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Impact of preformed and de novo anti-HLA DP antibodies in renal allograft survival

Dolores Redondo-Pachón [a], Julio Pascual [a,*], María J. Pérez-Sáez [a], Carmen García [b], Juan José Hernández [b], Javier Gimeno [c], Marisa Mir [a], Marta Crespo [a]

[a] Department of Nephrology, Institute Mar for Medical Research, Hospital del Mar, Barcelona, Spain
[b] Laboratori de Referencia de Catalunya, Barcelona, Spain
[c] Department of Pathology, Hospital del Mar, Barcelona, Spain

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ABSTRACT

The influence of antibodies against HLA-DP antigens detected with solid-phase assays on graft survival after kidney transplantation (KT) is uncertain. We evaluated with Luminex® the prevalence of pre- and posttransplant DP antibodies in 440 KT patients and their impact on graft survival. For 291 patients with available pretransplant samples, DP antibodies were present in 39.7% KT with pretransplant HLA antibodies and 47.7% with DSA. Graft survival of KT with pretransplant class-II DSA was worse than with non-DSA ($p = 0.01$). DP antibodies did not influence graft survival. Of 346 patients monitored post-KT, 17.1% had HLA class-II antibodies, 56% with DP antibodies. Class-II DSA was detected in 39%, 60.9% of them had DP antibodies. Graft survival was worse in patients with class-II DSA ($p = 0.022$). DP antibodies did not change these results. The presence of isolated DP antibodies was a rare event both pre- and posttransplantation (1.03 and 0.86%). The presence of pretransplant and posttransplant DSA is associated with a negative impact on graft survival. However, the presence of DP antibodies does not modify this impact significantly.

1. Introduction

One of the most relevant advances in organ transplantation has been the development of more sensitive assays to detect HLA antibodies, namely solid-phase immunoassays in Luminex® platform [1–3]. Multiple studies have shown a correlation between the information derived from these new techniques and clinical events in renal allograft recipients [4–8]. Currently, Luminex® Single Antigen (LSA) studies permit the assessment of antibodies against antigens HLA A, B, C, DR, DQ and DP. Classically, HLA DP and HLA C have been considered to be less immunogenic than HLA A, B, DR and DQ molecules [9]. Two pairs of genes codify HLA DP molecules, two for region A and two for region B. Only one of these pairs of genes (DPA1 and DPB1) codifies α- and β-chains and show many polymorphisms. The other pair of genes (DPA2 and DPB2) has a limited polymorphism, and the codified antigens are not expressed on cell surface. Until now, 132 alleles have been located in DPB1 exon 2, whereas 27 alleles are known for the less polymorphic DPA1 (DPA1*01–04) [10,11].

The influence of HLA DP antibodies detected in Luminex® platform on short and long-term graft survival is not well-known. We aimed to evaluate their prevalence in renal allograft recipients before and after kidney transplantation (KT) and their impact on graft survival.

2. Patients and methods

2.1. Patients

From January 2008 to March 2013, 440 renal allograft recipients transplanted between 1979 and 2012 and functioning for more than 3 months were included in the study. Transplantations were performed after negative CDC crossmatch. Internal review board approved the study. A database included demographics, donor type, number of previous transplantations, initial and maintenance immunosuppression, delayed or immediate graft function and acute rejection episodes. Graft function (serum creatinine, MDRD4 estimated glomerular filtration rate (GFR) and urinary protein/creatinine ratio) was recorded at the time of HLA antibody testing.

Pretransplant serum samples were available for 291 of the 440 patients included. A total of 346 patients had posttransplant serum samples studied and follow-up was completed until March 2013.

2.2. HLA antibodies determination and analyses

Serum samples were stored at −80 °C until use. Anti-HLA antibodies detection was performed retrospectively using Lifecodes-LifeScreen-

* Corresponding author at: Department of Nephrology, Passeig Maritim 25–29, 08003 Barcelona, Spain.
E-mail address: julpascual@gmail.com (J. Pascual).

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Deluxe kits (Gen-Probe, Stanford, CT) in Luminex platform, according to manufacturer instructions. The kit comprised 7 beads with class-I glycoproteins and 5 with class-II glycoproteins. Three microliters of beads were incubated with 40 μl of wash buffer and 12.5 μl of patient serum during 30 min and 3 washings were performed. Three microliters of goat anti-human IgG conjugated to phycoerytrin o PE and 22 μl of wash buffer in each well were incubated for 30 min. Samples were analyzed in Luminex 200 platform (Luminex, Austin, TX) using Bio-Plex Manager 6.0 (software for data acquisition) and the program MatchIt! Antibody v1.1.0.2 (Gen-Probe) as software for analysis. A sample was considered to be positive for anti-HLA antibodies if: 1) at least one of the 7 beads with class-I and/or at least one of the 5 beads with class-II were positive with score 3; 2) fulfilled established criteria for the negative internal control beads (CON1, 2 & 3), and 3) showed an MFI for the positive control above 3500. In addition, the kit's positive and negative control sera were included in each assay.

The identification of class-II specific IgG anti-HLA allo-antibodies was made with Lifecodes LSA™ Class-I and/or Class-II kits (Gen-Probe), according to manufacturer instructions. The kit LSA Class-I included 93 beads with class-I HLA molecules (HLA-A, B, C), and the kit LSA Class-II included 84 beads with class-II HLA molecules (HLA-DR, DQ, DP). Data were analyzed using MatchIt! (Gen-Probe). The MFI cut-off point was set at 1000 for positivity.

The donor specificity of anti-HLA antibodies was considered with the typing of HLA A, B, DRB and in some cases for C and DQB. In case of unavailability of DQB and C typing, specificity was assigned through linkage disequilibrium.

2.3. Statistical analysis

Normal continuous variables are expressed with means and standard deviation (SD), and non-normal variables are expressed with median and interquartilic range (IQR). Chi-square tests were used for comparing categorical variables. Continuous variables were assessed using non-parametric Mann–Whitney U tests or Student t tests depending on normality distribution. Survival analyses were performed using Kaplan–Meier curves with log-rank test comparisons.

The multivariate analysis was performed using the logistic and Cox regression analysis.

The studies were performed using software SPSS v.21. Significance was considered with a $p < 0.05$.

3. Results

3.1. Pretransplant antibodies

3.1.1. HLA DP antibodies in patients with pretransplant HLA antibodies

Pretransplant sera were available in 291 of the 440 KT recipients included in this study. HLA class-II IgG antibody screening was positive in 68 of them (23.3%). Twenty-seven (39.7%) had HLA DP antibodies in the LSA tests. Their characteristics compared with those patients with class-II positive screening but without HLA DP antibodies (n = 41) are shown in Table 1. Mean age in both groups was around 50 years, and they were predominantly women. Half of the patients had previously received another KT. Patients with HLA DP antibodies did not suffered biopsy-proven acute rejection episodes compared with 9.1% in the group of patients with pretransplant HLA class-II antibodies without DP specificities.

3.1.2. HLA DP antibodies in patients with pretransplant donor-specific anti-HLA antibodies (DSA)

Among the 68 patients who screened positive for HLA class-II antibodies, 36 (52.9%) had DSA. In 47.2% patients with DSA, we found HLA DP antibodies, while this percentage was only 31.2% in those patients with HLA non-DSA.

In the group of patients with DSA, no clinical or demographical differences were found among patients with HLA DP antibodies and those with HLA without DP antibodies, although the rate of delayed

Table 1
Demographic and clinical characteristics of studied patients distributed according to the presence or absence of donor-specific antibodies (DSA) and the presence or absence of HLA DP antibodies before kidney transplantation.

	No antibodies (n = 223)	Anti-HLA					
		No DSA (n = 32)			DSA (n = 36)		
		HLA DP antibodies (n = 10)	No HLA DP antibodies (n = 22)	p	HLA DP antibodies (n = 17)	No HLA DP antibodies (n = 19)	p
Recipient age (years, mean ± SD)	52.5 (±14.5)	49.2 (±15.2)	48.8 (±13.3)	0.86	52.3 (±14.03)	46.4 (±10.2)	0.95
Female recipient (%)	32.1%	42.9%	64.7%	0.19	66.7%	64.7%	1
Deceased donor (%)	91.1%	92.9%	100%	0.45	95.2%	93.3%	0.34
Expanded criteria donors (%)	34.4% 63/183	30% 3/10	11.1% 2/18	0.87	29.4% 5/17	36.3% 4/11	0.58
Donor age (years, mean ± SD)	50.1 (±16.3)	52.4 (±12.7)	50 (±6.3)	0.95	44.5 (±18.7)	49.3 (±17.2)	0.56
Cold ischemia time (hours: median [IQR])	15 (12–19.5)	16 (9–22)	15.5 (12–18)	0.13	16 (14–20)	13.5 (11–16)	0.08
Retransplantation (%)	8%	20%	13.6%	0.67	71.4%	80%	0.70
Peak PRA CDC > 5% (%)	11.7%	28.6%	17.6%	0.61	42.9%	40%	0.86
Pretransplant PRA CDC > 5% (%)	1%	0	0	–	26.7%	23.5%	0.57
HLA A/B/DR mismatch (mean ± SD)	4.1 (±1.2)	4 (±1.1)	3.9 (±1.2)	0.89	4.4 (±0.9)	3.6 (±1.6)	0.19
Antilymphocyte induction (%)	35.8%	20%	18%	0.72	35.2%	26.3%	0.17
Initial immunosuppression: Tacrolimus + mycophenolic acid	98.6%	100%	100%	–	100%	100%	–
Delayed graft function (%)	37.9%	42.9%	23.5%	0.45	61.9%	13.3%	0005
Acute rejection (%) (ACR/ABMR/Borderline)	7.2% (8/2/6)	0%	9.1% (2/0/0)	–	23.5% (2/1/1)	5.2% (0/1/0)	0.29
DSA class-I (n, %)							
Only class-I	4 (1.7%)	0	0	–	0	0	0.99
Class-I and -II	0	0	0		4 (23.5%)	2 (10.5%)	
Follow-up after KT [months: median (IQR)]	45 (26–66)	56 (28–97)	80 (52–103)	0.22	66 (40–86)	47 (23–82.5)	0.21

graft function was higher in patients with HLA DP antibodies (61.9% vs. 13.3%, p = 0.005).However, a multivariate analysis adjusted for donor age and cold ischemia time, made this significant difference in DGF to disappear (HR 4.9; p = 0.14; IC 95% 0.2–42.5).The incidence of biopsy-proven acute rejection according to Banff 2009 classification was numerically but not significantly higher in the group of patients with HLA DP antibodies (23.5 vs. 5.2%, p = 0.59). They were T cell-mediated (ACR, Banff 4 category), except for two acute antibody-mediated rejection episodes (ABMR, Banff 2 category) (one in a patient with HLA DP antibodies and one in a recipient without HLA DP antibodies).

Available donor DNA permitted typing for DP in 7 cases with pretransplant antibodies: for 5 patients in the group with HLA non-DSA, DP antibodies resulted to be non-donor-specific and none of them presented clinical ABMR (graft survival: 100%). The remaining two recipients with available donor DP typing resulted to have DP DSA, besides DQ and DR DSA; both presented ABMR.

3.1.3. Characteristics of DP antibodies

Median MFI of HLA DPB1* antibodies was similar in those with DSA and without DSA (3623 [IQR: 2067–15.736] vs. 3672 [IQR: 1683–7279]). The most frequently detected DPB1* specificity was DPB1*05:01, in 18.5% patients. In the subgroup with DSA, the most frequent specificity was DPB1*04:02 and in those with HLA non-DSA, it was DPB1*02:01. The distribution of specificities is depicted in Fig. 1A. Analysis of DP epitopes in patients with pretransplant DP antibodies showed that 84DEAV was the most frequent (19.5%), followed by 35LV (14.6%); and 127L, 55DE and 84 VG were present in 9.8% of the patients.

All 27 patients with pretransplant anti-HLA DP antibodies had prior sensitizing events (51.8% prior transplant, 26% blood transfusion and 24% pregnancy). We could not find any correlation between a specific sensitizing event and different epitopes.

In addition to HLA DP antibodies, other specificities were detected in 90% of patients (Fig. 2B). Only 3 patients had isolated HLA DP antibodies before KT. They had received blood transfusions without other sensitizing events. The median (IQR) MFI was 3059 (1937–3590). After KT, these DP antibodies disappear. At follow up, graft survival was 100%, and none of them suffered acute rejection episode.

3.1.4. Impact of pretransplant HLA DP antibodies on graft survival

Patients with pretransplant DSA showed worse censored-for-death graft survival than those with anti-HLA non-DSA (p = 0.01) after a median follow-up of 76.5 months (IQR 35–149.7). The presence or absence of HLA DP antibodies did not affect graft survival in patients with DSA (p = 0.54) and with HLA non-DSA (Fig. 2), neither the strength of DP antibodies did (we did not find any MFI cut-off for DP antibodies which correlated with graft loss).

A Cox regression analysis adjusted for retransplantation, recipient gender and pretransplant CDC PRA showed similar survival in patients with DSA despite the presence of HLA DP antibodies before kidney transplantation (Table 3).

3.2. Posttransplant antibodies

3.2.1. HLA DP antibodies in patients with posttransplant HLA antibodies

The analysis of posttransplant antibodies in 346 patients showed that 59 (17.1%) had a positive screening for HLA class-II IgG antibodies. Thirty-three of them (56%) with HLA DP antibodies. When we compared patients with HLA DP antibodies (n = 33) and those without (n = 26), there were no differences in age, gender, type of donor, retransplantation, HLA mismatches and pretransplant sensitization (Table 2). Biopsy-proven acute rejection rate was similar in both groups, being all T cell-mediated. No significant differences were detected in the proportion of patients with pretransplant DSA between those with posttransplant HLA DP antibodies and patients without HLA DP antibodies (42.4 vs. 35.6%, p = 0.81)).

3.2.2. HLA DP antibodies in patients with posttransplant DSA

Posttransplant assessment evidenced DSA in 23 patients (39%). The presence of HLA DP antibodies in this group was 60.9% vs. 52.8% in patients with anti-HLA non-DSA.

3.2.3. Characteristics of posttransplant DP antibodies

Posttransplant HLA DP antibodies showed a median MFI of 3116 (1814–4322). The most frequent DPB1* specificities were DPB1*17:01 (21.2%) followed by DPB1*13:01 and DPB1*18:01, present in 18.2% of patients. Similarly to pretransplantation, in most patients (91%) the presence of HLA DP antibodies was associated with other anti-HLA class-II DR and/or DQ specificities (Fig. 3A). Only 3 patients had isolated HLA DP antibodies. In 2 cases, they appeared de novo after transplantation. At the end of follow-up, those 3 patients had a functioning graft and had not suffered rejection episodes. Median anti-HLA DP MFI values was similar in patients with DSA and patients with anti-HLA non-DSA [2435 (1718–3869) vs. 2650 (1654–4289), respectively]. De novo posttransplant HLA DP antibodies were detected in 5 patients with DSA and 8 patients without DSA. In 6 patients we could not confirm the de novo appearance, as pretransplant sera were not available (Fig. 3B).

Analysis of DP epitopes revealed that 84DEAV was the most common target posttransplantation being present in 19% of patients with DP antibodies, same as before kidney transplantation. Epitopes 8V, 76I, 55DE and 35FV were detected in 14.3% patients respectively.

3.2.4. Impact of posttransplant HLA DP antibodies on graft survival

Death-censored graft survival was better in patients without DSA after a median follow-up of 48 (35–50.7) months (p = 0.022) after

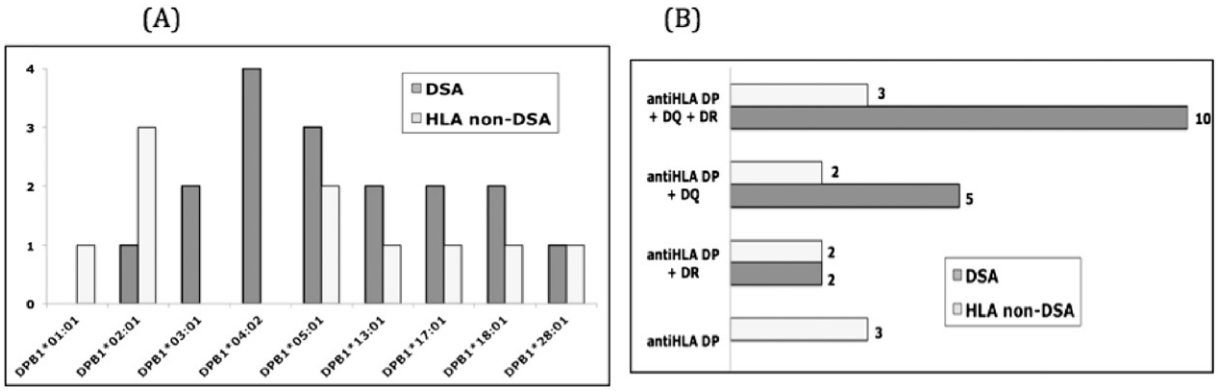

Fig. 1. (A) Pretransplant HLA-DP antibodies specificities (B) Detection of DP antibodies and other HLA class II antibodies (DR, DQ).

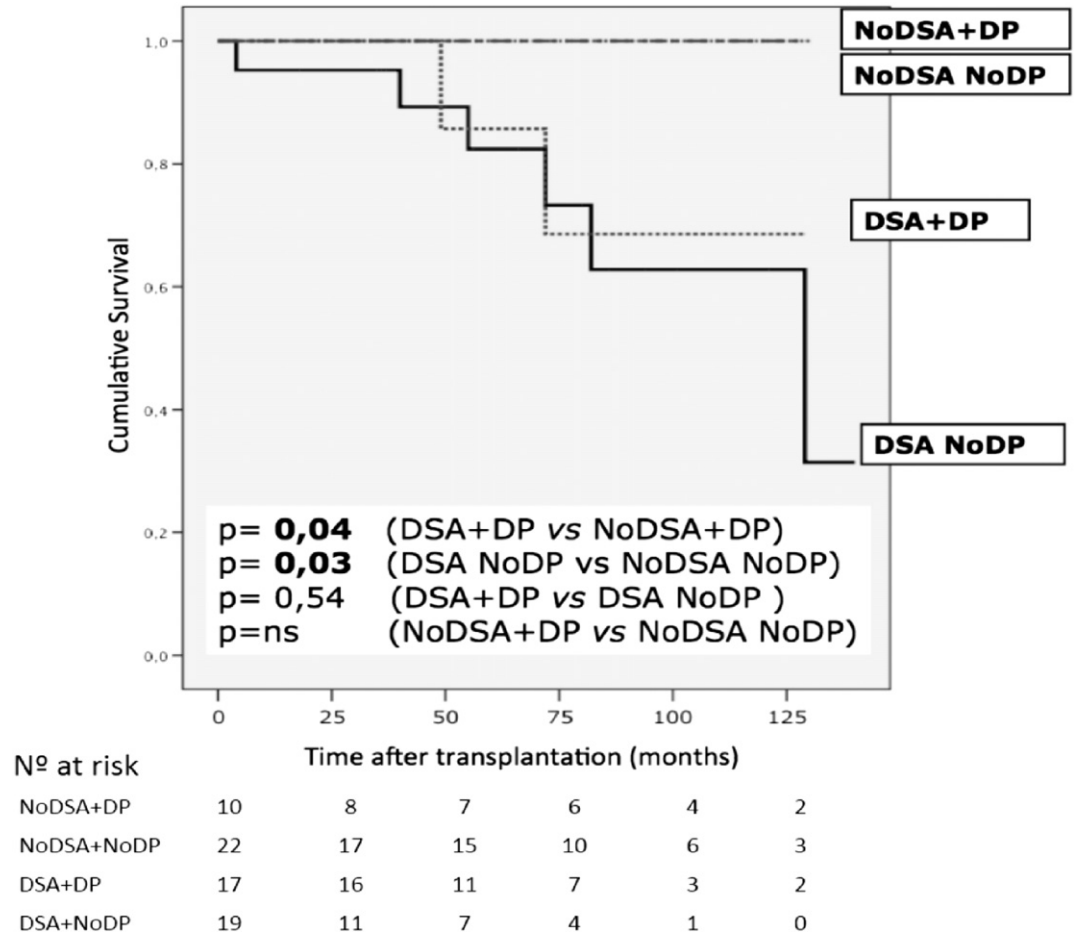

Fig. 2. Kidney allograft survival according to donor specific antibodies (DSA) and antiHLA DP antibodies detected pretransplantation.

HLA antibody testing. The presence of HLA DP antibodies did not modify the impact on graft-survival. Patients with DSA with or without HLA DP antibodies showed comparable graft survival ($p = 0.34$). Similarly, among those patients with non-DSA HLA antibodies, patients with and without HLA DP antibodies did not show differences in graft survival at the end of follow-up ($p = 0.50$) (Fig. 4). We did not find that strong compared to weak DP antibodies had a different impact on graft survival.

A Cox regression analysis adjusted for retransplantation, receptor gender and pretransplant CDC PRA showed no differences in graft survival between patients with DSA with or without HLA DP after the transplant (Table 3).

4. Discussion

In our experience, the prevalence of Luminex® detected anti-HLA DP antibodies is similar before and after KT (9.2% vs. 9.5% respectively). However, the prevalence of isolated anti-HLA DP is very low. Only 3 patients before and 3 patients after KT showed those antibodies without any other class-II specificities (1% vs. 0.9% respectively). In our study, 53% of patients with pretransplant anti-HLA antibodies had DSA, and almost half of them anti-HLA DP. In the posttransplant study, 39% of patients showed DSA, 60% of them being anti-HLA DP. We cannot provide information of weather these anti-HLA DP antibodies are against the donor. Our results suggest that these antibodies do not impact graft survival, neither in patients with DSA nor in those without DSA. Consequently, it is possible that the assessment of anti-HLA DP specificities would only be necessary in selected cases, for instance in those with suspicion of humoral rejection and apparently negative DSA.

Before LSA assessments became common practice, clinical studies including the detection of HLA DP antibodies were scarcely reported. Technical complexity for the detection of such antibodies with lymphocytotoxicity probably explains this fact. In the most relevant pre-Luminex study, the presence of HLA DP antibodies in sera from 505 patients included in a waiting list for KT showed a prevalence of 7.3% [12].

Despite the fact that solid-phase immunoassays allow precise detection of HLA antibodies, only few studies have reported their data on Luminex detected HLA DP antibodies. One of the first studies assessed 738 waitlisted patients with FlowPRA® beads. Anti-HLA class-II antibodies were detected in 23.1% of patients, a similar proportion to ours (23.3%) [13]. In this series of KT candidates, 12% of patients showed HLA DP antibodies. Two additional studies using Luminex® beads showed similar pretransplant prevalences [11,14].

The posttransplant impact of the presence of HLA DP antibodies has been even less frequently reported. Before the development of solid-phase assays, a large study in more than 3600 patients receiving a deceased-donor primary KT evaluated the influence of mismatches in HLA DPB locus in one-year graft survival. No differences were observed when comparing all patients with 0, 1 or 2 DPB mismatches [15]. The increased number of DPB mismatches only showed a negative influence in graft survival in the subgroup of 1305 retransplantations: the 345 KT recipients without DPB mismatches, showed one-year graft survival of 83%, significantly better than those with 1 or 2 mismatches. In a later study, undertaken with Luminex platform, the presence of HLA DP antibodies was more frequent in patients with graft rejection (19.5% vs. 5.1%, $p < 0001$), though the impact on survival was not reported [16].

Numerous studies have demonstrated that the presence of DSA, both pre- and posttransplantation is associated with a negative impact on renal allograft survival [17–20]. Our results confirm this negative

Table 2
Demographic and clinical characteristics of studied patients distributed according to the presence or absence of donor-specific antibodies (DSA) and the presence or absence of HLA DP antibodies after kidney transplantation.

	No antibodies (n = 287)	Anti-HLA					
		No DSA (n = 36)			DSA (n = 23)		
		HLA DP antibodies (n = 19)	No HLA DP antibodies (n = 17)	p	HLA DP antibodies (n = 14)	No HLA DP antibodies (n = 9)	p
Recipient age (years, mean ± SD)	48.8 (±14.2)	50.1 (±14.9)	48.9 (±15.6)	0.98	48.1 (±12.2)	48.3 (±12.4)	0.96
Female recipient (%)	34.1%	66.9%	56.5%	0.29	60%	61.9%	0.94
Deceased donor (%)	91.6%	100%	100%	–	100%	84.6%	0.43
Donor age (years, mean ± SD)	47.5 (±16.1)	40 (±22.9)	46.2 (±12.4)	0.13	42.1 (±16.2)	42.4 (±15.3)	0.83
Expanded Criteria Donors (%)	36.7% 85/231	40% 4/10	31.2% 5/16	0.12	22.2% 2/9	33.3% 3/9	0.41
Cold ischemia time (hours: median [IQR])	15 (12–20)	18 (15–18)	14 (12–20)	0.18	15.5 (14–16)	15 (9–17)	0.52
Retransplantation (%)	9.4%	23.1%	47.8%	0.14	60%	61.5%	0.92
Peak PRA CDC > 5% (%)	12%	23.1%	26.1%	0.84	30%	61.5%	0.21
Pretransplant PRA CDC > 5% (%)	1.8%	0	0	–	0	13.3%	0.07
HLA A/B/DR mismatch (mean ± SD)	3.7 (±1.4)	4.1 (±0.98)	3.04 (±1.5)	0.02	3.9 (±1.1)	3.9 (±1.2)	0.19
Antilymphocyte induction (%)	27.1%	15.7%	23.5%	0.46	35.7%	33.3%	0.98
Initial immunosuppression: Tacrolimus + mycophenolic acid	84.3%	94.7%	88.3%	0.31	78.5%	88.8%	0.27
Delayed graft function (%)	41.4%	53.8%	47.8%	0.83	50.1%	53.6%	0.85
Acute rejection (%) (ACR/ABMR/Borderline)	5.9% (9/2/6)	0	23.5% (3/0/1)	–	14.3% (0/2/0)	22.2% (2/0/0)	0.59
Pretransplant DSA (%)	6.8%	44.1%	46.6%	0.91	83.3%	85.7%	0.97
Posttransplant DSA class-I (n, %)							
Only class-I	2 (0.7%)	0	1 (5.9%)	–	0	0	–
Class-I and -II	0	0	0		0	0	
Follow-up after HLA test [months: median (IQR)]	48 (40–51)	48 (29–52)	47 (44–51)	0.61	45.5 (30–48)	29 (26–34)	0.87

impact, both for pre- and posttransplant DSA. Donor HLA DP typing is not usually performed, and consequently, it is difficult to know whether or not the IgG antibodies detected with single antigen studies are directed specifically against the donor antigens. As a result, the potential clinical relevance, the relationship with humoral rejection, and the impact on graft survival are not easy to assess, as it has more easily been analyzed for class-II anti-HLA DR and DQ [21–23]. Two reports have evaluated the impact of the presence of DSA against HLA DP compared with anti-HLA DP non-DSA. Three of 6 patients with pretransplant donor-specific anti-HLA DP, without any other DSA, developed antibody-mediated rejection, compared with only 1 of 15 patients with non-donor specific anti-HLA DP ($p = 0.02$). The main concern of this latter study, in addition to the low number of patients, is that no data was reported on the impact of humoral rejection on graft survival or graft loss [24]. In a recent publication, Bachelet et al. included 24 patients with only DP DSA within a selected cohort of 199 sensitized recipients. They found that patients with pretransplant DP and/or C DSA had worse graft survival compared to patients with no DSA, but similar to survival of recipients with other DSA. No information was provided on survival for patients with only DP DSA, but they showed a higher rate of clinical ABMR [25]. Other authors have described isolated cases of ABMR in patients with donor specific HLA DP antibodies without other DSA [26–28].

The main limitation of our study is the absence of information regarding donor HLA-DP typing, resulting in the impossibility of identifying true donor-specificity of anti-HLA DP antibodies. Besides, patients were included in the study only if their kidney grafts survived over three months. LSA tests were not performed for all patients included

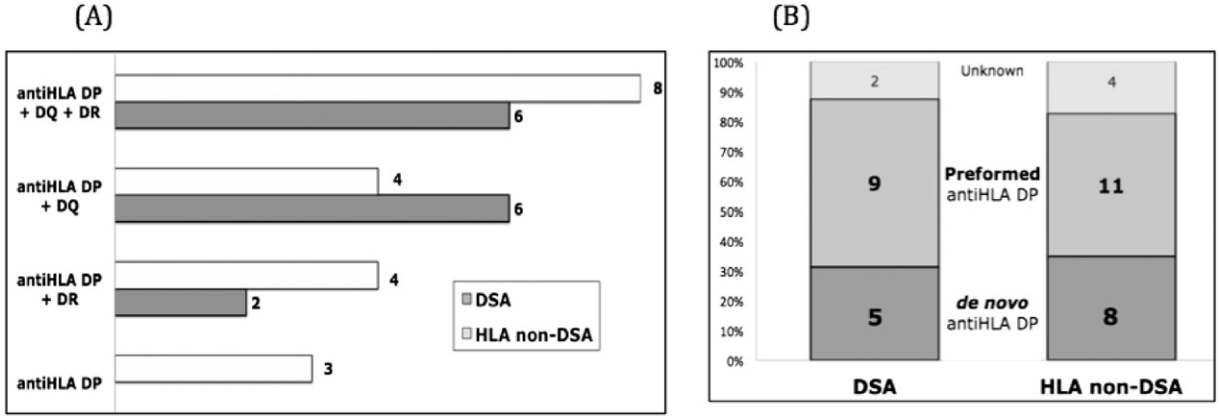

Fig. 3. (A) Detection of DP antibodies and other HLA class II antibodies (DR, DQ). (B) Preformed and de novo antiHLA-DP antibodies in patients with donor specific antibodies (DSA) and non-donor specific antibodies (HLA non-DSA).

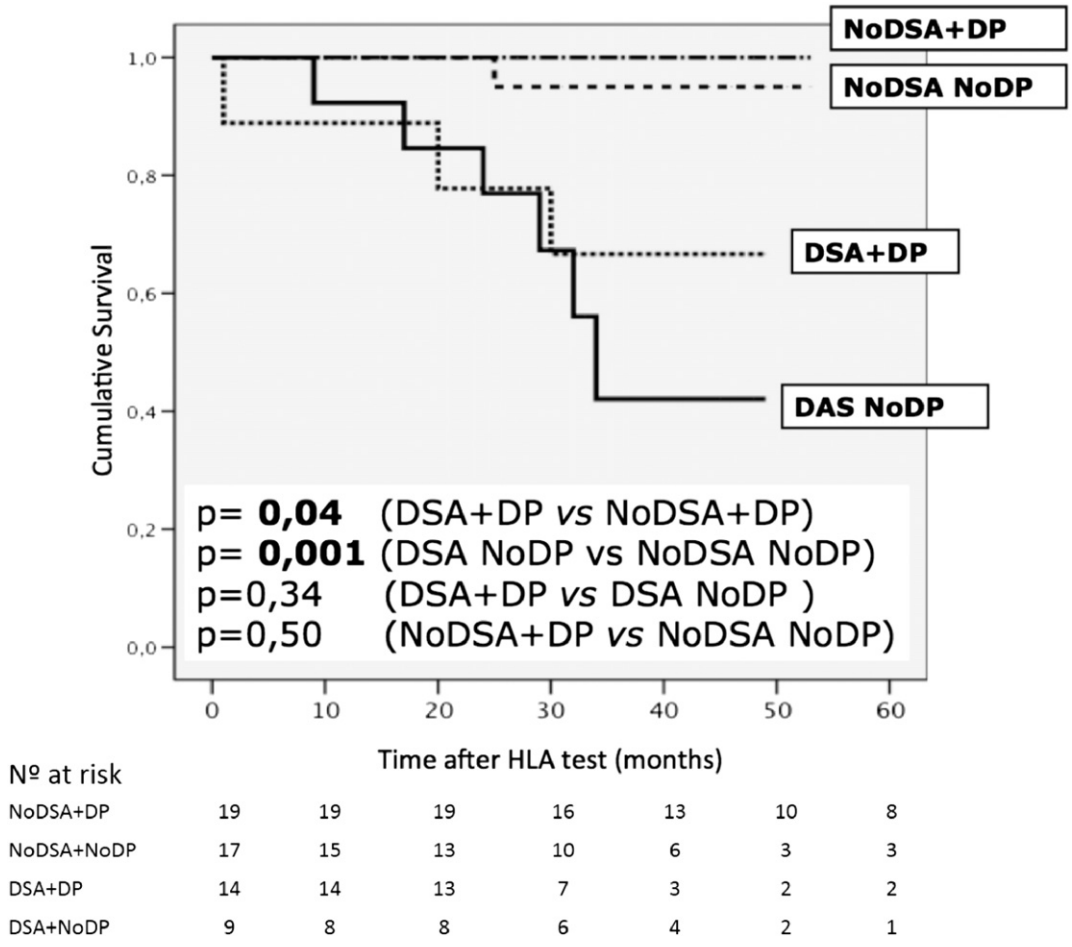

Fig. 4. Kidney allograft survival according to donor specific antibodies (DSA) and antiHLA DP antibodies detected posttransplantation.

in the study but only for those who had a positive HLA class-II screening result before or after transplantation, thus assuming sera which screened negative should not show significant DP antibodies.

5. Conclusions

In our population, approximately 10% of KT recipients show anti-HLA DP antibodies in single antigen studies, both pre- and posttransplantation. The presence of pretransplant and posttransplant DSA are both associated with a negative impact on graft survival. However, the presence of HLA DP antibodies combined with DSA does not modify this impact significantly. Nevertheless, DP antibodies have been identified in cases of antibody-mediated rejection. In cases of humoral rejection with undetected HLA A, B, C, DR or DQ DSA, HLA DP antibodies, as well as other non-HLA antibodies, should be assessed. Larger studies with KT donors well typed for HLA DP are needed to confirm our results.

Table 3
Cox regression analysis for graft survival (death-censored) comparing DSA patients with and without HLA DP antibodies before and after transplantation.

		Pre-transplantation		
		HR	IC 95%	p
Retransplantation	1.59	0.18	13.8	0.67
Female recipient	1.05	0097	11.39	0.96
Pretransplant PRC CDC	0.99	0.93	1.05	0.81
Delayed graft function	4.9	0.2	42.5	0.14

Contributors

DRP, JP and MC designed research the study, performed the study, analyzed data and wrote the paper; CG and JJH performed the antibody tests; JG did the pathology studies; MJPS and MM collected data. All authors reviewed the manuscript draft and approved the final version.

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