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Impact of *de novo* donor-specific anti-HLA antibodies on grafts outcomes in simultaneous pancreas–kidney transplantation

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Summary

De novo donor-specific antibodies (dDSA) relevance in simultaneous pancreas–kidney (SPK) transplantation has been scarcely investigated. We analyzed dDSA relationship with grafts outcomes in a long-term follow-up SPK-transplanted cohort. In 150 patients that received SPK transplant between 2000 and 2013, post-transplant anti-human leukocyte antigen (HLA) antibodies were screened and identified using Luminex-based assays in sera collected at 3, 6, and 12 months, then yearly. dDSA were detected in 22 (14.7%) patients at a median 3.1 years after transplant. Pretransplant anti-HLA sensitization (OR = 4.64), full HLA-DR mismatch (OR = 4.38), and previous acute cellular rejection (OR = 9.45) were significant risk factors for dDSA. dDSA were significantly associated with kidney (in association with acute rejection) and pancreas graft failure. In dDSA+ patients, those with at least one graft failure presented more frequently dDSA against class II or I + II ($P = 0.011$) and *locus* DQ ($P = 0.043$) and had a higher median dDSA number ($P = 0.014$) and strength ($P = 0.030$). Median time between dDSA emergence and pancreas and kidney graft failure was 5 and 12 months, respectively. Emergence of dDSA increased the risk of grafts failure in SPK-transplanted patients. Full HLA-DR mismatch was associated with dDSA emergence. dDSA characteristics might help identify patients at a higher risk of graft failure.

Introduction

Simultaneous pancreas–kidney (SPK) transplantation has become the mainstay treatment in selected patients with insulin-dependent diabetes mellitus (IDDM) and end-stage renal failure, liberating them both from insulin and dialysis [1,2]. Improvements in immunosuppression protocols have allowed SPK grafts recipients to experience an incidence of acute rejection (AR) similar to kidney-only transplantation [3]. However, medium-term graft attrition rates in SPK transplantation remain significant [4,5], calling for an improvement in graft monitoring. Pancreas graft immunological surveillance has been pursued using several biomarkers and pathological evaluations [5]. In kidney transplantation, there is wider experience and knowledge about graft pathology evaluation and easily available biomarkers (e.g., creatinine, proteinuria) that allow a closer monitoring of graft function and lesion [6]. Nevertheless, undiagnosed immune-mediated injury has been shown to be responsible for many cases of late kidney graft failure [7,8].

In the past decade, screening of antibodies against human leukocyte antigens (HLA) by solid-phase assays after transplantation, particularly for *de novo* donor-specific antibodies (dDSA), has been used to track recipient alloimmune reactivity in organ transplantation [9]. Improvements in these solid-phase assays have allowed detection

and specification of anti-HLA antibodies to be done with greater precision and reproducibility [10]. Several published studies have shown that post-transplant emergence of dDSA is associated with lower graft survival in kidney [11–13], heart [14], and liver transplantation [15]. In pancreas transplantation, only two long-term series have addressed this issue [16,17]. However, its importance is evident given that alloimmune sensitization after SPK transplantation is fairly stronger than in kidney-only transplants, as a larger amount of immunogenic tissue is transplanted (kidney, exocrine and endocrine pancreatic tissues, and a segment of donor duodenum) and because it is performed frequently with a poorer HLA matching for logistic reasons, resulting in a higher incidence of AR [18].

Thus, we decided to analyze in our cohort of SPK-transplanted patients, which factors were related with formation of dDSA, their association with AR occurrence, and dDSA potential role as predictors of kidney and/or pancreas graft failure. Furthermore, dDSA characteristics were detailed in search of a potential association with grafts outcomes.

Materials and methods

Patients

All 165 consecutive adult patients who received a SPK transplant in our unit between May 2000 and December 2013 were investigated. Fifteen patients who experienced pancreas and/or kidney graft failure from surgical reasons within the first 15 days after surgery were excluded, as our objective was to study dDSA emergence and impact in SPK transplantation in the long term. Hence, only the remaining 150 patients were considered for our analyses. Pancreas transplants were performed using a systemic-enteric drainage. All patients were transplanted with a negative pretransplant T- and B-lymphocyte complement-dependent cytotoxicity crossmatch in current and peak sera and without the presence of preformed DSA. The Institutional Review Board at Centro Hospitalar do Porto approved this study.

Anti-HLA antibodies screening and specification

Anti-HLA antibodies screening was performed before transplantation in the last pretransplant sera and after transplantation at 3, 6, 12 months, then yearly post-transplant, and when clinically indicated. During follow-up, a median of nine screening samples (range 4–18) per patient was obtained in comparison with a projected median of eight samples (range 4–18) (Wilcoxon's paired test $P = 0.594$). This objective was not fulfilled in only 20 patients (13.3%), with 15 patients missing one sample and 5 two samples. In 18 patients (12%), the number of samples was above the anticipated (nine with one, five with two, and four with three samples more).

All anti-HLA antibodies screenings were performed by Luminex, prospectively since 2006 and retrospectively, for the purpose of this study, in patients transplanted before 2006. Anti-HLA antibodies were tested by multiplex microsphere based on Luminex Xmap® Technology (LABScreen® Mixed kit; OneLambda Inc., Canoga Park, CA, USA). To determine the specificity of the HLA antibodies, single-antigen bead (SAB) assays (LabScreen Single Antigen Beads®; OneLambda Inc.) were performed in patients with a positive screening. The sera used for the SAB assay were the same used for the screening. Mean fluorescence intensity (MFI) of each bead was measured using LABScan™100 flow analyzer (Luminex®, Austin, TX, USA). To account for a possible complement interference or prozone effect, when the positive control bead was inappropriately low, the sample was retested after a 1:8 dilution. The analysis was performed using HLA fusion® software (OneLambda Inc.), and a cutoff for a positive reaction was set in MFI value of $\geq$1000.

HLA typing and dDSA assignment

Donor and recipient were typed before transplant in *loci* HLA-A*, B*, and DR* using polymerase chain reaction (PCR) amplification with specific sequence primers (SSP; Olerup SSP® low resolution HLA typing kits, Stockholm, Sweden). Donor and recipient HLA-Cw*, HLA-DQ* and HLA-DP* antigens were also typed by SSP DNA-typing, when the recipient was sensitized against antigens from these *loci*. High resolution was performed in those cases in which it was necessary to establish whether the anti-HLA antibodies were dDSA.

In every patient, dDSA antigenic targets were identified through the comparison of donor–recipient HLA mismatch to the antibody profile in each patient' sample. All dDSA detected during patients' follow-up were cumulatively recorded, considering each studied sample, and taken into account when analyzing the number of dDSA and their HLA class and *locus*. For dDSA MFI analysis, we selected, within those against the same antigenic target (bead), the one with the highest MFI value of all the values observed longitudinally. Then, in each patient, we analyzed the MFI of the highest ranked dDSA bead and the MFI sum of all detected dDSA beads.

Induction protocol and maintenance immunosuppression

Per protocol, all patients received induction therapy using a polyclonal antithymocyte globulin [ATG Fresenius® (Fresenius Biotech GmbH, Gräfelfing, Germany), 3 mg/kg for 5–7 days]. All enrolled recipients had similar triple maintenance immunosuppression, consisting of oral tacrolimus, mycophenolate mofetil (MMF), and prednisolone.

Tacrolimus was started at the dose of 0.1–0.15 mg/kg/day, and the dose was adjusted to maintain a trough level in whole blood between 8 and 12 ng/ml during the first month, between 7 and 10 ng/ml during 2–3 months after transplant and between 5 and 8 ng/ml thereafter. MMF was started at the dose of 2000 mg/day, with the dose decreasing to 1000–1500 mg/day during the first month, depending on white blood cells count. Methylprednisolone was administered intravenously at doses of 500, 250, and 125 mg/day on the day of transplantation, day 1–2 and day 3–4 after the operation, respectively. Oral prednisolone was started on day 5 after the operation at the dose of 20 mg, being then tapered to 5–10 mg/day within 2–3 months after transplant. Steroids were completely withdrawn in 80 (53.3%) patients at 6 months post-transplant.

Data collection and outcomes

Data regarding recipient and donor characteristics, and pre- and post-transplantation variables were collected retrospectively. Delayed kidney graft function was defined as dialysis requirement in the first week post-transplant. Grafts biopsies were performed for cause only. Kidney graft biopsy was done when serum creatinine rose by more than 20% compared with previous measurements and/or when increased levels of proteinuria were detected. Pancreas graft biopsy was undertaken when an elevation of pancreatic enzymes was detected, in the presence of a preserved endocrine function. Estimated glomerular filtration rate (eGFR) was evaluated using the 2006 MDRD equation. All patients were followed up from time of transplant until death, graft failure, or December 31, 2014. Graft survival was analyzed considering each graft separately. All grafts survival analyses considered graft failure censored for death with a functioning graft.

Rejection diagnosis and treatment

Kidney graft rejection was defined as biopsy-proven acute rejection (BPAR), with specimens being evaluated by light microscopy and immunofluorescence staining for C4d and classified according to Banff classification as updated in 2013 [19].

Pancreas graft rejection was defined as BPAR (specimens were evaluated by light microscopy and immunofluorescence staining for C4d) and classified according to Banff classification as updated in 2011 [20]. Noteworthy, pancreas graft biopsies are performed in our center only since 2006.

Banff grade I acute cellular rejection (ACR) was treated with pulse steroids (500 mg MP for 3 days) and increased maintenance immunosuppression. All other ACRs were treated with ATG. Antibody-mediated rejection (AMR) was also treated with pulse steroids, intravenous immunoglobulin 2 g/Kg (maximum 140 g) divided in 2–4 doses associated with plasmapheresis (at least 3–5 sessions), and rituximab (single-dose of 375 mg/m^2). Patients with dDSA emergence but without signs of graft dysfunction received no specific treatment, besides optimization of tacrolimus (trough level 8–10 ng/ml) and MMF dose.

Statistical analysis

Continuous data were described using mean and standard deviation (SD) or median and interquartile range (IQR), and categorical data were expressed as number (and percentages). Categorical data including demographic, clinical, and immunological features and dDSA detection were compared using Pearson χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared with Student's *t*-test or Mann–Whitney *U*-test, as appropriate. Logistic regression analysis was used to determine significant associations between studied variables and dDSA appearance, using a multivariable model that included variables presenting $P \leq 0.1$ in the univariable analysis (recipient age, pretransplant anti-HLA sensitization, AR in any graft and DR HLA mismatches). Graft survival curves were done using Kaplan–Meier method and compared by log-rank test.

A two-sided *P*-value <0.05 was considered as statistically significant. Statistical analyses were performed using SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics

In our sample of 150 SPK grafts recipients, anti-HLA antibodies were detected post-transplant in 39 (26.0%) patients, with 22 (14.7%) of them having *de novo* antibodies directed against their donor HLA molecules (dDSA+). The proportion of patients with missing screening samples was similar between dDSA− (18/128) than in dDSA+ (2/22, both patients had one missing sample) patients ($P = 0.739$).

Patient, donor, and transplant characteristics are given in Table 1. dDSA+ patients were more frequently sensitized against HLA class I ($P = 0.035$) or II ($P = 0.041$) before transplantation. A trend toward a full HLA-DR mismatch ($P = 0.065$) and younger age ($P = 0.070$) in dDSA+ patients was also noticeable.

Post-transplant outcomes

Clinical outcomes after transplantation are detailed in Table 2. Studied population mean follow-up was 7.4 years, with no significant differences between groups. dDSA

Table 1. Baseline characteristics.

	Total (*N* = 150)	dDSA− (*N* = 128)	dDSA+ (*N* = 22)	*P*-value
Recipient				
Age (years), mean ± SD	34.9 ± 6.1	35.3 ± 6.0	32.5 ± 6.4	0.070
Female gender, *n* (%)	78 (52.0)	66 (51.6)	12 (54.5)	0.796
Years of IDDM, mean ± SD	24.0 ± 5.9	24.2 ± 6.0	22.8 ± 5.6	0.287
Previous blood transfusions, *n* (%)	44 (29.3)	39 (30.5)	5 (22.7)	0.461
Previous pregnancies, *n* (%)	28 (18.7)	25 (19.5)	3 (13.6)	0.767
Previous dialysis technique				
Hemodialysis, *n* (%)	111 (74.0)	96 (75.0)	15 (68.2)	0.408
Peritoneal dialysis, *n* (%)	33 (22.0)	28 (21.9)	5 (22.7)	
Preemptive, *n* (%)	6 (4.0)	4 (3.1)	2 (9.1)	
Months on dialysis, median (IQR)*	23.5 (16.0–36.0)	24.5 (17.0–36.0)	19.0 (12.0–44.0)	0.352
Peak panel reactive antibody >5%, *n* (%)	10 (6.7)	8 (6.3)	2 (9.1)	0.641
Anti-HLA antibodies pretransplant, *n* (%)				
Class I				
Undetected	136 (90.7)	119 (93.0)	17 (77.3)	0.035
Third party	14 (9.3)	9 (7.0)	5 (22.7)	
Class II				
Undetected	144 (96.0)	125 (97.7)	19 (86.4)	0.041
Third party	6 (4.0)	3 (2.3)	3 (13.6)	
Donor				
Age (years), mean ± SD	27.8 ± 10.3	27.7 ± 10.3	28.5 ± 10.2	0.731
Female gender, *n* (%)	65 (43.3)	55 (43.0)	10 (45.5)	0.828
Transplant				
ABDR HLA mismatches, *n* (%)				
0–4	72 (48.0)	60 (46.9)	12 (54.5)	0.506
5–6	78 (52.0)	68 (53.1)	10 (45.5)	
AB HLA mismatches, *n* (%)				
0–2	43 (28.7)	37 (28.9)	6 (27.3)	0.876
3–4	107 (71.3)	91 (71.1)	16 (72.7)	
DR HLA mismatches, *n* (%)				
0–1	68 (45.3)	62 (48.4)	6 (27.3)	0.065
2	82 (54.7)	66 (51.6)	16 (66.7)	
Cold ischemia time (h), mean ± SD	14.5 ± 5.9	14.4 ± 5.8	14.8 ± 6.3	0.768
Delayed kidney graft function, *n* (%)	20 (13.3)	15 (11.7)	5 (22.7)	0.177

dDSA, *de novo* donor-specific antibody; SD, standard deviation; IDDM, insulin-dependent diabetes mellitus; IQR, interquartile range; HLA, human leukocyte antigen; h, hours.

*N = 144, after exclusion of patients who received a preemptive transplant.

detection timing ranged from 0.1 to 10.0 years post-transplant (median 3.1 years) (Fig. 1). All dDSA were detected before occurrence of failure in any of the grafts.

The occurrence of BPAR in any graft was more common in dDSA+ (40.9%) in comparison with dDSA− (9.4%) patients (P = 0.001). Median time from transplant until first BPAR was 3.8 months, with no observable difference between dDSA− and dDSA+ patients (P = 0.413). All dDSA+ patients who experienced BPAR (n = 9) had dDSA detected at the time or after AR (median 2.4 months, range 0–24.6).

The impact on clinical outcomes of steroid withdrawal at 6 months after transplant is presented in Table 3. No significant difference between patients on or withdrawn from steroids was noticeable in terms of dDSA emergence (P = 0.263) or graft failure (P = 0.413). Expectedly, patients with early BPAR (<6 months) were more frequently kept on steroids (P = 0.003). Later occurring BPAR episodes (>6 months) were similar between groups (P = 0.751).

In a multivariable logistic regression analysis, after exclusion of patients who experienced AMR (n = 5), pretransplant anti-HLA sensitization (OR = 4.64, P = 0.023), full HLA-DR mismatch (OR = 4.38, P = 0.028), and biopsy-proven ACR in any graft (OR = 9.45, P = 0.002) were shown to be significantly associated with dDSA (Table 4).

Kidney graft outcomes

Biopsy-proven acute rejection in the kidney graft (Table 2) occurred more frequently in dDSA+ (36.4%) than in dDSA− (7.0%) patients (P = 0.001). Noteworthy, Banff

Table 2. Patient and grafts outcomes.

	Total (*N* = 150)	dDSA− (*N* = 128)	dDSA+ (*N* = 22)	*P*-value
Patients follow-up time (years), mean ± SD	7.4 ± 3.7	7.5 ± 3.7	7.1 ± 3.6	0.642
Patients with BPAR in any graft, *n* (%)	21 (14.0)	12 (9.4)	9 (40.9)	0.001
Months until first BPAR, median (IQR)*	3.8 (0.6–14.6)	2.6 (0.4–13.3)	9.1 (0.8–24.1)	0.413
BPAR in kidney graft, *n* (%)	17 (11.3)	9 (7.0)	8 (36.4)	0.001
ACR, *n*	16	9	7	
Banff grade 1, *n*	7	6	1	
Banff grade 2/3, *n*	9	3	6	
Acute AMR, *n*	4†	0	4†	
Second BPAR in kidney graft, *n* (%)	5	1	4	
eGFR at last visit (ml/min), mean ± SD‡	58.5 ± 19.7	59.7 ± 19.8	48.9 ± 16.3	0.035
Kidney graft failures, *n* (%)	11 (7.3)	6 (4.7)	5 (22.7)	0.011
Causes				
Infection, *n*	2	2	0	^
BKV nephropathy, *n*	1	1	0	
Rejection, *n*	6	1	5	
Unknown, *n*	2	2	0	
BPAR in pancreas graft, *n* (%)	8 (5.3)	3 (2.3)	5 (22.7)	0.002
ACR Banff grade 1, *n*	1	1	0	
ACR Banff grade 2/3, *n*	6	2	4	
Acute AMR, *n*	1	0	1	
C-peptide at last visit (ng/ml), mean ± SD§	3.02 ± 1.49	3.07 ± 1.52	2.41 ± 0.99	0.078
Pancreas graft failures, *n* (%)	23 (15.3)	14 (10.9)	9 (40.9)	0.001
Causes				
Infection, *n*	3	3	0	
Rejection, *n*	7	2	7	
Vascular, *n*	2	3	1	
Unknown, *n*	4	3	1	
Pancreatic fistula, *n*	3	3	0	
Patient deaths, *n* (%)	8 (5.3)	5 (3.9)	3 (13.6)	0.094
Causes				
Infection, *n*	4	3	1	
Myocardial infarct, *n*	2	2	0	
Digestive bleeding, *n*	1	0	1	
Stroke, *n*	1	0	1	

dDSA, *de novo* donor-specific antibody; SD, standard deviation; BPAR, biopsy-proven acute rejection; IQR, interquartile range; ACR, acute cellular rejection; AMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate by MDRD formula.

*$N = 21$.

†One patient presented AMR without ACR. In the other three cases, ACR was classified concomitantly (2 grade 2, 1 grade 1).

‡$N = 131$ (117 dDSA−, 14 dDSA+), after exclusion of kidney graft failures and patient deaths.

§$N = 119$ (109 dDSA−, 10 dDSA+), after exclusion of pancreas graft failures and patient deaths.

grade 2/3 ACR was more common in dDSA+ (6/7) than in dDSA− patients (3/9). A higher proportion of dDSA+ patients (4/8 vs. 1/9) had a second episode of kidney graft BPAR.

Acute AMR occurred early after transplant in two patients, with good response to treatment, and no effect on graft survival. The other two patients experienced acute AMR after the first year in a noncompliance setting, with dismal consequences for them as they lost both their grafts (in one patient, a pancreas graft biopsy was performed showing a ACR grade 1).

Death-censored kidney graft survival according to dDSA presence is shown in Fig. 2. At 8-year follow-up, kidney graft survival was 97.3% in dDSA− and 76.2% in dDSA+ patients ($P = 0.001$). Causes of kidney graft failure are presented in Table 2. All graft failures ($n = 5$) in dDSA+ patients were deemed as rejection driven in comparison with only one (out of 6) in dDSA− patients. However, no significant association was observed between kidney graft failure and dDSA detection, if BPAR occurrence status was taken into consideration through a stratified analysis (Table 5). Nonetheless, kidney graft eGFR at last visit was

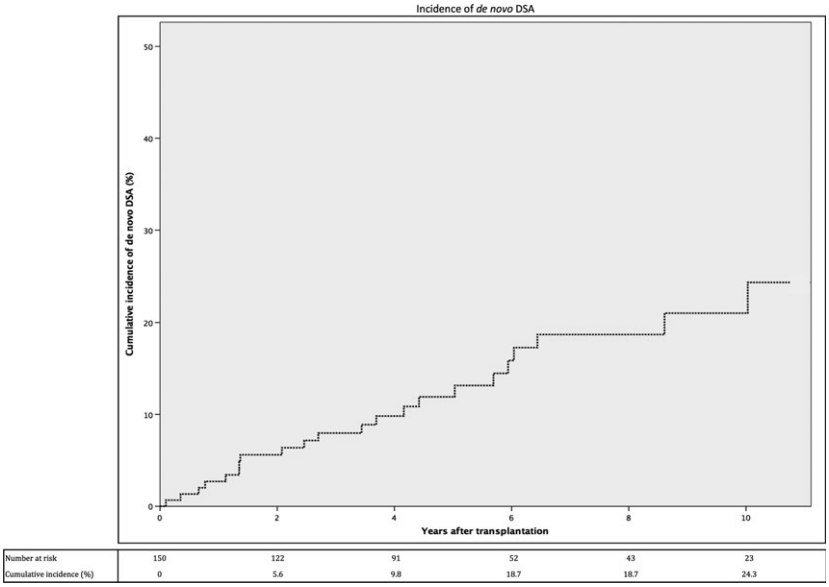

Figure 1 Cumulative incidence of *de novo* donor-specific anti-HLA antibodies by Kaplan–Meier curve. Median years until *de novo* DSA detection = 3.1 (IQR 1.3–5.8; min = 0.1; max = 10.0).

Table 3. Association between steroid withdrawal at 6 months and clinical outcomes.

	Total (N = 150)	No steroids (N = 80)	On steroids (N = 70)	P-value
dDSA+, *n* (%)*	20 (53.4)	13 (16.5)	7 (10.1)	0.263
Patients with BPAR in any graft				
Before 6 months, *n* (%)	11 (7.3)	1 (1.3)	10 (14.3)	0.003
After 6 months, *n* (%)	10 (6.7)	6 (7.5)	4 (5.7)	0.751
Failure of any graft, *n* (%)	28 (18.7)	13 (16.3)	15 (21.4)	0.413
Kidney graft failure, *n* (%)	23 (15.3)	13 (16.3)	10 (14.3)	0.739
Pancreas graft failure, *n* (%)	11 (7.3)	6 (7.5)	5 (7.1)	0.933

dDSA, *de novo* donor-specific antibody; BPAR, biopsy-proven acute rejection.
*Two patients with dDSA appearance before the 6th month post-transplant were excluded, one from each group.

Table 4. Multivariable logistic regression analysis for predictors of *de novo* DSA.*

	OR (95% CI)	P-value
Recipient age, per year	0.948 (0.868–1.034)	0.226
Presence of anti-HLA antibodies pretransplant	4.636 (1.237–17.376)	0.023
DR HLA mismatches 2 (vs. 0–1)	4.384 (1.168–16.452)	0.028
Biopsy-proven ACR in any graft	9.450 (2.345–38.080)	0.002

CI, confidence interval; HLA, human leukocyte antigen; ACR, acute cellular rejection.
*N = 145 (five patients were excluded given that they experienced antibody-mediated rejection with concomitant detection of dDSA).

significantly lower in dDSA+ patients (P = 0.035) (Table 2).

Pancreas graft outcomes

The occurrence of BPAR in the pancreas graft (Table 2) occurred more frequently in dDSA+ (22.7%) than in dDSA− (2.3%) patients (P = 0.002). Most BPARs were graded as Banff 2/3, both in dDSA− (2/3) and in dDSA+ (4/5) patients. Noteworthy, one dDSA+ patient had acute AMR in the pancreas graft only, early after transplant, which was successfully treated; dDSA was only detected 2 weeks after rejection.

Death-censored pancreas graft survival according to dDSA presence is shown in Fig. 3. At 8-year follow-up, pancreas graft survival was 89.1% in dDSA− and 47.4% in dDSA+ patients (P < 0.001). Causes of pancreas graft failure are presented in Table 2. Seven of nine graft failures in dDSA+ patients were deemed as rejection driven in comparison with only 2 (out of 14) in dDSA− patients. Interestingly, an association between pancreas graft failure and dDSA detection, even in the absence of BPAR occurrence (P = 0.043), was observed (Table 6). Moreover, a trend for lower C-peptide levels in dDSA+ patients was noticeable at the end of follow-up (P = 0.078) (Table 2).

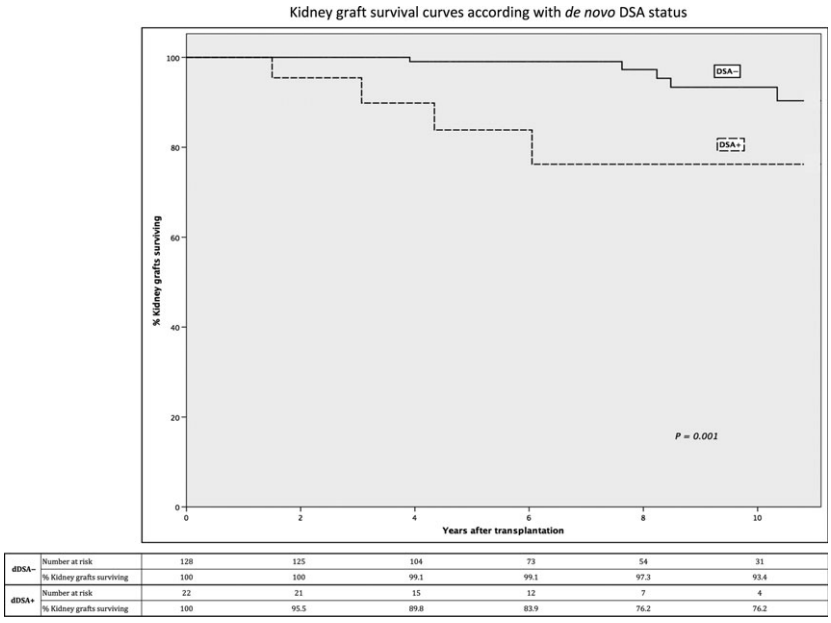

Figure 2 Death-censored kidney graft survival in patients with *de novo* donor-specific anti-HLA antibodies (DSA+, $n = 22$) and those without (DSA−, $n = 128$).

Table 5. Association between BPAR, *de novo* DSA, and kidney graft outcomes.

No BPAR ($N = 133$)	Graft functioning ($N = 129$)	Graft failed ($N = 4$)	*P*-value
dDSA−	116 (89.9%)	3 (75.0%)	0.363
dDSA+	13 (10.1%)	1 (25.0%)	
BPAR ($N = 17$)	**Graft functioning ($N = 10$)**	**Graft failed ($N = 7$)**	***P*-value**
dDSA−	6 (60.0%)	3 (42.9%)	0.637
dDSA+	4 (40.0%)	4 (57.1%)	

dDSA, *de novo* donor-specific antibody; BPAR, biopsy-proven acute rejection.

Patient outcomes

Patient death occurred more frequently in dDSA+ ($n = 3$, 13.6%) than in dDSA− ($n = 5$, 3.9%) group ($P = 0.094$). dDSA appearance preceded patient death by 2.9–4.2 years. Infectious ($n = 4$) and vascular ($n = 2$) events were the more common causes of death.

Graft failure and dDSA characteristics

Comparison of dDSA characteristics in patients with at least one graft lost ($n = 10$) and those with both grafts functioning ($n = 12$) is shown in Table 7. Of the 32 dDSA detected, 21 (66%) were against HLA class II molecules, 12 (38%) anti-DQ and 8 (25%) anti-DR. The presence of dDSA against HLA class II and I+II was more common in patients with ≥1 graft failure ($P = 0.011$), as was dDSA against HLA *loci* DQ ($P = 0.043$) and DR ($P = 0.074$). An increasing median MFI of the highest dDSA bead ($P = 0.030$), of the sum of all dDSA beads ($P = 0.017$), and of the number of dDSA present ($P = 0.014$) was observed in patients with ≥1 graft failure. Steroid withdrawal at 6 months post-transplant had no impact on grafts outcome in dDSA+ patients ($P = 0.675$).

Time from transplantation to dDSA onset was similar between groups ($P = 0.742$). Most dDSA+ ($n = 20$) patients remained with at least one dDSA detectable throughout the serial follow-up screening. Two dDSA+ patients came to have dDSA with a MFI persistently below the threshold level (but above 500), one after three consecutive yearly positive samples, and the other after five positive yearly samples; none of them experienced BPAR or graft failure.

All pancreas graft failures in dDSA+ patients occurred within 2–13 months (median 5 months) after dDSA detection. All kidney graft failures in dDSA+ patients occurred within 3–30 months (median 12 months) after dDSA detection.

Discussion

Our study about dDSA relationship with grafts outcomes in SPK-transplanted patients has the longest follow-up time (mean 7.4 years) published to date, presenting critical data about the risk factors for dDSA emergence and its effect on

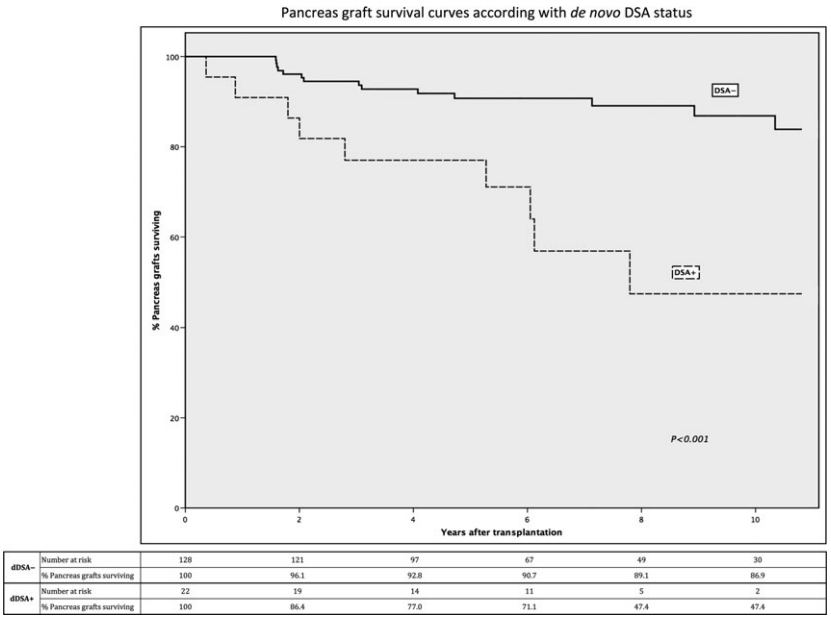

Figure 3 Death-censored pancreas graft survival in patients with *de novo* donor-specific anti-HLA antibodies (DSA+, $n = 22$) and those without (DSA−, $n = 128$).

Table 6. Association between BPAR, *de novo* DSA, and pancreas graft outcomes.

No BPAR ($N = 142$)	Graft functioning ($N = 124$)	Graft failed ($N = 18$)	*P*-value
dDSA−	112 (90.3%)	13 (72.2%)	0.043
dDSA+	12 (9.7%)	5 (27.8%)	
BPAR ($N = 8$)	Graft functioning ($N = 3$)	Graft failed ($N = 5$)	*P*-value
dDSA−	2 (66.7%)	1 (20.0%)	0.464
dDSA+	1 (33.3%)	4 (80.0%)	

dDSA, *de novo* donor-specific antibody; BPAR, biopsy-proven acute rejection.

SKP grafts. We detected 22 patients (14.7%) with dDSA at a median time of 3.1 years post-transplant. In one series with 167 pancreas graft recipients (152 patients also received a kidney graft), 15.6% developed dDSA at a median of 1 year post-transplant [16]. Another study reported that 12.8% of SPK grafts recipients developed dDSA between months 1 and 35 after transplant [17]. Cumulative incidence of dDSA in our cohort was similar to these studies, although with a comparably later onset time.

Additionally, we are able to discern risk factors for dDSA appearance like pretransplant anti-HLA sensitization and previous episodes of ACR, as several other studies have shown [12,13]. Immunosuppression minimization has also been associated with donor-specific sensitization, mainly related with the use of tacrolimus-free regimens [21]. Notably, in our cohort, steroid withdrawal at 6 months had no impact on *de novo* allosensitization or grafts outcomes. Another dDSA predictor in our cohort was full HLA-DR mismatch. Interestingly, most of our dDSA were against DQ (39%) and DR (26%) molecules, and their presence was associated with graft failure. Donor–recipient DQ typing in all patients was not possible given the costs involved (it was only performed in patients with anti-DQ antibodies), hampering our ability to analyze HLA-DR and HLA-DQ mismatches as a predictor of dDSA as others have shown [22]. The *loci* within the DR-DQ region present a tight linkage and high linkage disequilibrium as shown by a study done in a population with European ancestry [23]. We can only speculate that those patients transplanted with full HLA-DR mismatch had also a high degree mismatch in HLA-DQ *locus* with their donor.

De novo DSA association with kidney graft failure was mainly related to AR occurrence, a particularly harmful and synergistic adverse combination, as several studies have shown [11,24]. Two forms of kidney graft AR (ACR and AMR) were present in our patients and their association with dDSA is distinct. AMR is intrinsically associated with the presence of DSA. Alternatively, the association between ACR occurrence and later development of dDSA may be related with the degree of microcirculatory inflammation present at the time of the ACR, particularly the sensitizing effect of upregulated HLA proteins expression in the peritubular capillaries [21]. Moreover, histopathological analysis of vascular rejection biopsies showed that concomitant presence of peritubular capillaritis was very common

Table 7. Comparison of *de novo* DSA characteristics according to the occurrence of graft failure.

	No graft failure (N = 12)	≥1 graft failure* (N = 10)	P-value
dDSA by HLA class			
DSA class I, *n* (%)	7 (58.3)	0	0.011
DSA class II, *n* (%)	4 (33.3)	6 (60.0)	
DSA class I + II, *n* (%)	1 (8.3)	4 (40.0)	
dDSA by HLA *locus*			
Anti-HLA-A, *n*	3	1	0.594
Anti-HLA-B, *n*	3	2	1.0
Anti-HLA-Cw, *n*	2	1	1.0
Anti-HLA-DR, *n*	2	6	0.074
Anti-HLA-DQ, *n*	4	8	0.043
Anti-HLA-DP, *n*	0	1	0.455
Number of dDSA, median (IQR)	1 (1–2)	2 (2–5)	0.014
Highest MFI dDSA bead, median (IQR)	2790 (1125–5283)	8988 (3241–15058)	0.030
MFI sum of all dDSA beads, median (IQR)	3205 (1125–6220)	11923 (4088–50734)	0.017
Steroid withdrawal at 6 months, *n*	7	7	0.675
Time (years) from transplantation to first dDSA detection, median (IQR)	2.9 (1.2–5.4)	3.1 (1.2–6.0)	0.742
Months from dDSA detection until pancreas graft failure, median (range)		5.4 (1.9–13.3)	
Months from dDSA detection until kidney graft failure, median (range)		11.6 (3.1–30.2)	

dDSA, *de novo* donor-specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity; IQR, interquartile range.
*Five patients had pancreas graft failure, one kidney graft failure and four lost both grafts.

(around 90%) [25]. In our cohort, 2 (out of 4) dDSA+ patients who experienced AR and kidney graft failure had ACR (and no AMR) previously to dDSA appearance. Furthermore, the presence of dDSA, even without clinically evident AR, can ensue a chronic active antibody-mediated injury, resulting in an insidious process of graft dysfunction and shortened graft half-life [26,27]. Interestingly, we showed that kidney graft function was significantly lower in dDSA+ than in dDSA− patients at the end of follow-up.

The impact of dDSA in pancreas graft outcomes is far less studied. The scarcity of tools for an adequate surveillance of pancreas function and the difficulty in obtaining tissue samples for histological evaluation jeopardize the analysis of the dynamics of antibody-mediated pancreas graft injury [28]. In the two studies published about dDSA impact in pancreas graft failure, one has no information about pancreatic histology [17] and the other refers only three cases of pancreas graft BPAR [16]. Herein, we present eight cases of BPAR in the pancreas graft, in which a non-significant (given the small numbers involved) association between dDSA and graft failure was detected, with 4/5 cases of BPAR resulting in failure in dDSA+ in contrast with 1/3 in dDSA− patients. Furthermore, in patients with no BPAR episode, dDSA was significantly associated with graft failure (5 failures in 17 dDSA+ and 13 failures in 125 dDSA− patients, $P = 0.043$), with all cases in dDSA+ patients corresponding to transplants performed after 2005 and no graft failure happened before the end of 2009. As pancreas graft biopsies were started in our center in 2006, we believe that these results raise the possibility of a dDSA deleterious effect on pancreas graft similar to the chronic active antibody-mediated injury described in the kidney graft [27]. Naturally, only per protocol or a lower clinical threshold for pancreas graft biopsies could truly elucidate this hypothesis. Besides, in patients with pancreas graft functioning at the end of follow-up, a trend for lower levels of C-peptide in dDSA+ patients was present.

The characteristics of anti-HLA antibodies have been associated with early events after kidney transplantation (e.g., AMR) in the context of preformed DSA [29–31]. Clinical correlations of these characteristics in dDSA with graft outcomes have been less analyzed. In kidney transplantation, the presence of dDSA against HLA class II or I + II or complement-binding dDSA has been associated with poorer graft survival [11,13,32], while others demonstrated that same deleterious effect for dDSA against HLA-DQ [22]. In SPK transplantation, no association between graft outcomes and dDSA MFI [16] has been shown, while the presence of dDSA against HLA class I+II was associated with graft failure [17]. It was noticeable that presence of dDSA against HLA class II or both classes was more common in patients with graft failure, as was the prevalence of dDSA against HLA-DQ and DR *loci*. Furthermore, median dDSA number and MFI values were significantly higher in patients with graft failure. These results should be considered with caution given the small sample of dDSA+ patients

involved. Nonetheless, these observations about dDSA characteristics correlation with graft failure seem to mimic the better-understood relationship between preformed DSA characteristics and graft outcomes [29–31]. Overall, grafts failures occurred within 2–30 months after DSA detection indicating that, at least in some patients, a clinical intervention directed against DSA would have been feasible. Unfortunately, the management of dDSA outside an episode of acute AMR is still undetermined. Some have reported the use of high-dose intravenous immunoglobulin with or without rituximab in patients with chronic kidney graft dysfunction and detectable DSA with limited [33] or even null effect [34].

We recognize that this study has limitations. First, given its long-term retrospective design, changes in patients' clinical management occurred resulting in some data biases. Second, given the schedule of anti-HLA antibodies surveillance (yearly after the first year), temporal relationship between DSA formation and graft failure could not be accurately determined. Third, the known limitations of standardization across studies in SAB assays and the lack of a designated MFI threshold for dDSA positivity should be considered while interpreting our results [10]. Fourth, no information about compliance with immunosuppression was available for this study, a known risk factor for *de novo* DSA formation [13]. Lastly, dDSA complement-fixing ability was not studied in this cohort. It has been shown that the detection of complement-binding DSA after transplantation by C1q Luminex assay pertains a significant adverse effect on kidney graft survival [32]. However, recently, Schaub *et al.* [35] demonstrated a strong relationship between anti-HLA antibodies MFI and C1q assay positivity, arguing that C1q binding ability correlated essentially with the strength of the antibody.

In conclusion, we consider that our results demonstrate a strong association between *de novo* DSA and kidney and pancreas graft failure in SPK transplantation, in close relationship with AR occurrence. Improved HLA-DR (and probably HLA-DQ) matching may have a preventive role for dDSA emergence. Analysis of dDSA characteristics might select patients particularly at risk for graft failure, although further studies are necessary. Nevertheless, only new and efficacious therapeutic strategies would clearly change the adverse prognosis associated with *de novo* DSA emergence.

Authorship

JM: study concept and design, acquisition of data and patient recruitment, statistical analysis, analysis and interpretation of data and manuscript drafting. LSM: acquisition of data and patient recruitment, analysis and interpretation of data and critical revision of the manuscript for important intellectual content. ST: technical support and laboratory analysis and analysis and interpretation of data. LD: acquisition of data and patient recruitment and critical revision of the manuscript for important intellectual content. IF: statistical analysis and analysis and interpretation of data. IB: critical revision of the manuscript for important intellectual content. AC-H: acquisition of data and patient recruitment, critical revision of the manuscript for important intellectual content and study supervision. AC: study supervision.

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