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Antibody-mediated rejection (AMR) after pancreas and pancreas–kidney transplantation

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Summary

Antibody-mediated rejection (AMR) requires specific diagnostic tools and treatment and is associated with lower graft survival. We prospectively screened C4d in pancreas (n = 35, in 27 patients) and kidney (n = 33, in 21 patients) for cause biopsies. Serum amylase and lipase, amylasuria, fasting blood glucose (FBG) and 2-h capillary glucose (CG) were also analysed. We found that 27.3% of kidney biopsies and 43% of pancreatic biopsies showed C4d staining (66.7% and 53.3% diffuse in peritubular and interacinar capillaries respectively). Isolated exocrine dysfunction was the main indication for pancreas biopsy (54.3%) and was followed by both exocrine and endocrine dysfunctions (37.1%) and isolated endocrine dysfunction (8.6%). Laboratorial parameters were comparable between T-cell mediated rejection and AMR: amylase 151.5 vs. 149 U/l (P = 0.075), lipase 1120 vs. 1288.5 U/l (P = 0.83), amylasuria variation 46.5 vs. 61% (P = 0.97), FBG 69 vs. 97 mg/dl (P = 0.20) and 2-h CG maximum 149.5 vs. 197.5 mg/dl (P = 0.49) respectively. Amylasuria values after treatment correlated with pancreas allograft loss (P = 0.015). These data suggest that C4d staining should be routinely investigated when pancreas allograft dysfunction is present because of its high detection rate in cases of rejection.

Introduction

Survival rates of pancreas allografts have increased in recent years [1–3]. This is mainly attributed to a decrease in technical failure and immunologic loss [1], as well as to the improvement of immunosuppressive regimen [3]. However, acute pancreas allograft rejection, which is related to transplantation modality [4], is still a subject of major concern after transplantation because of its association with pancreas loss [4–7].

Most of the pancreatic acute rejections are T-cell mediated rejections (TCMRs), although acute antibody-mediated rejection (AMR) has been appreciated as an important cause of graft loss, inasmuch as it has a worse prognosis and requires different forms of therapy [8]. The diagnostic criteria include morphologic evidence of acute tissue injury, immunopathologic evidence for antibody action that includes C4d deposition, and serologic evidence of circulating anti-donor antibodies against human leukocyte antigens (HLA) or other anti-donor endothelial antigens [9]. The impact of AMR on pancreas transplantation has been recently studied and is associated with 30–46% of pancreas allograft loss [10,11].

We report herein the occurrence of AMR in pancreas and pancreas–kidney transplantation in for-cause biopsies prospectively screened for C4d, describe the pattern of deposition of C4d and the outcome of pancreas and kidney allografts. We also correlate both AMR and TCMR to laboratorial parameters, such as serum amylase and lipase and amylasuria.

Patients and methods

From August 2006 through December 2008, 78 biopsies of either kidney ($n = 36$) or pancreas allografts ($n = 42$) from 38 patients were performed. These patients were previously submitted to simultaneous pancreas–kidney transplantation (SPKT; $n = 28$), pancreas-after-kidney transplantation (PAKT; $n = 7$), or pancreas transplantation alone (PTA; $n = 3$). In four out 28 SPKT patients, both kidney and pancreatic biopsies were performed.

Initial immunosuppressive regimen was started on day 2 after transplantation and included: tacrolimus 0.15 mg/kg/day and adjusted according to the period after transplantation (serum levels of 10–15 ng/ml in the first 30 days, 8–10 ng/ml between 31 and 90 days and subsequently 5–10 ng/ml), methylprednisolone (500 mg intraoperative, 250 mg in the first day, and 125 mg in the second day) followed by prednisone 1 mg/day with tapering until 5 mg/day, and mycophenolate mofetil 2 g/day or mycophenolate sodium 1.44 g/day. In SPKT, induction with polyclonal antibodies was not routinely employed, except in cases of retransplantation, panel reactive antibody (PRAs) greater than 20%, or when delayed kidney allograft function was present (defined as necessity of dialysis in the first week post-transplantation). On the other hand, in PAKT and in PTA induction with thymoglobulin 1.5 mg/kg/day for 10 days was employed in all patients according to peripheral lymphocytes or CD3 counts.

In SPKT, exocrine pancreatic drainage was enteric ($n = 13$) or in the bladder ($n = 15$). In PAKT and PTA, bladder drainage was exclusive. Either iliac vein or vena cava anastomosis was performed in all cases, except in two SPKT patients who had portal drainage. Donation after cardiac death, donor age >45 years, and positive family history of diabetes in first-degree relatives were exclusion criteria for donation.

In seven cases of SPKT, live kidney was synchronously transplanted. In PAKT, kidney transplantation was previously performed with live ($n = 6$) or deceased ($n = 1$) donors. In one PAKT patient, SPKT was previously performed with live donor kidney (mother), but he presented pancreatic vascular thrombosis during the first week and was submitted to a second pancreas transplantation.

The kidney allograft biopsy criteria included delayed graft function or increase in serum creatinine. The pancreas allograft biopsy criteria included an increase in serum amylase (reference 21–100 U/l) and/or lipase (reference 30–300 U/l) irrespective of whether the same is associated or not with a decrease in amylasuria (≥50% from baseline). Amylasuria was determined in 8-h samples collected through the night. Hyperglycemia of unknown cause was also a biopsy indication. After an overnight fast of at least 8 h, venous blood was sampled for baseline values of plasma glucose (glucose-oxidase method, reference 60–99 mg/dl). Two-hour capillary glucose (CG) was also performed. When available, glycosylated hemoglobin (HbA1c, reference 4.2–6%) and basal C-peptide (reference 0.9–7.1 ng/ml) were determined by high-performance liquid chromatography and enzyme-linked immunosorbent assay (ELISA) respectively.

Laboratorial parameters were determined pre- and post-treatment, which corresponded to the samples that were collected at the time of the biopsy indication and after immunosuppressive treatment (samples collected before hospital discharge or in the first medical appointment).

Histologic analyses

The analysis of light microscopy was performed to diagnose acute TCMR according to Banff 2005 (updated in Banff 07) [9,12] for kidney allografts and Drachenberg *et al.* for pancreas allografts [6]. Acute AMR diagnosis was based on Banff 2007 criteria [9]. If there were no donor-specific antibodies (DSAs) or these data were unknown, identification of histologic features of AMR was considered as suspicious for acute or chronic AMR, particularly if there was graft dysfunction.

Briefly, the formalin-fixed biopsies were embedded in paraffin, serially sectioned at 3–4 μm thickness and stained with hematoxylin–eosin (HE), Masson's trichrome, and periodic acid-Schiff.

C4d staining was routinely performed for all pancreas and kidney allograft biopsies and was the inclusion criteria in this study. Indirect immunofluorescence on cryostat sections used a mouse monoclonal anti-human C4d antibody (Quidel, San Diego, CA, USA) at 1:40 dilution, followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA) [13]. C4d staining in the kidney and pancreas was interpreted as positive when a linear staining along peritubular capillary basement membranes or interacinar capillaries was evident in >10 of them. When this pattern was observed in >50% of capillaries, this staining was considered diffuse positive; <50% was considered focally positive.

Donor specific antibody

When C4d detection was positive, we investigated circulating antibodies. These circulating antibodies searched included HLA (Human Leukocyte Antigens) and Major-histocompatibility-complex class I-related A (MICA). The Luminex® microsphere-based assay, which uses a panel

of color-coded microspheres coated with HLA antigens to determine percent PRA and to identify antibody specificities, was employed to detect levels of circulating antibody [14]. Briefly, for each patient sample, a total of 10 μl of serum was mixed between two wells, each containing either HLA class I- or class II-coated microspheres. Sera and microspheres were incubated for 30 min on a 96-well membrane filter plate. The specimens were then washed three times. A phycoerythrin (PE)-conjugated anti-human IgG was then added to each well and incubated for 30 min. All incubations were performed at room temperature, in the dark, on a rotating platform. The LABScan™ 100 flow analyser was used for bead and data acquisition. This instrument is a minidigital processing flow analyser that incorporates two lasers. When the fluid stream passes through the lasers, the first beam classifies the beads by HLA type. The second beam scans each bead for PE-labeled anti-human IgG bound to the HLA molecules. Luminex MICA single-antigen bead assay was also performed.

Rejection treatment

The treatment of pancreas acute rejection was determined by its grade: grade I was treated with methylprednisolone pulse (500 mg/day for 3 days) and nonresponsive acute rejections or grades II and III were treated with thymoglobulin 1–1.5 mg/kg/day or OKT3 2.5–5 mg/day for 10 days according to the number of peripheral lymphocytes or to CD3 counts. Kidney acute rejection was treated with methylprednisolone pulse (500 mg/day for 3 days) for grades IA to IIA and with thymoglobulin for grades IIB and III (7–10 days).

Kidney allograft outcome was defined as total recovery (serum creatinine ≤20% in comparison to baseline values), partial recovery (serum creatinine >20% in comparison to baseline values) and graft loss (return to dialysis). Pancreas allograft outcome was defined as improvement or no improvement of serum enzymes and amylasuria and euglycemia, partial function (hyperglycemia and normal C-peptide) and graft loss (hyperglycemia and low C-peptide).

All subjects were informed about the study protocol and written consents were obtained from all participants. The Brazilian Committee of Ethics and Research approved the study.

All results are reported mean ± SD, unless otherwise indicated. Statistical analysis was performed by SPSS 12.0 (Chicago, IL, USA). Fisher's exact *t*-test and ANOVA were performed for numerical variables and Pearson's chi-squared test for categorical variables. Correlation was performed using Pearson coefficient. The receiver operating characteristic curve demonstrated the area under the curve between acute rejection diagnosis or pancreas loss and laboratorial data (serum amylase and lipase and amylasuria). To determine such of those factors which were associated with C4d detection and with acute rejection, all putative factors that were univariably associated at $P \leq 0.3$ were entered simultaneously in a backward binary logistic regression model with those factors analysed as the dependent variable. Results were reported as 95% CI. The statistical analysis was assumed significant if $P < 0.05$.

Results

Twenty-five (60.5%) out of 38 patients were male subjects. Average age, diabetes history and time on dialysis were 34.8 ± 9.9 years, 20.9 ± 5.8 years, 34.1 ± 24.3 months respectively. Induction therapy was performed in 24 patients (63.2%) with thymoglobulin ($n = 18$) or OKT3 ($n = 6$). Pretransplant PRA (determined by ELISA assay) was as follows: 0–10% patients (92.1%), 10–50% – one patient (2.6%), 50–80% – 0 patient and >80% – two patients (5.3%). Mean follow-up after kidney and pancreatic biopsies were 12.7 ± 9 months (median 12.7 months) and 12.7 ± 8.5 months (median 10.2 months) respectively.

Kidney biopsies

The kidney biopsies were performed after 293.3 ± 535 days (median 64 days). From the 36 kidney biopsies performed in 21 patients, three were related to the same episode of acute rejection. In this way, 33 biopsies were separately analysed.

As demonstrated in Table 1, there were 15 episodes of acute kidney allograft rejection that included TCMR ($n = 7$, 46.7%), AMR ($n = 5$, 33.3%), and suspicious for AMR ($n = 3$, 20%) in 11 patients, which represented 52.4% (11/21) of patients submitted to kidney biopsy and 45.5% (15/33) of the biopsies performed in the period. In addition, 24.2% (8/33) of the biopsies showed AMR and suspicious for AMR.

C4d staining was positive in 27.3% ($n = 9$) of the biopsies (diffuse labeling in 66.7% of the cases in peritubular capillaries). More than two-thirds of the cases exhibited total recovery after the anti-rejection treatment, immunosuppressive regimen adjustment, or antimicrobial therapy. Moreover, we did not find correlation between C4d labeling and kidney allograft survival.

Pancreatic biopsies

The pancreatic biopsies were performed after 566 ± 682.3 days (median 192 days). Forty-two biopsies from

Table 1. Kidney allograft biopsies histology, C4d labeling, antibody detection and outcome (n = 33).

	C4d			Antibody detection				Outcome		
Histology	Negative	Focal	Diffuse	Negative	HLA	MICA	N/A	TR	PR	Graft loss
Normal (n = 2)	2	0	0	0	0	0	2	2	0	0
ATN (n = 7)	7	0	0	1	0	0	6	7	0	0
Acute AMR (n = 5) ATN + capillaritis: 3; ATN: 2	1	0	4	0	3	2	0	3	2	0
Suspicious for acute AMR (n = 3) ATN: 1; Borderline: 1; IA: 1	0	3	0	0	0	0	3	2	1	0
Acute TCMR + AMR (n = 7) IA: 5; IB: 1; IIA: 1	6	1	0	1	0	0	6	6	1	0
IF/AT (n = 3) Grade I: 2; grade II: 1	3	0	0	0	0	0	3	0	3	0
Pyelonephritis (n = 2)	1	0	1	0	0	0	2	1	0	1
Other (n = 4)	3	0	1	0	0	0	4	3	1	1
	Negative: 24 (72.7%) Positive: 9 (27.3%) – Focal: 3 (33.3%) – Diffuse: 6 (66.7%)			Negative: 2 (6.1%) HLA: 3 (9.1%) MICA: 2 (6.1%) N/A: 26 (78.7%)				PR: 8 (24.2%) TR: 24 (72.7%) Graft loss: 1 (3.3%)		

ATN, acute tubular necrosis; AMR, antibody mediated rejection; TCMR, T-cell mediated rejection; IF/AT, interstitial fibrosis/atrophy tubular; HLA, human leukocyte antigens; MICA, major-histocompatibility-complex class I-related A; N/A, not available; PR, partial recovery; TR, total recovery.

27 patients were analysed. Seven biopsies represented the same episode of acute rejection, therefore 35 pancreatic biopsies were analysed (27 with bladder drainage and 8 with enteric drainage). Altered exocrine pancreatic function was the main indication for biopsy (54.3%), followed by both hyperglycemia and exocrine dysfunction (37.1%) and by isolated hyperglycemia (8.6%).

Rejection was present in 20 patients, which was 74.1% (20/27) of the patients submitted to pancreatic biopsy. In addition, rejection was diagnosed in 71.4% (25/35) of the pancreatic biopsies performed. Fifty percent (17/35) of the biopsies showed AMR and suspicious for AMR. In addition, 17 out of 25 (68%) the biopsies with rejection were AMR (Table 2). Fifteen out of 17 were acute AMR (Figs 1a–c and 2a,b), whereas the other two were chronic active AMR. C4d staining was positive in 43% of all pancreatic biopsies (53.3% diffuse in interacinar capillaries). Antibodies to MICA were detected in five out of eight acute AMR episodes.

In four SPKT cases from the same deceased donor, either pancreas or kidney dysfunction was present and a biopsy was indicated for both grafts, which means that 14.3% (4/28) of SPKT presented synchronous allograft rejection. In one patient with pancreas and kidney dysfuction, the biopsy disclosed mild changes for the pancreas (ductular ectasia and septal fibrosis) and was normal for the kidney. However, the incidence of synchronous rejection after SPKT was probably underestimated as the biopsies were only performed when clinical dysfunction was observed. In relation to the transplant modality, almost 65% of both acute and chronic AMR rejection occurred in patients submitted to PTA, PAKT or SPKT with living kidney donor.

On average, acute AMR of either pancreas or kidney allograft (n = 5 patients) was treated with a mean of 6.8 sessions of plasmapheresis (range: 3–11 sessions) and 2.2 doses of intravenous immunoglobulin (IVIg) 1 g/kg (range: 1–4 doses).

Laboratorial parameters

Laboratorial parameters considering both acute TCMR and AMR (TCMR + AMR, suspicious for AMR and chronic active AMR) either pre- or post-treatment were as follows: amylase 292.7 ± 526.5 U/l (median 156 U/l) vs. 104.1 ± 60.5 U/l (median 82 U/l) (P = 0.075), lipase 1362.1 ± 1275.4 U/l (median 1120 U/l) vs. 377.3 ± 224.1 U/l (median 406.5 U/l) (P = 0.0003) and amylasuria 1271.3 ± 1214.1 U/h (median 625 U/h) vs. 1966.5 ± 1423 U/h (median 1680 U/h) (P = 0.004). Thus, at the time of acute pancreas rejection the values of serum amylase and lipase were 1.5 times and four times greater than the reference respectively. There was significant correlation between serum amylase and lipase for the diagnosis of rejection (R^2 = 0.74, P < 0.0001).

When we compared acute TCMR (n = 8) and TCMR associated with AMR (n = 17) in relation to pancreatic enzymes pre- and post-treatment as well as regarding endocrine function, there were no significant differences: Amylase pre (U/l) 178.9 ± 95 (median 151.5) vs. 338.4 ± 651.7 (median 149) (P = 0.075); amylase post

Table 2. Pancreas allograft biopsy histology, C4d labeling, antibody detection, and outcome (n = 35).

	C4d labeling			Antibody detection				Outcome Exocrine			Outcome Endocrine		
Histology	Negative	Focal	Diffuse	Negative	HLA	MICA	N/A	Normal	Reduced NE	Amylasuria IE	Normal	Partial	Graft loss
Acute TCMR (n = 8)													
All of them grade I	8	0	0	1	0	0	7	7	0	1	7	0	1
Acute TCMR + AMR (n = 9)													
Grade I: 3 Grade II: 4 Grade III: 2*	2	2	5	1	0	5	3	2	3	4	5	1	3
Suspicious for acute AMR (n = 6)													
Normal: 1 Grade I: 3 Grade II: 1 Other: 1	0	5	1	2	0	0	4	6	0	0	4	1	1
Chronic active AMR (n = 2)	0	0	2	1	0	0	1	1	0	1**	1	1	0
Other (n = 10)													
Indeterminate: 4 Chronic rejection grade I: 3 Ductular degenerative changes: 2 Normal: 1	10	0	0	1	0	0	9	10	0	0	9	1	0
	Negative: 20 (57.1%) Positive: 15 (42.9%) – Focal: 7 (46.7%) – Diffuse: 8 (53.3%)			HLA: 0 MICA: 5 (14.3%) Negative: 6 (17.1%) N/A: 24 (68.6%)				Normalized Amylasuria: 26 (74.3%) Reduced Amylasuria: 9 (25.7%) – Normalized enzymes: 3 (8.6%) – Increased enzymes: 6 (17.1%)			Normal: 26 (74.3%) Partial: 4 (11.4%) Graft loss: 5 (14.3%)		

TCMR, T-cell mediated rejection; AMR, antibody-mediated rejection; HLAM, human leukocyte antigens; MICA, major-histocompatibility-complex class I-related A; N/A, not available; NE, normalized serum enzymes; IE, increased serum enzymes.
*One case with acute AMR associated with chronic rejection grade III.
**Only increased enzymes (enteric conversion in the end of the first year because of persistent hematuria).

(U/l) 90.9 ± 40.6 (median 80) vs. 92.6 ± 67.2 (median 69) (P = 0.95); lipase pre (U/l) 1169 ± 670.8 (median 1120) vs. 1288.5 ± 1553.6 (median 721) (P = 0.83); lipase post (U/l) 355.5 ± 213.6 (median 296.5) vs. 284.8 ± 228.4 (median 258) (P = 0.47); amylasuria pre (U/h) 1509.3 ± 1311.5 (median 1137) vs. 1395.2 ± 1484.7 (median 767.5) (P = 0.87), amylasuria post (U/h) 2153.7 ± 1277.8 (median 1860.5) vs. 2201.3 ± 1926.7 (median 1558.5) (P = 0.95), amylasuria variation pre (%) 45 ± 41.1 (median 46.5) vs. 44.1 ± 49.6 (median 61) (P = 0.97); fasting plasma glucose (mg/dl) 96.6 ± 66.7 (median 69) vs. 143 ± 88.4 (median 97) (P = 0.20); minimum 2-h CG (mg/dl) 105.5 ± 28.5 (median 99) vs. 136.1 ± 73.5 (median 109) (P = 0.27) and maximum 2-h CG (mg/dl) 182.4 ± 91.8 (median 149.5) vs. 213.7 ± 106.5 (median 197.5) (P = 0.49).

The receiver-operating characteristic (ROC) curve for serum amylase and lipase and the diagnosis of pancreas allograft rejection demonstrated an area under the curve (AUC) of 0.55 and 0.73 (P = 0.62, 95% CI: 0.33–0.77 and P = 0.025, 95% CI: 0.55–0.91 respectively). In addition, the ROC curve for amylasuria values and amylasuria variation and rejection diagnosis demonstrated an AUC of 0.24 (P = 0.036, 95% CI: 0.04–0.44) and 0.72 (P = 0.06, 95% CI: 0.53–0.91) respectively. ROC curve for amylasuria values after treatment and pancreas allograft loss showed AUC of 0.17 (P = 0.015, 95% CI: 0.03–0.32). For values less than 150 U/h the sensibility and the specificity were 83% and 96% respectively.

Uni- and multivariate analysis did not show a correlation between C4d and the other variables: amylase and lipase before treatment (P = 0.68 and P = 0.39), amylase and lipase after treatment (P = 0.96 and P = 0.97), amylasuria before treatment (P = 0.42), variation of amylasuria (P = 0.41), pancreas allograft loss (P = 0.23) or PTA (P = 0.2).

When we compared indeterminate inflammatory infiltrate to grades I–III of pancreas rejection, C4d detection

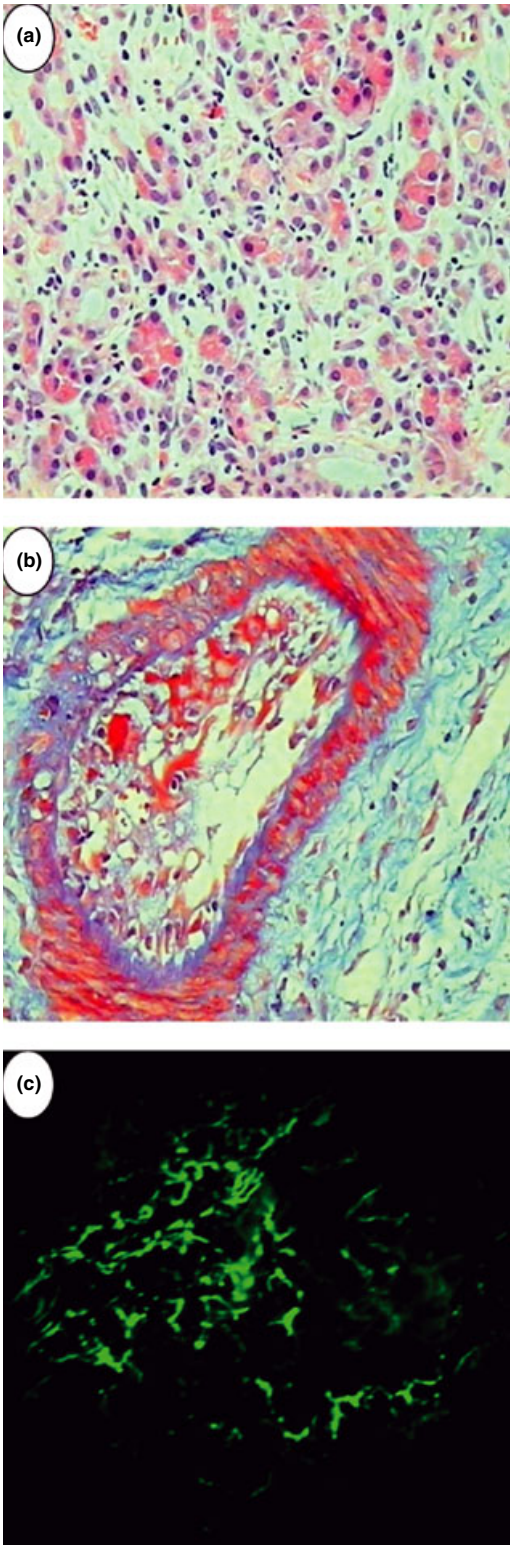

Figure 1 Acute TCMR (grade II) + AMR in SPKT. (a) Acinar inflammatory infiltrate of lymphocytes and eosinophils; (b) moderate arteritis (Hematoxilin & Eosin, magnification ×200–400); (c) diffuse C4d in interacinar capillaries (Immunofluorescence, magnification ×200).

was absent in all cases of the indeterminate grade ($P = 0.031$), which suggest that this grade may be really associated with mild immunologic activation.

Discussion

Acute AMR has no distinguishing clinical features but typically occurs earlier after transplantation and causes rapid functional deterioration. However, AMR can also occur much later, particularly in the setting of reduced immunosuppression or noncompliance. Acute and chronic AMR in kidney transplantation have been extensively studied, although the coverage in the literature of AMR in other organs is poor. It seems that most AMR episodes after pancreas transplantation can be diagnosed in a similar way as in isolated kidney transplantation, with the triad of allograft dysfunction, C4d positivity and detectable DSA in recipient serum [11].

Antibody-mediated rejection of pancreas allograft was reported initially in case reports of SPKT and PAKT [15,16]. Subsequently, the incidence of AMR after SPKT was reported to be 15.3% and in most of the cases both pancreas and kidney allografts presented AMR, although only in two cases C4d was positive in pancreas allograft [10].

We report here the incidence of AMR that was observed in almost 68% of the cases in the three modalities of pancreas transplantation. C4d staining was present in 43% of all pancreatic biopsies. Diffuse C4d staining pattern was present in the interacinar capillaries in more than 50% of the cases, which is in conformity with other reports with the same follow-up [11]. In addition, we did not detect islet staining for C4d in the cases of AMR.

In our center, almost 200 pancreas transplants were performed between August 2002 and December 2008, but C4d detection has been only routinely investigated since August 2006. Some of the cases reported here with C4d staining and allograft dysfunction were undoubtedly AMR episodes, despite the lack of DSA at the time of diagnosis. According to the literature, it is reported that antibodies to donor HLA class I or II antigens are present in 88–95% of the patients who have C4d deposition and acute graft dysfunction [17].

C4d positivity predicts either worse allograft survival or early graft dysfunction, which is reported by several studies that include all solid organ transplantation [10,12,17,18]. However, we did not find a correlation between C4d staining and allograft survival rates. Our incidence of pancreas allograft loss was 33.3% for cases of acute AMR (23.5% if the cases suspicious for acute AMR and chronic AMR are included), which was lower than other reports [10,11]. This difference could be explained by the shorter time of follow-up and the higher doses of

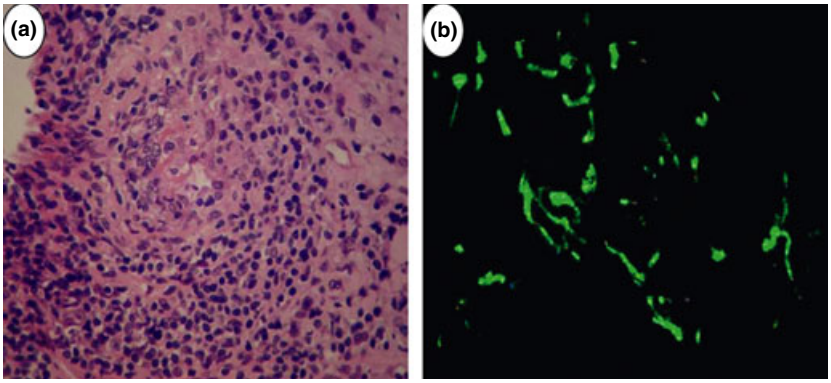

Figure 2 Acute TCMR (grade III) + AMR in PTA. (a) Arteritis with fibrinoid necrosis (Hematoxilin & Eosin, magnification ×400); (b) diffuse C4d in interacinar capillaries (Immunofluorescence, magnification ×200).

intravenous immunoglobulin associated with plasmapheresis that we used here. It has been reported that late AMR (>3 months) was the main risk factor for pancreas allograft loss, female gender was the only risk identified for AMR, and other known factors, such as HLA A, B or DR mismatches, did not show correlation with AMR [10]. On the other hand, our cases were predominantly male subjects and the pancreas allocation was not determined by HLA in Brazil.

It is a matter of debate if the outcome of the cases of diffuse C4d staining is worse than the focal ones, but it seems that even focal staining is associated with decreased allograft survival and elevated rates of acute cellular rejection [19]. Our data did not permit the establishment of a relation between focal versus diffuse C4d staining and prognosis, and further studies are required. In addition, the cases of 'suspicious for acute AMR' could be associated with a different clinical evolution and prognosis [20]. Although the significance of C4d in the absence of histologic evidence of tissue injury is unknown, C4d deposition also occurs in 2–26% of histologically normal grafts, the higher frequency being found in HLA-presensitized patients [19], as it was found in our two HLA-presensitized patients. In these cases, there are two potential outcomes: accommodation or evolution to acute AMR, as reviewed by Kozakowski and Regele [21].

The occurrence of chronic pancreas AMR has rarely been reported. Our three patients presented fibrosis, diffuse or focal C4d staining, and variable clinical evolution (in one instance, the pancreas was still functioning; in another, there was loss of the pancreas, and in yet another, pancreas was with partial function). There is no consensus on the treatment for such cases. In addition, in chronic kidney AMR, the diagnostic criteria are based on histologic evidence [9] and it seems that C4d in peritubular capillaries is only predictive of kidney outcome in the acute setting, as opposed to late acute rejection [22]. In pancreas allografts, it remains to be elucidated if chronic AMR is associated with specific histologic alterations.

The antibodies to major-histocompatibility-complex class I-related chain (MICA) also predict either early or late graft failure, even in the absence of measurable HLA antibodies [23–25]. MICA antigens are surface glycoproteins with functions related to innate immunity and they are expressed in several cells, including endothelial and epithelial cells, but not peripheral lymphocytes. It is reported that antibodies against MICA alleles are present in 11.4% of the patients before kidney transplantation [23]. In patients with functioning kidney transplants, 24% of those with HLA antibodies have MICA/B antibodies and 19% of those without HLA antibodies have MICA/B antibodies, in comparison with 21% of detection in pregnant women [24]. Surprisingly, the four patients here (5 biopsies) with pancreas rejection and MICA antibodies were men. In addition, it has been reported that the presence of both antibodies in the kidney allograft is associated with the worst survival [25]. Interestingly, MICA antibodies are not related to blood transfusions and its association with reduced graft survival was more evident in recipients with good HLA matching [26]. Both in acute and chronic pancreas allograft rejection, MICA has already been detected in acini and islets [27]. Additionally, MICA antibodies are recognized by NKG2D receptors on activated natural killers and CD8+ T cells, which may function as a bridge between innate and adaptive immunity after transplantation (reviewed in 28). Furthermore, the mechanism by which MICA antibodies were produced remains to be elucidated. One limitation of our study, however, was the lack of proof of donor specificity because donor DNA was not available to test against MICA antigens.

The increase of serum pancreatic enzymes and their correlation with each other, as well as the decrease of amylasuria for the diagnosis of pancreas rejection that we observed herein were in accordance with similar data reported in the literature [29–31]. In our study, lipase had more specificity and sensitivity than amylase for the diagnosis of acute pancreas rejection. However, it was not possible to differentiate between cellular rejection and

AMR based on laboratorial parameters neither in relation to exocrine nor to endocrine dysfunctions. The lack of amylasuria increase after the biopsy was correlated to pancreas loss. This suggests that severe acinar injury secondary to the inflammatory insult may be subsequently associated with pancreatic endocrine impairment. However, it is a matter of debate whether patients with lower amylasuria levels have a worse pancreas allograft in comparison to patients with higher levels.

In conclusion, our study showed the occurrence of AMR after the three modalities of pancreas transplantation. The high frequency of C4d staining in pancreas allograft reinforces the necessity of its investigation in all cases of pancreatic rejection, inasmuch as it may require specific treatment that may predict graft survival.

Authorship

EBR, DMACM: designed the study. EBR, DMACM, MCRC, IA, FC, TG, MP: collected the data and performed the study. MAT: performed the antibodies analyses. EBR, MCRC: analysed the data. EBR, MCRC: wrote the paper.

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