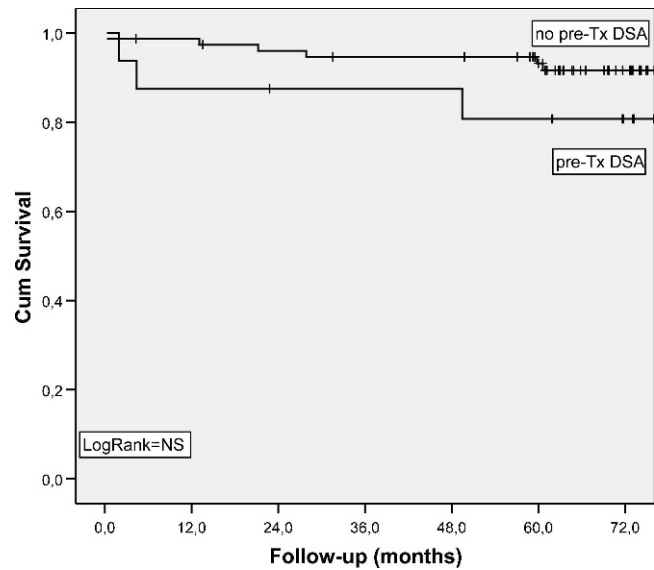


Figure 2 - Death-censored graft survival curves for the pre-Tx DSA group as compared to the non-DSA group.

the first study to analyze pre- and post-Tx DSA data for the same patient population and correlate these dynamics with long-term patient outcome. A large effort was made to collect blood from patients who returned to dialysis and to search for frozen serum samples collected from patients prior to death. We acknowledge that, in many cases, the post-Tx serum samples were collected in a cross-sectional analysis many years following transplantation and that this factor may explain the long-term profile of low-level pre-Tx DSA.

Patients who either completely cleared the DSA post-transplant or who demonstrated decreased levels of DSA exhibited similar long-term outcomes as compared to DSA-negative patients. Therefore, the simple presence of pre-Tx DSA does not impact transplant outcome, and it seems that it is critical for outcome that the DSA level remains above a certain cutoff value post-transplantation.

Patients with DSA who survived the first six months post-transplant presented a similar long-term outcome as patients without DSA. These results are consistent with those of previous studies, although these studies failed to identify the reasons behind the observed improved outcome. It is possible that a mean 6-year follow-up period may not be sufficient for drawing definitive conclusions (5,10).

In summary, this study revealed that the presence of pre-Tx DSA was initially associated with the occurrence of AMR, and this condition may lead to graft loss in low-risk renal patient populations transplanted with a negative CDC crossmatch. Our data also suggest that early posttransplant DSA monitoring can improve the understanding of DSA dynamics. Moreover, pre-Tx DSAs are likely only problematic when these concentrations remain positive after transplantation and above a certain threshold. However, a larger post-Tx analysis of pre-Tx DSA-positive patients would be necessary to prove this hypothesis.

AUTHOR CONTRIBUTIONS

David-Neto E was responsible for the manuscript writing and the intellectual and scientific content of the study. Agena F, Souza PS, Ventura CG and Rodrigues H were responsible for the generation, collection and assembly of the data. Panajotopoulos N, Lemos FC, Castro

MCR, Nahas WC and Kalil JE critically revised the intellectual content of the manuscript. David DSR performed the revision of renal biopsies.

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