TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Duodenal leaks after pancreas transplantation with enteric drainage – characteristics and risk factors

Vinzent N. Spetzler,[1] Nicolas Goldaracena,[1] Max A. Marquez,[1] Sunita K. Singh,[2] Andrea Norgate,[1] Ian D. McGilvray,[1] Jeffrey Schiff,[2] Paul D. Greig,[1] Mark S. Cattral[1] and Markus Selzner[1]

1 Multi Organ Transplant Program, Department of Surgery, Toronto General Hospital, Toronto, ON, Canada
2 Multi Organ Transplant Program, Department of Medicine, Toronto General Hospital, Toronto, ON, Canada

Keywords
complication after transplantation, duodenal Leaks, pancreas after kidney transplantation, pancreas transplantation, simultaneous pancreas–kidney transplantation.

Correspondence
Markus Selzner MD,
Toronto General Hospital, NCSB 11C-1244,
585 University Avenue, Toronto, ON
M5G2N2, Canada.
Tel.: 1 416 340 5230;
fax: 1 416 340 5242;
e-mail: markus.selzner@uhn.ca

Conflicts of interest
The authors declare no conflict of interests.

Received: 27 October 2014
Revision requested: 27 November 2014
Accepted: 29 January 2015
Published online: 23 February 2015

doi:10.1111/tri.12535

Summary

Pancreas–kidney transplantation with enteric drainage has become a standard treatment in diabetic patients with renal failure. Leaks of the graft duodenum (DL) remain a significant complication after transplantation. We studied incidence and predisposing factors of DLs in both simultaneous pancreas–kidney (SPK) and pancreas after kidney (PAK) transplantation. Between January 2002 and April 2013, 284 pancreas transplantations were performed including 191 SPK (67.3%) and 93 PAK (32.7%). Patient data were analyzed for occurrence of DLs, risk factors, leak etiology, and graft survival. Of 18 DLs (incidence 6.3%), 12 (67%) occurred within the first 100 days after transplantation. Six grafts (33%) were rescued by duodenal segment resection. Risk factors for a DL were PAK transplantation sequence (odds ratio 3.526, $P = 0.008$) and preoperative immunosuppression (odds ratio 3.328, $P = 0.012$). In the SPK subgroup, postoperative peak amylase as marker of preservation/reperfusion injury and recipient pretransplantation cardiovascular interventions as marker of atherosclerosis severity were associated with an increased incidence of DLs. CMV-mismatch constellations showed an increased incidence in the SPK subgroup, however without significance probability. Long-term immunosuppression in PAK transplantation is a major risk factor for DLs. Early surgical revision offers the chance of graft rescue.

Introduction

Pancreas–kidney transplantation is the standard treatment for patients with type 1 diabetes mellitus and end-stage renal failure [1–4]. Over the last three decades of pancreas transplantation, 5-year patient and graft survival have improved significantly to 85% and 75% [5]. The improved outcome of pancreas transplantation is mainly due to improved immunosuppression and advances in surgical technique [3,4]. Although simultaneous pancreas–kidney (SPK) transplantation remains the most common method, pancreas after kidney transplantation (PAK) has become more frequent in part because of the increasing popularity of live kidney donation.

Despite this success, graft rejection and technical complications still account for a considerable number of pancreatic graft losses [3–6]. Duodenal leaks of the graft stump (DL) are among the most common surgical complications with an incidence of 4–10%, resulting in pancreatic graft loss in most of the cases [6–9]. The surgical practice for the drainage of the pancreatic duct changed over time, duodenoenterostomy having replaced duodenocystostomy as the predominantly used technique in most centers [4,10]. Most literature on DLs after pancreas transplantation dates back more than a decade and is focused on duodenocystostomies, or reports only a small number of cases [8,9,11–14]. Little effort has been made revealing the pathophysiology and risk factors for DLs in patients with duodenoenterostomy.

In order to develop strategies to prevent and treat DLs after pancreas transplantation, we systematically reviewed the database of one of North America's largest pancreas

transplantation programs, focusing on risk factors, attributing causes, and treatment regimes.

Patients and methods

Study population

All pancreas transplantations at Toronto General Hospital performed between January 2002 and April 2013 ($n = 284$) were prospectively entered into our transplant database and analyzed retrospectively. Organ recipients had at least 12 months of follow-up after pancreas transplantation. Both SPK and PAK procedures were included in the database.

The study was approved by the University Health Network's Research Ethics Board (IRB# 13-6912).

Surgical procedure

All organ recoveries and transplantations were performed according to our standard protocol with systemic venous drainage and enteric drainage of exocrine pancreas secretion [15,16]. The exocrine drainage of the pancreas graft was performed by an enteric anastomosis using a Roux-en-Y reconstruction in all cases. The anastomoses were approximately 5 cm in length and hand sewn in two layers. Both distal and proximal ends of the donor duodenum were shortened until sufficient blood supply of the cutting surface was apparent (approximate duodenal length 8–10 cm) and closed using a gastrointestinal stapler device. During the SPK procedures, the kidney was anastomosed and reperfused before the pancreas. Antibiotic prophylaxis included cefazolin i.v. for 48 h for the recipient only. The majority of kidney graft recipients in the PAK group was transplanted elsewhere and referred to our center for PAK. Nineteen procedures were retransplantations after previous pancreas graft loss (all PAK). Only two SPK transplantations were performed as secondary transplantation (both after kidney graft failure). Six pancreas grafts were recovered after cardiac death (DCD).

Organ allocation in collaboration with our organ procurement organization was based on recipient-to-donor AB0 compatibility and waiting time. All patients had a negative anti-human globulin complement-dependent cytotoxic T-cell cross-match at the time of transplantation. Presence of donor-specific antibodies with a negative cross-match was not a contraindication to transplant.

Immunosuppression

As induction therapy, thymoglobulin was given to the vast majority (80%) of patients at a dosage of 1–1.5 mg/kg/day for 3–5 days according to their immunologic risk status (donor-specific antibodies, panel-reactive antibodies, secondary transplant). The remaining patients received either basiliximab or no induction therapy in very few cases. Patients receiving basiliximab were administered an i.v. dose of 20 mg within 2 h prior to transplant surgery and a second dose on the fourth day after transplant.

All patients received 500 mg of i.v. methylprednisolone intra-operatively, followed by a rapid taper from 200 to 20 mg/day on day 5. The oral prednisone dosage was reduced to 5 mg/day by 6 months and maintained between 2.5 and 5 mg/day thereafter. Tacrolimus (target level of 10–14 μg/l at day 7 and 5–10 μg/l at 6 months) and mycophenolate mofetil (MMF, 500 mg twice a day) were initiated on postoperative days 2–5. In patients where donor-specific antibodies were identified, intravenous immunoglobulin (IVIg) was given at a dose of 1 g/kg pretransplant.

Pathologically confirmed, steroid resistant acute rejection episodes were treated with thymoglobulin. Humoral rejections were treated with steroids, thymoglobulin, plasmapheresis, intravenous immune globulin, and rituximab as indicated.

Cytomegalovirus prophylaxis

Cytomegalovirus prophylaxis was provided by ganciclovir and/or valganciclovir for 3–6 months in mismatch scenarios with seronegative recipients (D+/R−), while cases with seropositive recipient mismatches (D−/R+) and seropostive matches (D+/R+) received prophylaxis for 3 months. Ganciclovir initial dose was 5 mg/kg i.v. daily, followed either by an oral ganciclovir dose of 1 g three times a day or 900 mg of oral valganciclovir per day, whenever the patient could tolerate oral medications. Doses of both ganciclovir and valganciclovir were adjusted according to renal function.

Identifying DLs

All diagnoses of DL were preceded by symptoms of acute peritonitis. CT scan presentation of free gas and retained fluid around the pancreatic graft were either treated with a first attempt of interventional drainage or, if an intestinal leak was suspected, directly by explorative laparotomy. If a leak of the graft duodenum was confirmed during laparotomy, either the whole pancreas graft was removed if the surgeon rated the situation as not preservable, or the affected duodenal segment was mobilized and resected. The resection site was stapled off only, without additionally oversewing the stapler line. The Roux anastomosis was not resolved and redone.

Time until leak was defined as days from transplantation to diagnostic confirmation of a DL. Findings of imaging studies and laparotomies were documented and the reports retrospectively analyzed. Surgical specimens were evaluated

using histology for signs of graft infection (especially CMV), rejection, and ischemia. Samples of the retained intra-abdominal fluid were sent for microbiological examination.

Donor and recipient data

Data collection for both donor and recipient included gender, age at donation/transplantation, weight, height, body mass index (BMI), CMV status, Epstein–Barr virus (EBV) status, AB0 status, and cause of donor death. Anamnestic recipient data also included transplantation sequence (SPK versus PAK), previous pancreas transplantations (retransplantation), immunosuppression therapy prior to pancreas transplantation, panel-reactive antibodies, history of cardiovascular interventions, duration of diabetes and dialysis prior to transplantation, and type of dialysis. Serum high-density lipoproteins (HDL), low-density lipoproteins (LDL), and triglyceride levels were evaluated as markers of metabolic dysfunction prior to transplantation. Preoperative diabetes control was evaluated by serum HbA_{1c} levels.

Transplantation data

Procedure-related data were collected for: type of donation (neurological determination of death versus death after cardiocirculatory arrest/DCD), pancreas cold ischemic time (CIT), time of implantation (warm ischemia time, WIT), procedure time, blood loss, number of transfused packed red blood cell units, and induction of immunosuppressant therapy.

Follow-up

Follow-up data covered follow-up time, hospitalization time, complications and infections (bacterial, viral, and fungal), laboratory values for reperfusion injury and graft function (both pancreas and kidney), date and treatment of rejection (diagnosed by biopsy, Maryland classification for pancreas grafts [17]), cardiovascular events, and time and cause of both graft loss and death. Graft loss was defined as return to insulin dependency [18].

Statistical analyses

Statistical data analysis was performed with SPSS (SPSS for Windows 20.0, Chicago, IL, USA). Statistical differences were assessed using a chi-squared test in categorical variables and a Mann–Whitney test in continuous variables. A *P*-value below 0.05 was considered statistically significant. Subgroup analysis was performed for both the SPK and PAK groups and for subgroups sorted by time of DL occurrence. Only significant differences were reported for subgroup analyses.

To determine the impact of each significant risk factor by means of odds ratio, a stepwise logistic regression model was performed. Significant factors from binary logistic regression analysis were added to a multivariate logistic regression analysis. Certain factors such as age, BMI, CMV status, or variables that proved to be significant in chi-squared or Mann–Whitney subgroup analyses were forced into a multivariate analysis model. Odds ratio and confidence intervals were reported for significant variables only.

Results

Study group

Between January 2002 and April 2013, 284 pancreas transplantations were performed including 191 SPK (67.3%) and 93 PAK (32.7%). Anamnestic donor and recipient data are listed in Table 1.

Eighteen patients (6.3%) developed a DL throughout their entire follow-up time, 13 (4.6%) occurring within the first year. Of these 13 cases, 8 patients lost their pancreas graft due to the DL (2.8% of all grafts). The overall 1-year pancreas graft loss rate was 10.9% (31 cases) with a 1-year patient mortality of 1.8% (5 deaths). Accordingly, a quarter (26%) of all 1-year graft losses was due to DLs. Therefore, DLs are the leading 1-year graft loss cause, followed by graft pancreatitis (6 cases, 19.4%), vascular thrombosis (5 cases, 16.1%), and acute rejection (4 cases, 12.9%). The yearly incidence of DLs related to the year of transplantation ranged between 0% and 14.8%. However, there was no era effect detectable.

DL characteristics

The majority of DLs occurred 'early', within the first 100 days after transplantation (12 cases, 67%). The remaining DLs occurred 'late' and are distributed within several years of follow-up without another temporal culmination (see Fig. 1).

Only 1 DL was at the duodeno-jejunal anastomosis. Seventeen DLs were located either close or directly at the proximal or distal stapler line of the duodenal graft. In most cases, a clear cause of the DL was not identified. All pathology specimens showed different degrees of nonspecific peripancreatic, periduodenal, and mucosal inflammation. Signs of microvascular circulation disorder were present in the pathological specimens of most DLs (focal ulceration, microthrombi, mucosal necrosis).

Within the DL group, 12 (66.7%) patients lost their graft, while in 6 (33.3%) cases, the graft was rescued by mobilization and resection of the affected duodenal segment. None of the DLs patients died, all of them recovered quickly after re-operation, and only two of them were admitted to ICU (in both cases for less than 2 days).

Intra-abdominal culture swabs obtained during laparotomy for the DL demonstrated typical intestinal contamination.

Table 1. Donor and recipient characteristics as well as procedure-associated factors for both the duodenal leak (DL) group and the control group (numbers shown as percentage for categorical variables and mean ± standard deviation for continuous values). Characteristics were similar in both groups. Only the cold ischemia time (CIT) was significantly longer in the control group than in the DL group.

		All (n = 284)	DL (n = 18)	Control (n = 266)	P
Donor					
Gender	% female	37.7	33.3	38	0.69
Age	Mean years	27.5 ± 10.7	25.9 ± 10.8	27.6 ± 10.7	0.48
BMI	Mean kg/m^2	23.5 ± 3.7	24.2 ± 4.4	23.5 ± 3.6	0.62
CMV status	% positive	43.3	38.9	43.6	0.70
Recipient					
Gender	% female	34.9	38.9	34.6	0.71
Age	Mean years	43.8 ± 8.0	43.7 ± 9.8	43.8 ± 7.9	0.88
BMI	Mean kg/m^2	25.4 ± 3.8	25.3 ± 3.7	25.4 ± 3.8	0.76
CMV status	% positive	40.1	50.0	39.5	0.38
Years of diabetes	Mean years	31.6 ± 7.6	32.6 ± 9.9	31.6 ± 7.5	0.87
Procedure					
Retransplant	%	6.7	5.6	6.8	0.84
DCD	%	1.8	0	1.9	0.56
CIT	Mean min	587.1 ± 153.2	506.9 ± 81.6	592.3 ± 155.3	0.03
WIT	Mean min	32.9 ± 10.3	30.4 ± 6.7	33.0 ± 10.6	0.40
Time of hospital stay	Mean days	11.6 ± 9.4	9.4 ± 4.1	11.7 ± 9.6	0.20

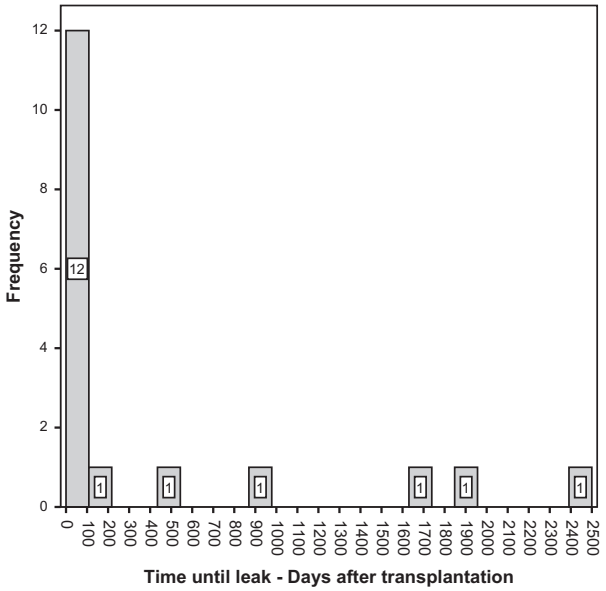

Figure 1 Histogram of the days until leak; most duodenal leaks (DLs) occurred within the first 100 days (12 cases). The remaining 6 DLs were distributed over the subsequent 7 years without another temporary culmination.

Four of 18 patients presented extended spectrum β-lactamase (ESBL) bacteria and one vancomycin-resistant enterococcus (VRE).

Patients with DL were compared with the patients without DL (control group). The DL group did not show an impaired early graft function. The mean fasting serum glucose level on the 7th postoperative morning was 5.2 ± 0.9 mmol/L in the DL group compared with 5.7 ± 1.3 mmol/L in the control group (P = 0.19). Glycemic control (HbA_{1c}) and metabolic function markers (LDL, HDL, triglyceride) 3 months post-transplantation have limited value because 66.7% of all DL had already occurred. However, no inferiority was detectable in the remaining 6 patients of the DL group compared with the control group at this time point.

Risk factors

Donor and recipient characteristics such as age, BMI, and gender were similarly distributed between DL cases and control patients (see Table 1). Duration of diabetes prior to transplantation (DL 32.6 ± 9.9 years vs. control 31.6 ± 7.5 years, P = 0.87) and pretransplant HbA_{1c} levels as a marker of diabetes control (DL 8.4 ± 0.9% vs. control 8.4 ± 1.5%, P = 0.63) were comparable in both groups. Likewise, pretransplant serum HDL, LDL, and triglyceride levels as markers of metabolic dysfunction were similar.

In the recipient population, cardiovascular interventions prior to transplantation as marker of severity of arteriosclerosis did not have a significant effect on DLs (DL 37.5% vs. control 24.1%, P = 0.23). Nevertheless, they showed a significantly higher rate in the DL patients when analyzing SPK cases only (DL 57.1% vs. control 23.5%, P = 0.04).

There were no significant differences in duration of the transplant procedure, intra-operative blood loss and number of transfusions, hospitalization time, as well as in-hospital and follow-up complications (see Table 1).

Immunosuppression and rejection

The whole study population received a standardized immunosuppression therapy after pancreas transplantation. Only the induction therapy varied, with thymoglobulin as the most frequent option (80%). There was no significant difference in the frequency of thymoglobulin induction therapy comparing the DL and control groups (DL 94.4% vs. control 78.5%, $P = 0.11$).

The PAK group had a higher occurrence rate of DLs than the SPK group (PAK 11.8%/11 of 93 vs. SPK 3.7%/7 of 191; χ^2: $P = 0.008$, log reg.: $P = 0.01$, odds ratio=3.5, CI: 1.3–9.4; see Fig. 2). At the same time, 61.1% of all DL patients had received PAK transplantation, compared with only 30.8% in the control group. Similarly, 61.1% of all DL patients had received immunosuppression therapy before pancreas transplantation, compared with only 32.1% in the control group (χ^2 $P = 0.01$, log reg.: 0.02, odds ratio: 3.3, CI: 1.2–8.9). As described above, all but two secondary transplants were performed as PAK. Accordingly, the higher rate of preoperative immunosuppression therapies in the DL group strongly correlates with the rate of PAK transplantations. Hence, PAK transplantation and immunosuppression prior to pancreas transplantation cannot be considered as independent variables.

The interval between kidney and pancreas transplantation in the PAK group (time of immunosuppression before the pancreas transplantation) was 2034.22 ± 1376.3 days in average. All PAK patients were treated with a calcineurin inhibitor (either cyclosporin or tacrolimus) before pancreas transplantation. As only 75 of 93 PAK patients (80.6%) received MMF therapy and 71 (76.3%) steroid therapy before pancreas transplantation, the rates of these two immunosuppressive agents are correspondingly lower than the calcineurin inhibitor rates in both groups. There was no significant difference in DL occurrence between patients receiving either MMF or steroids and their therapy naïve counterparts in the PAK subgroup, which could have clarified the effect on DLs of each drug independently.

Although resulting in an increased immunologic risk, the rate of pancreas retransplantation seems to be equally distributed in both groups (DL 5.6% vs. control 6.8%, $P = 0.84$; see Table 1). In addition, the peak panel-reactive antibody values had a similar distribution in both groups (DL 26.5 ± 32.4% vs. control 25.5 ± 30.7%, $P = 0.84$). Accordingly, there was no difference in pancreas rejection rates within the first 100 days (DL 11.1% vs. control 8.3%, $P = 0.67$). In addition, only 1 of 12 duodenal specimens showed signs of rejection (C4d-positive vasculitis). Otherwise, there were no signs of lymphocyte infiltration or crypt injury/inflammation. Due to the peripancreatic inflammatory reaction, serum amylase levels as an indicator of rejection were difficult to interpret at the time of DL and did not correspond with the observations in the pancreatic specimens.

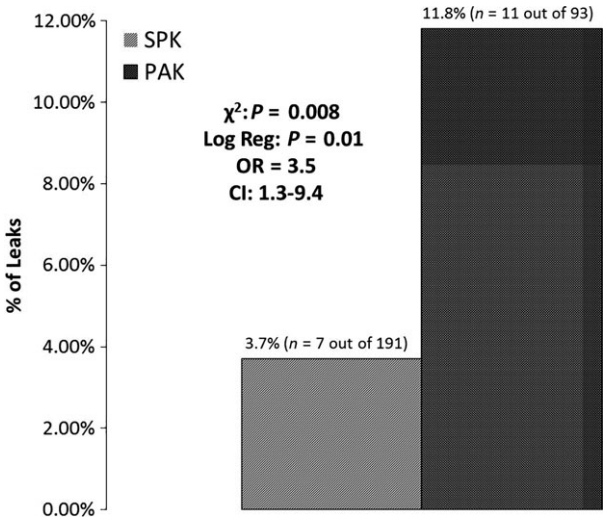

Figure 2 Influence of the sequence of pancreas to kidney transplantation; duodenal leaks (DLs) occurred in only 3.7% of all simultaneous pancreas–kidney transplantations (SPK), whereas 11.8% of all patients receiving a pancreas after kidney transplantation (PAK) developed a DL (χ^2-test: $P = 0.008$; log. regression: $P = 0.01$, Odds Ratio = 3.5, CI: 1.3–9.4).

Reperfusion injury

Peak serum amylase levels after transplantation as a marker of pancreas reperfusion injury [19] did not show a significant difference between both groups (DL 447.1 ± 247.3 U/L vs. control 400.85 ± 349.8 U/L, $P = 0.11$). However, in the 'early leak' subgroup (DL 492.0 ± 218.6 U/L vs. control 399.9 ± 348.3 U/L, $P = 0.03$) and the SPK subgroup (DL 586.8 ± 187.8 U/L vs. control 414.6 ± 362.8 U/L, $P = 0.02$), patients with DL had higher peak amylase levels than the control group (see Fig. 3).

None of the DCD pancreas recipients developed a DL. Additionally, CIT as a risk factor for reperfusion injury was lower in patients with DL (mean DL 506.9 ± 81.6 min vs. control 592.3 ± 155.4 min, $P = 0.03$). This paradox effect was confounded by a procedural-related shorter CIT in PAK cases compared with the SPK group (PAK 534.7 ± 143.0 min vs. SPK 615.1 ± 151.5 min, $P < 0.001$). The time of implantation (WIT) was comparable in both groups (mean DL 30.4 ± 6.7 min vs. control 33.0 ± 10.6 min, $P = 0.4$).

Cytomegalovirus

Five of 18 DLs (27.8%) occurred after a D−/R− CMV transplant constellation compared with 36.2% in the control group ($P = 0.47$). No difference was present between both groups regarding D+/R− mismatches when looking at

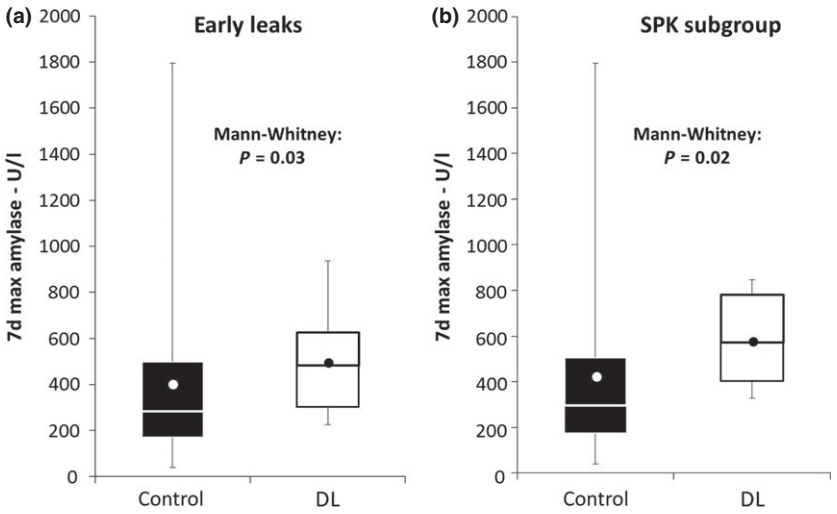

Figure 3 (a) & (b) Describe two different subgroup analysis. Role of reperfusion injury in duodenal leaks (DLs); peak amylase levels within 7 days after transplantation illustrated as boxplots (box 25th to 75th percentile; line-separation: median; point: mean value; whiskers: minimum and maximum). Amylase levels served as surrogate marker of graft reperfusion injury. The control group's peak amylase levels were significantly lower in both the simultaneous pancreas–kidney transplantation subgroup (SPK) as well in the 'early leaks' subgroup (leaks within 100 days after transplantation).

the entire study group (DL 27.8% vs. control 24.4%, $P = 0.75$; Fig. 4a). However, in the SPK subgroup alone, there was a trend toward a higher rate of D+/R− CMV mismatches in DL patients (DL 57.1% vs. control 26.6%, $P = 0.08$; see Fig. 4b). However, this trend was not significant.

Signs of viral infections including CMV were not detected in any of the pathology specimens. In addition, only two of 18 DL patients (11.1%) presented a CMV seroconversion during their entire follow-up time, however without direct temporal correlation to the onset of the DL. Both cases occurred in the 'early leak' group.

Likewise, no connection was apparent between EBV infection rates and DLs.

Kidney function

Duration of dialysis before transplantation did not have a significant impact on the incidence of DLs. Pretransplant serum creatinine levels were significantly higher in the DL group (DL 337.0 ± 320.8 μmol/L vs. control 518.5 ± 350.9 μmol/L, $P = 0.03$), most likely related to the functioning kidney graft in the PAK patients. Three month after transplantation, there was no apparent relation between the occurrence of DLs and kidney function. At the time of DL, none of the patients were back on dialysis.

Multivariate analysis

In the binary logistic regression analysis, immunosuppression prior to transplantation (any immunosuppression as well as the individual immunosuppressant agents) and transplant sequence (PAK versus SPK) remained the only significant variables of our study group (see Fig. 2). Because of their close correlation, these two variables were not included to multivariate analyses at the same time, but only immunosuppression prior to transplantation was used in further comparisons. In addition to the immunosuppression status prior to transplantation, factors that have shown to have potential influence on DL occurrence in earlier studies (donor and recipient age and BMI, CMV mismatch) and factors that have presented significant results in the subgroup analyses shown above (amylase peak, cardiovascular intervention) were forced into several different multivariate analysis approaches. Only the preoperative immunosuppression proved to be a significant risk factor. When comparing the influence of the individual immunosuppressant agents in a separate multivariate analysis, none of the agents proved to have an independent effect on DL occurrence.

Discussion

Outcomes of pancreas transplantation have progressively improved over the last decades, now offering excellent graft function and patient survival [3]. Our data coincide with this trend, presenting a 1-year graft and patient survival of 89.1% and 98.2%. However, a considerable number of pancreas grafts are still lost due to supposedly avoidable causes such as DLs. In the presented study, DLs were accountable for a quarter of all 1-year graft losses. Most DLs occur within 100 days after transplantation, but we have also seen later DLs up to 7 years after transplantation. Similar to ear-

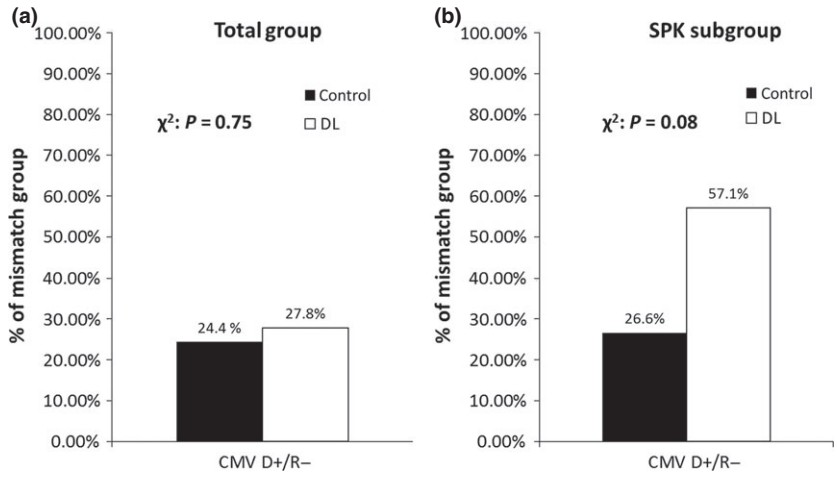

Figure 4 CMV-mismatch constellation; (a) In the total study group, rates of CMV mismatches were similar in both study groups; (b) In the simultaneous pancreas–kidney transplantation subgroup (SPK), CMV mismatches seemed to have a higher frequency in the duodenal leak group (DL), however without demonstrating a significant difference (χ^2-test: $P = 0.08$).

lier studies [6–8,20], a considerable number of grafts (33.3%) was rescued by duodenal resection.

Our data analysis included both PAK and SPK transplant sequences. The most striking finding of the analysis was that PAK patients have more than 3 times increased odds to develop a DL when compared to SPK patients. Correspondingly, immunosuppressive therapies given before pancreas transplantation showed a similar risk pattern and appear to be the causative factor for higher DL rates in the PAK group. As these substances are given in combination after kidney transplantation, it was impossible to identify a risk for a single substance alone. Steroids, calcineurin inhibitors, as well as MMF application prior to pancreas transplantation have all shown to have a significant impact on DL occurrence in our study population, but they were mostly given simultaneously and in PAK cases only. The striking effect of long-term immunosuppression on DL occurrence apparently confounded other potential risk factors when analyzing the whole study population.

When the SPK subgroup was analyzed separately and, thus, the risk factors of the PAK subgroup were avoided, peak amylase levels after pancreas transplantation, as marker of graft preservation/reperfusion injury, and previous cardiovascular interventions, as marker of severity of vascular disease in the recipient, were identified as additional risk factors for DLs. Furthermore, SPK transplantation in CMV-mismatch constellations was associated with a higher rate of DLs, approaching significant probability.

Unfortunately, the histological analysis of our duodenal specimens did not clarify the cause of DLs. Neither CMV infections nor graft rejections were histologically associated with DLs. Mainly, unspecific vascular changes such as microthrombosis or circumscribed ischemic mucosal areas were detected. Certainly, long-term immunosuppressant therapy, reperfusion injury, and vascular disease of the recipient could attribute to these findings. However, these changes could also be related to a wide variety of other factors.

Two groups have reported their experiences with DLs after pancreas transplantation using primary duodenoenterostomy within the past 15 years. Different to our analysis, Singh *et al.* found anamnestic donor characteristics such as age >30 years and BMI >30 kg/m^2 to be significant risk factors in the occurrence of peripancreatic fluid collection after SPK transplantation [7]. However, only 25% of these cases were confirmed DLs. Also, their study included both enteric-drained and bladder-drained pancreas transplantations. Heredia *et al.* reported graft rejection and CMV infections to be the main causes of DLs in their SPK series [20]. Unfortunately, the authors did not specify their CMV prophylaxis and diagnostic criteria. Additionally, their postoperative immunosuppression regime was rather heterogeneous.

Although PAK has proven to be an excellent alternative to SPK with similar overall graft survival and function [21], PAK presents a significant risk factor for the incidence of DLs. The causative differences to SPK involve mainly the immunosuppressive therapy before transplantation. Our data did not suggest the contribution of other possible causes that could explain a higher risk of DLs in PAK transplantation, including increased immunologic risk as well as exceeding CMV exposure due to the preceded kidney transplantation.

Immunosuppressive agents are generally considered to have a negative effect on wound and intestinal healing. For

long-term steroids and MMF application concomitant to intestinal surgery, a negative and dose-dependent effect on anastomotic stability has been shown in animal [22–27] and clinical [28,29] studies. Thus, a causative connection between these two substances and DLs appears plausible. Differently, CNIs (both cyclosporine and tacrolimus) have been associated with impaired wound healing but not with anastomotic leakage [30–34]. However, these studies were conducted as peri- and postoperative drug application models only. The effect of long-term CNI treatment on intestinal anastomosis healing has not been studied adequately so far.

Another factor that might contribute to DL occurrence independently from PAK transplantation was reperfusion injury of the pancreatic graft measured by peak amylase levels. Assuming that the reperfusion injury of the pancreas graft predicts the injury of its duodenal attendant as well, this finding is consistent with the study of Wasserberg *et al.*, showing that anastomotic stability decreases with increasing reperfusion injury in a rat bowel transplantation model [35].

Apart from the unsatisfactory results from our histological analysis, our study was limited by a rather small case number and the retrospective study design. To compensate for these shortcomings, all of our patients were derived from a single center with a homogenous treatment protocol in a modern era of transplantation.

In summary, our data indicate that PAK transplantation along with long-term immunosuppressive therapy prior to the pancreas transplantation plays a pivotal role in the development of DLs. Other factors such as preservation/reperfusion injury, cardiovascular disease of the graft recipient, and CMV-mismatch constellations appear to contribute to the overall risk of DL occurrence. In terms of DL management, an early surgical intervention with resection of the leaking graft duodenum part presents a viable option to successfully preserve affected pancreas graft.

Authorship

VNS: participated in research design, participated in the performance of the research, participated in data analysis, participated in writing the paper. NG: participated in the performance of the research, participated in writing the paper. MAM: participated in research design, participated in data analysis. SKS, AN and JS: participated in the performance of the research (patient follow-up). IDM, PDG and MSC: participated in the performance of the research (performing surgery). MS: participated in research design, participated in writing the paper, participated in the performance of the research (performing surgery).

Funding

The authors have declared no funding.

References

1. Robertoson RP, Davis C, Larsen J, Stratta R, Sutherland DE. Pancreas transplantation for patients with type 1 diabetes: American Diabetes Association. *Diabetes Care* 2000; **23**: 117.
2. Meltzer S, Leiter L, Daneman D, *et al.* 1998 clinical practice guidelines for the management of diabetes in Canada. Canadian Diabetes Association. *CMAJ* 1998; **159** (Suppl 8): S1.
3. Gruessner RW, Gruessner AC. The current state of pancreas transplantation. *Nat Rev Endocrinol* 2013; **9**: 555.
4. Gruessner AC. 2011 update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the International Pancreas Transplant Registry (IPTR). *Rev Diabet Stud* 2011; **8**: 6.
5. Gruessner AC, Gruessner RW. Pancreas transplant outcomes for United States and non United States cases as reported to the United Network for Organ Sharing and the International Pancreas Transplant Registry as of December 2011. *Clin Transpl* 2012; 23.
6. Manrique A, Jimenez C, Lopez RM, *et al.* Relaparotomy after pancreas transplantation: causes and outcomes. *Transplant Proc* 2009; **41**: 2472.
7. Singh RP, Vrakas G, Hayek S, *et al.* Clinically significant peripancreatic fluid collections after simultaneous pancreas-kidney transplantation. *Transplantation* 2013; **95**: 1263.
8. Gunasekaran G, Wee A, Rabets J, Winans C, Krishnamurthi V. Duodenoduodenostomy in pancreas transplantation. *Clin Transplant* 2012; **26**: 550.
9. Reddy KS, Stratta RJ, Shokouh-Amiri MH, Alloway R, Egidi MF, Gaber AO. Surgical complications after pancreas transplantation with portal-enteric drainage. *J Am Coll Surg* 1999; **189**: 305.
10. Monroy-Cuadros M, Salazar A, Yilmaz S, McLaughlin K. Bladder vs enteric drainage in simultaneous pancreas-kidney transplantation. *Nephrol Dial Transplant* 2006; **21**: 483.
11. Hakim NS, Gruessner AC, Papalois BE, *et al.* Duodenal complications in bladder-drained pancreas transplantation. *Surgery* 1997; **121**: 618.
12. Connolly JE. Pancreatic whole organ transplantation. *Surg Clin North Am* 1978; **58**: 383.
13. Douzdjian V, Abecassis MM, Cooper JL, Smith JL, Corry RJ. Incidence, management and significance of surgical complications after pancreatic transplantation. *Surg Gynecol Obstet* 1993; **177**: 451.
14. Steurer W, Bonatti H, Obrist P, *et al.* Incidence of intraabdominal infection in a consecutive series of 40 enteric-drained pancreas transplants with FK506 and MMF immunosuppression. *Transpl Int* 2000; **13**(Suppl 1): S195.

15. Martin X, Petruzzo P, Dawahra M, *et al.* Effects of portal versus systemic venous drainage in kidney-pancreas recipients. *Transpl Int* 2000; **13**: 64.
16. Cattral MS, Bigam DL, Hemming AW, *et al.* Portal venous and enteric exocrine drainage versus systemic venous and bladder exocrine drainage of pancreas grafts: clinical outcome of 40 consecutive transplant recipients. *Ann Surg* 2000; **232**: 688.
17. Drachenberg CB, Papadimitriou JC, Klassen DK, *et al.* Evaluation of pancreas transplant needle biopsy: reproducibility and revision of histologic grading system. *Transplantation* 1997; **63**: 1579.
18. Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. *Clin Transplant* 2005; **19**: 433.
19. Tamura K, Manabe T, Kyogoku T, Andoh K, Ohshio G, Tobe T. Effect of postischemic reperfusion on the pancreas. *Hepatogastroenterology* 1993; **40**: 452.
20. Heredia EN, Ricart MJ, Astudillo E, *et al.* Pancreas transplantation with enteric drainage: duodenal segment leak. *Transplant Proc* 2002; **34**: 215.
21. Bazerbachi F, Selzner M, Marquez MA, *et al.* Pancreas-after-kidney versus synchronous pancreas-kidney transplantation: comparison of intermediate-term results. *Transplantation* 2013; **95**: 489.
22. Phillips JD, Kim CS, Fonkalsrud EW, Zeng H, Dindar H. Effects of chronic corticosteroids and vitamin A on the healing of intestinal anastomoses. *Am J Surg* 1992; **163**: 71.
23. Baca B, Ozben V, Boler DE, *et al.* Effect of corticosteroid dose and duration of administration on colonic anastomosis. *Inflamm Bowel Dis* 2010; **16**: 2162.
24. Polat A, Nayci A, Polat G, Aksoyek S. Dexamethasone down-regulates endothelial expression of intercellular adhesion molecule and impairs the healing of bowel anastomoses. *Eur J Surg* 2002; **168**: 500.
25. Del Rio JV, Beck DE, Opelka FG. Chronic perioperative steroids and colonic anastomotic healing in rats. *J Surg Res* 1996; **66**: 138.
26. Sikas N, Imvrios G, Takoudas D, Gakis D, Papanikolaou V. Mycophenolate mofetil impairs the integrity of colonic anastomosis. *J Surg Res* 2006; **134**: 168.
27. Zeeh J, Inglin R, Baumann G, *et al.* Mycophenolate mofetil impairs healing of left-sided colon anastomoses. *Transplantation* 2001; **71**: 1429.
28. Stumpf M, Junge K, Wendlandt M, *et al.* Risk factors for anastomotic leakage after colorectal surgery. *Zentralbl Chir* 2009; **134**: 242.
29. Wagner OJ, Egger B. Influential factors in anastomosis healing. *Swiss Surg* 2003; **9**: 105.
30. Schaffer M, Fuchs N, Volker J, Schulz T, Kapischke M, Viebahn R. Differential effect of tacrolimus on dermal and intestinal wound healing. *J Invest Surg* 2005; **18**: 71.
31. Schaffer MR, Fuchs N, Proksch B, Bongartz M, Beiter T, Becker HD. Tacrolimus impairs wound healing: a possible role of decreased nitric oxide synthesis. *Transplantation* 1998; **65**: 813.
32. Raptis D, Mantzoros I, Pramateftakis MG, *et al.* The effects of tacrolimus on colonic anastomotic healing in rats. *Int J Colorectal Dis* 2012; **27**: 299.
33. Kiyama T, Tajiri T, Tokunaga A, Yoshiyuki T, Barbul A. Tacrolimus enhances colon anastomotic healing in rats. *Wound Repair Regen* 2002; **10**: 308.
34. Guatelli R, Koh IH, Neto AB, Gallupo MT, Goldenberg S, Barone B. Effect of cyclosporine A on the healing process of ileal anastomosis in rats. *Transplant Proc* 1996; **28**: 2589.
35. Wasserberg N, Tzakis AG, Santiago SF, Ruiz P, Salgar SK. Anastomotic healing in a small bowel transplantation model in the rat. *World J Surg* 2004; **28**: 69.