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

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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-

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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