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Late impact of preformed anti-HLA antibodies on kidney graft outcome

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1. Introduction

Kidney transplantation is the treatment of choice for most chronic kidney disease patients. It is associated with increased patient survival and better quality of life [1].

The advances in organ preservation, surgical techniques, immunosuppressive medications, identifica and treatment of rejection and infections had improved overall graft survival in the last decades [2].

Controlling the immunological response is a key factor for a successful kidney transplant [3]. The identification of the HLA System and its role in the immune reactions was a major step in understanding the importance of donor and recipient compatibility [4,5].

In the last 40 years, there have been advances in the crossmatch techniques with the development of more sensitive methods, such as flow cytometry [6]. In the last decade, the solid-phase assays have allowed a more precise detection of anti-HLA antibodies [7,8].

There is a historical debate about which component of the immune response is more important in the rejection process: cells or antibodies. Although there is not a definitive answer, in the last 20 years there is growing evidence of the important role of the antibodies on graft failure [9,10]. A variety of studies have shown controversial results regarding DSA presence when identified at lower levels with more sensitive techniques [11–15].

2. Objective

The aim of this study was to evaluate the impact of preformed DSA on long term graft outcomes.

3. Material and methods

We retrospectively evaluated a cohort of adult recipients of kidney or simultaneous kidney and pancreas transplants performed at our Institution from January 2009 to December 2012, with follow-up until June 2016. During the study period, 857 adult kidney transplants were performed. Were excluded from the analysis 223: 125 pediatric transplants, 20 recipients of other solid organs except for pancreas, 19 surgical losses, 8 deaths within the first month from cardiovascular causes, 4 primaries non-function, and 47 with incomplete immunological data. The study was approved by the local Ethical Committee.

Data collected included clinical and demographic characteristics, immunosuppressive treatment, calculated panel reactive antibodies (cPRA), preformed DSA, donor type, and rejection episodes.

All patients were tested for anti-HLA antibodies using Luminex Single Antigen Beads with One Lambda Class I and Class II kits (Canoga Park, CA, USA). Sera were submitted to heat treatment before testing. Pre-transplant cPRAs were stratified as zero (0%), low (1–49%), intermediate (50–79%) and high (> 80%). Pre-transplant anti-HLA antibodies were analyzed by mean fluorescence intensity (MFI) levels. DSA was considered positive if it was an IgG antibody and the normalized intensity via single antigen bead was 1000 MFI or above. DSA intensity was stratified in 3 levels: 1000–2000, 2001–5000 and above 5000 MFI, according to the highest value. We considered antibodies against HLA-A, B, C, DR, DQ in the analysis.

PRA calculation was based on the HLA profile of a reference cohort of > 2000 actual kidney donors from our region using antibodies against HLA-A, B, C, DR, DQ with MFI above 1000. The cPRA was expressed in percentages and the individual antibodies in MFI.

Crossmatching was performed by complement-dependent lymphocytotoxicity (CDC) and flow cytometry. Only pre-transplant serum was considered in this analysis, however, we performed historic and current sera crossmatching for all patients. B cell positive flow cytometry crossmatch (FCXM) transplants were performed only in cases when the recipient had an urgency to receive a kidney, they were all CDC negative.

Deceased donors were categorized as standard (SCD) or expanded criteria (ECD), according to UNOS classification [16]. Delayed graft function (DGF) was defined as the need for dialysis in the first post-

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transplant week.

Per protocol, transplants performed with a deceased donor with any DSA level and/or with cPRA > 50% and/or estimated cold ischemia time above 24 h received rabbit antithymocyte globulin (ATG) induction and the remaining received basiliximab. Living donor recipients received induction therapy with DSA presence and/or 3 or more HLA mismatches. Maintenance immunosuppression included a calcineurin inhibitor, mycophenolic acid derivative and prednisone taper starting at the time of transplantation to reach 5 mg at 3 months.

Rejection episodes were for-cause biopsy-proven and classified according to 2007 Banff criteria [17]. Borderline infiltrates were not considered rejection episodes for the analyses. Cellular mediated rejection (TCMR) episodes were treated with methylprednisolone or ATG, according to Banff grade or steroid resistance. Antibody mediated rejection (AMR) episodes were treated with a total of 10 sessions of every other day plasmapheresis and subsequent intravenous immunoglobulin.

Statistical analysis: continuous variables were analyzed by Student's *t*-test or nonparametrical Mann–Whitney *U* test. Categorical data were analyzed with Chi square or Fisher's exact test. Graft survival in kidney transplant recipients with pre-transplant HLA class I and II DSA were compared using the Kaplan–Meier method and the log-rank tests. Data are expressed as means ± standard deviation or medians with interquartile range (IQR) as appropriate. The associations of donor, recipient, transplant parameters and immunological factors with graft loss were assessed in univariate regression analyses. Cox proportional hazards analysis was used to estimate the survival difference magnitude. The variables with a significance level of $p < 0,25$ in the univariate analyses were selected for inclusion in the multivariate analyses. A backward model selection method (threshold of $p = 0.05$) was used to reach a final regression model. Graft loss was defined as the return to dialysis, censored by death with a functioning graft. Statistical analyses were performed using SPSS® version 20 (SPSS Inc., Chicago, IL, USA).

4. Results

The clinical and demographic characteristics of the 634 patients are presented in Table 1. Preformed DSAs were identified in 91 (14.3%) patients, 42 (46.1%) were anti-HLA class I (A, B, C) and 64 (70.3%) anti-class II (DR, DQ). The DSA group had a higher proportion of female patients (60.4% vs. 40.1%, $p < 0.0001$), second transplant (20.9% vs. 5.7%, $p < 0.0001$), more induction therapy (93.4% vs. 64.2%; $p < 0.001$) and B cell positive FCXM (24.2% vs. 0.7%, $p < 0.0001$).

There were 436 recipients (68.7%) with a positive cPRA class I or class II, 11.4% above 80%.

There were 230 acute rejection episodes during follow-up, 213 (92.6%) TCMR and 17 (7.4%) AMR, with no differences between DSA and non-DSA groups (29.7% vs. 34.3% for TCMR and 4.4% vs. 2.4% for AMR, respectively; $p = 0.42$).

The risk factors for acute rejection were ECD (HR 2.05 95%CI 1.45–2.89; $p < 0.001$), DGF (HR 2.16 95%CI 1.58–2.90; $p < 0.001$), 4 mismatches in locus A/B (HR 2.38 95%CI 1.28–4.45; $p = 0.006$), one and two mismatches in DR (HR 1.43 95%CI 1.05–1.93; $p = 0.02$ and HR 2.10 95%CI 1.42–3.09; $p < 0.001$, respectively), PRA class I 1–49% (HR 1.57 95%CI 1.19–2.08; $p = 0.001$), PRA class II of 1–49% (HR 1.60 95%CI 1.21–2.13; $p = 0.001$), B positive FCXM (HR 1.89 95% CI 1.02–3.49; $p = 0.04$), class I and class II DSA sum above 5000 MFI (sum of all identified DSA) (HR 1.83 95%CI 1.02–3.30; $p = 0.04$).

The following independent predictors of acute rejection were identified: one or two mismatches in DR (HR 1.76 95% CI 1.20–2.57; $p = 0.004$ and HR 2.14 95% CI 1.36–3.38; $p = 0.001$, respectively) and DGF (HR 2.06 95% CI 1.50–2.83; $p < 0.001$).

From the 17 cases of AMR, nine (52.9%) occurred after the first year of follow-up. Five cases were non-adherent patients and one patient presented persistent low levels of immunosuppression due to recurrent vomiting caused by diabetic gastroparesis.

Table 1
Baseline characteristics and transplant outcomes according to DSA status.

	All patients (n = 634)	DSA group (n = 91)	Non-DSA group (n = 543)	p
Age, years (min-max)	47 (18–80)	46 (18–69)	48 (18–80)	0.34
Female Gender (%)	273 (43.1)	55 (60.4)	218 (40.1)	< 0.0001
Caucasian (%)	338/402 (84.1)	46/59 (78.0)	292/343 (85.3)	0.28
Cause of ESRD (%)				0.36
GN	76 (11.9)	9 (9.9)	67 (12.3)	
DM	88 (13.8)	14 (15.3)	74 (13.6)	
Hypertension	102 (16.0)	10 (10.9)	92 (16.9)	
Other	368 (58.0)	58 (63.7)	310 (57.1)	
Transplant number (%)				< 0.0001
1	579 (91.3)	69 (75.8)	510 (93.9)	
2	50 (7.9)	19 (20.9)	31 (5.7)	
3	3 (0.5)	2 (2.2)	1 (0.2)	
4	2 (0.3)	1 (1.1)	1 (0.2)	
Type of donor (%)				0.77
SCD	287 (45.3)	41 (45.1)	246 (45.3)	
ECD	178 (28.1)	28 (30.8)	150 (27.6)	
Living	169 (26.7)	22 (24.1)	147 (27.1)	
CIT[a] (%)				0.002
< 12 h	13/450 (2.9)	1/64 (1.6)	12/386 (3.1)	
12-24 h	226/450 (50.2)	20/64 (31.3)	206/386 (53.4)	
> 24 h	211/450 (46.9)	43/64 (67.2)	168/386 (43.5)	
DGF[b] (%)	214/336 (63.7)	38/54 (70.4)	176/282 (62.4)	0.28
HLA mismatch, median (IQR)				
Class I (A, B)	2 (2–3)	2 (1–3)	2 (2–3)	0.02
Class II (DR)	1 (0–1)	1 (0–1)	1 (0–1)	0.07
PRA class I (%)				< 0.0001
0	282 (44.5)	10 (11.0)	272 (50.1)	
1–49	281 (44.3)	46 (50.5)	235 (43.3)	
50–79	38 (6.0)	19 (20.9)	19 (3.5)	
> 80	33 (5.2)	16 (17.6)	17 (3.1)	
PRA class II (%)				< 0.0001
0	314 (49.5)	10 (11.0)	304 (56.0)	
1–49	265 (41.8)	42 (46.2)	223 (41.1)	
50–79	31 (4.9)	20 (22.0)	11 (2.0)	
> 80	24 (3.8)	19 (20.8)	5 (0.9)	
DSA quantity (%)	91 (14.2)			
1		63 (69.2)		
2		19 (20.9)		
3		6 (6.6)		
4		3 (3.3)		
DSA Class (%)				
Class I (A ± B ± C)		27 (29.7)		
Class II (DR ± DQ)		49 (53.8)		
Class I and II		15 (16.5)		
DSA class I highest MFI (%)				
1000–2000		21/42 (50.0)		
2001–5000		16/42 (38.1)		
> 5000		5/42 (11.9)		
DSA class II highest MFI (%)				
1000–2000		28/64 (43.8)		
2001–5000		18/64 (28.1)		
> 5000		18/64 (28.1)		
Induction therapy	N = 434	N = 85	N = 349	< 0.0001
Anti-CD25	280 (64.5)	22 (25.9)	258 (73.9)	
ATG	149 (34.3)	58 (68.2)	91(26.1)	
R + PP	1 (0.2)	1 (1.2)	0	
ATG + IVIg + PP	4 (0.9)	4 (4.7)	0	
B Positive crossmatch	26 (4.1)	22 (24.2)	4 (0.7)	< 0.0001
eGFR (CKD-EPI)				
1 year		43.3 ± 21.5	47 ± 17.6	0.09
5 years		54.4 ± 22.8	50.3 ± 20.8	0.36

(*continued on next page*)

Table 1 (*continued*)

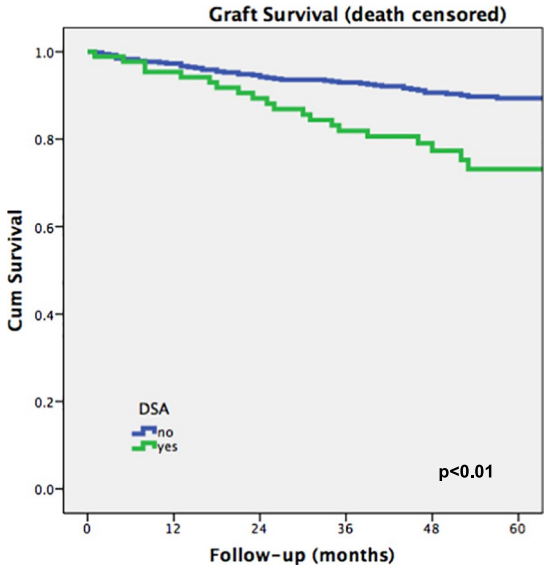
	All patients (n = 634)	DSA group (n = 91)	Non-DSA group (n = 543)	p
Rejection (%)				
No rejection	404 (63.7)	60 (65.9)	344 (63.4)	0.42
TCMR	213 (33.6)	27 (29.7)	186 (34.3)	
AMR	17 (2.7)	4 (4.4)	13 (2.3)	
Death (%)	66 (10.3)	7 (7.7)	59 (10.7)	0.49
Graft loss (%)	77 (12.1)	20 (22.0)	57 (10.5)	0.005
Median follow-up, mo (IQR)	53 (42–69)	48 (30–60)	54 (43–70)	0.004[c]

ESRD: end stage renal disease. GN: glomerulonephritis. DM: diabetes mellitus.
SCD: standard criteria donor. ECD: extended criteria donor.
CTI: cold ischemia time.
DGF: delayed graft function.
HLA: human leucocyte antigen. DSA: donor specific antibody.
Induction: Anti-CD25 = basiliximab or daculizumab. ATG = antithymocyte globulin.
R + PP = rituximab + plasmapheresis. ATG + IVIg + PP = ATG + Intravenous immunoglobulin + plasmapheresis.
eGFR: estimated glomerular filtration rate (ml/min/1.73m^2).
TCMR: T-cell mediated rejection. AMR: antibody mediated rejection.
IQR: interquartile range 25–75.
[a] Data available from 450 patients (of 465 deceased donors).
[b] Data available from 336 patients (of 465 deceased donors).
[c] Mann-Whitney U test.

The median follow-up after transplantation was 53 (IQR 42–69) months.

Patient survival in DSA and non-DSA groups was similar at one (93.2% vs. 94.1%), three (93.2% vs. 90.1%) and five years (89.5% vs. 88.8%) post-transplant (p = 0.5).

Death-censored graft survival at 5 years was inferior in the DSA group (73.2% vs. 89.4% in non-DSA group; p < 0.0001) (Fig. 1).



Time (months)	12	36	60
No-DSA group			
No at risk	482	436	209
Survival (%)	97.3	93.0	89.4
DSA group			
No at risk	80	65	22
Survival (%)	95.4	81.9	73.2

Fig. 1. Death censored kidney graft survival according to the presence of donor specific antibodies (DSA). Graft survival at 5 years was inferior in the DSA group.

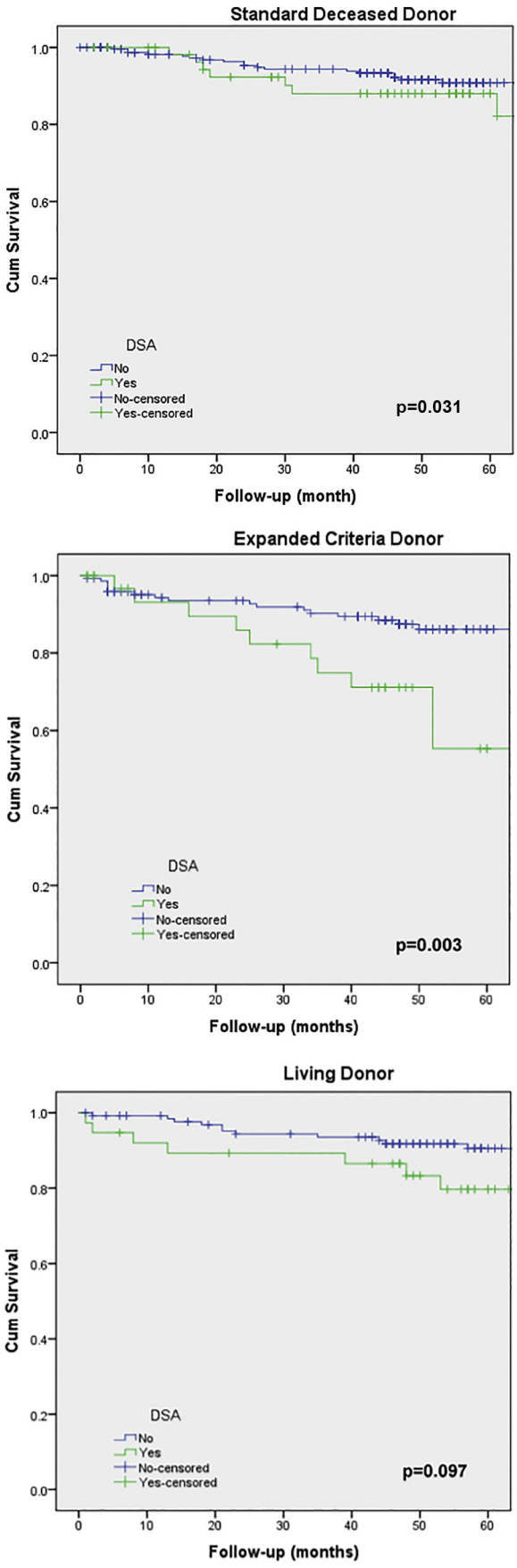


Fig. 2. Death censored kidney graft survival according to type of donor and DSA status. The graft survival was significantly lower in the DSA group (green line) for SCD and ECD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Factors associated with graft loss in univariate analyses.

	No of patients/events	Hazard ratio (95% CI)	p
Age	634/77	0.99 (0.98 to 1.01)	0.70
Gender			
Female	273/40	1	
Male	361/37	1.49 (0.95 to 2.33)	0.081
Type of donor			
Living	169/19	1	
SCD	287/27	0.94 (0.52 to 1.70)	0.86
ECD	178/31	1.95 (1.10 to 3.46)	0.022
Cold ischemia time[a]			
< 12 h	13/17	1	
12-24 h	226/20	1.42 (0.19 to 10.63)	0.73
> 24 h	211/35	3.04 (0.41 to 22.43)	0.27
DGF[b]			
No	122/20	1	
Yes	214/26	1.37 (0.67 to 2.80)	0.38
HLA mismatch			
Class I (A and B)			0.43
0	41/6	1	
1	97/8	0.56 (0,19 to 1.63)	
2	206/20	0.71 (0.28 to 1.72)	
3	161/19	0.90 (0.35 to 2.26)	
4	109/19	1.45 (0.57 to 3.67)	
Class II (DR)			0.34
0	232/23	1	
1	280/35	1.25 (0.73 to 2.11)	
2	100/12	1.39 (0.69 to 2.81)	
DSA			
No	543/57	1	
Yes	91/20	2.41 (1.44 to 4.03)	0.001
DSA number			
0	543/57	1	
1	63/14	2.40 (1.33 to 4.31)	0.003
≥2	28/6	2.45 (1.05 to 5.69)	0.037
PRA Class I			
0	282/34	1	
1–49%	281/31	1.02 (0.62 to 1.67)	0.91
50–79%	38/5	1.10 (0.43 to 2.83)	0.83
> 80%	33/7	2.22 (0.98 to 5.04)	0.056
PRA Class II			
0	314/33	1	
1–49%	265/35	1.47 (0.91 to 2.39)	0.11
50–79%	31/3	1.09 (0.33 to 3.57)	0.88
> 80%	24/6	2.94 (1.22 to 7.06)	0.015
B Flow Crossmatch			
Negative	608/72	1	
Positive	26/5	1.92 (0.77 to 4.78)	0.15
Rejection			
No rejection	404/25	1	
Cellular rejection	213/45	3.61 (2.21 to 5.89)	< 0.01
Humoral rejection	17/7	7.69(3.31 to 17.85)	< 0.01

SCD: standard criteria donor. ECD: extended criteria donor.
CTI: cold ischemia time.
DGF: delayed graft function, defined as need of dialysis in the first post-operative week.
HLA: human leucocyte antigen.
DSA: donor specific antibody.
PRA: panel reactive antibodies.
[a] Data available from 450 patients (of 465 deceased donors).
[b] Data available from 336 patients (of 465 deceased donors).

Stratifying by donor type, DSA impacted significantly in ECD (60.9% DSA vs. 84.3% no DSA; p = 0.028) and SCD (80.7% vs. 90.3%, p = 0.046) graft survival. Among living donors, there was a trend towards a lower survival (71.8% vs. 90.6%, p = 0.051) (Fig. 2).

There was no difference in eGFR at 1 year (43.3 ± 21.5 vs 47 ± 17.6 ml/min/1.73m^2, p = 0.09) and 5 years (54.4 ± 22.8 1 vs 50.3 ± 20.8 ml/min/1.73m^2, p = 0.36) in DSA and non-DSA, respectively.

In the univariate analyses, risk factors for graft loss (Table 2) were ECD, DSA presence, class II PRA > 80% and acute rejection episodes (TCMR or AMR).

Table 3
Factors associated with graft loss in multivariate analyses.

	Hazard ratio (95% CI)	P
PRA Class I		
0	1	
1–49%	0.81 (0.48 to 1.36)	0.42
50–79%	0.81 (0.29 to 2.23)	0.69
≥80%	2.44 (0.98 to 6.06)	0.054
DSA		
No	1	
Yes	2.21 (1.24 to 3.95)	0.007
Rejection		
No rejection	1	
TCMR	3.99 (2.41 to 6.59)	< 0.001
AMR	8.41 (3.53 to 20.03)	< 0.001

SCD: standard criteria donor. ECD: extended criteria donor.
PRA: panel reactive antibodies.
DSA: donor specific antibody.
TCMR: T-cell mediated rejection.
AMR: antibody mediated rejection.

In the multivariate analyses, DSA presence and acute rejection episodes were associated with decreased graft survival (Table 3).

One year graft survival was worse in patients with DSA sum (Class I + II) > 5000 compared to MFI between 1000 and 5000 and without DSA (86.3% vs. 90.2% vs. 91.4%, respectively, p = 0.041).

Among 26 patients with a positive B FCXM, 17 had DSA class I + II MFI > 5000, 5 between 1000 and 5000 and 4 without known DSA. Five of the 17 (29%) with sum above 5000 had graft loss after the first year post-transplantation.

The DSA presence impacted graft survival regardless of a pre-transplant negative FCXM. Graft survival was lower at 5 years in DSA with negative FCXM (DSA: 72.2% vs non-DSA: 89.3%, p = 0.002). In the DSA with positive B FCXM group, the difference did not reach statistical significance (DSA: 74.8%, vs non-DSA: 100%; p = 0.352), probably due to the small sample size.

During the follow-up time, there were 19 graft failures in the DSA group (20.9%), 11 (57.9%) from immunological causes (7 acute rejections and 4 chronic allograft nephropathy), 6 from mix causes (BKV, recurrent pyelonephritis and chronic lesions in biopsy), one malakoplakia and multiple myeloma. In the non-DSA group, there were 57 (10.5%) graft failures, 23 (40.3%) from immunological causes (p = 0.302).

5. Discussion

In our cohort of 634 kidney transplant recipients, 91 (14.3%) had preformed DSA, which is similar to other studies (6.7 to 14%) [12,15,18–22]. The majority of DSA was anti-HLA class II (53.8%). We found a higher prevalence of sensitized patients, with PRA class I or II > zero (68.7%) compared to other reports of 20–25% [19,22].

We had four B positive FCXM in the Non-DSA group, they were cases with a probably not identified DSA. HLA phenotyping did not include subtypes C and DQ in about 38% of deceased donors since we receive organs from various centers that do not perform this evaluation.

We did not evaluate DSA source, but since there is a majority of women and recipients with a previous transplant in the DSA group, pregnancy and exposure to HLA antigens from a previous donor could be an explanation. We do not investigate if the antibodies are complement fixing or not.

The association of DSA and rejection episodes is well documented. Several studies have demonstrated an increased risk of AMR but not TCMR [18,20,23], especially with higher MFI levels [11,22]. One study found a higher incidence of both types of rejection [19]. In our study, there was an overall high incidence of acute rejection episodes

independently of DSA. However, the acute rejection was associated with DGF and mismatches in HLA DR locus in the multivariate analysis. Our incidence of DGF, as in other Brazilian centers [24], is much higher than other Countries [21,25]; this may have contributed to the overall increased incidence of acute rejection. Wissing et al. demonstrated that DGF increased acute rejection in > 2-fold, although with borderline significance. They also found that a higher number of HLA mismatches independently predicted acute rejection (OR 1.65 for each increase in the number of mismatches; 95% CI: 1.15 to 2.38; $p = 0.007$), and that episodes in the first year after transplantation were associated with a significantly lower death-censored graft survival (63.5% vs. 91.2%; $p < 0.0001$) [25].

Although previous studies suggested the association between PRA > 0% and increased risk of acute rejection and graft loss [26–28], we believe that in our study, the correlation between class I and class II PRA > 80% and graft loss may be because of DSAs not recognized at the time of transplant probably due to antibodies anti-HLA C and DQ. As stated above 38% of donors came from centers who do not perform these typings.

We found an elevated rate of late AMR, often associated with non-adherence and low levels of immunosuppression. This is in accordance with Gupta et al., that evaluated 23 patients with late AMR, 20 of them with reduced immunosuppression before the onset of rejection (17% for non-adherence and 69% for a clinical recommendation) [29].

Our study showed the deleterious effects of preformed DSA on graft survival, with a marked reduction at 5-years follow-up, which is in accordance to other studies [11–13,15,30,31], even with low MFI levels [20].

One year graft survival was worse in patients with DSA sum (Class I + II) > 5000. The current experience in our service shows that MFI sum or isolated above 5000 are associated with positive flow crossmatch and only exceptionally transplanted nowadays. Until 2010 the positive flow crossmatch was not an absolute contraindication to transplant, after one analysis of those data presented at the Banff meeting 2013 we abandoned all CDC cross and start to rely exclusively on the FCXM.

Malheiro et al. demonstrated a poorer graft survival only in patients with DSA and AMR (68.8%), compared with patients with DSA and no AMR (96.0%) and those without DSA (94.9%) [22]. Graft losses were mainly attributed to immunologic causes and infections. This could be due to a direct effect of the antibodies and related to the immunosuppression side effects.

Recipients of kidneys from ECD with DSA had even worse graft survival (60.9% in 5 years), showing that the immunological risk can cause more damage to an older graft, as discussed by Aubert *et. al,* that found a 4.4-fold increased risk of graft loss compared with recipients of ECD without DSA and a 5.6-fold increased risk compared with all other transplants [21]. Among living donors, there was a trend towards a lower graft survival. Recently, Kamburova et al. demonstrated that the presence of preformed DSA class I or class II is a risk factor for graft loss in deceased donor transplant, but not in living donor transplant. Although, in the presence of combined DSAs class I and class II, there seems to be an association with an increased risk for graft failure [30].

The patient survival was not inferior in the DSA group, it was even better than expected Brazilian annual death rate of 18.2% of dialysis patients [32].

Although there are limitations inherent to retrospective analyses, the strengths of this study are the large patient number and long follow up period.

In conclusion, our study showed that the presence of preformed DSA can have deleterious effects on the kidney allograft, especially from expanded criteria deceased donors, even in cases with negative pre-transplant flow cytometry crossmatch. The immunosuppression needed to overcome the immunologic damage of those antibodies can increase the risk of infections and toxic effects on the patient and the graft. It is important to identify those antibodies prior to transplantation and balance the risks and benefits of the procedure in an individual basis.

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Conflict of interests

No conflicts of interest.

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