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Local Postoperative Graft Inflammation in Pancreas Transplant Patients With Early Graft Thrombosis

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Background. Graft thrombosis is the main cause of early graft loss following pancreas transplantation, and is more frequent in pancreas transplant alone (PTA) compared with simultaneous pancreas-kidney (SPK) recipients. Ischemia-reperfusion injury during transplantation triggers a local thromboinflammatory response. We aimed to evaluate local graft inflammation and its potential association with early graft thrombosis. **Methods.** In this observational study, we monitored 67 pancreas-transplanted patients using microdialysis catheters placed on the pancreatic surface during the first postoperative week. We analyzed 6 cytokines, interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8, interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein 1β (MIP-1β), IL-10, and the complement activation product complement activation product 5a (C5a) in microdialysis fluid. We compared the dynamic courses between patients with pancreas graft thrombosis and patients without early complications (event-free) and between PTA and SPK recipients. Levels of the local inflammatory markers, and plasma markers C-reactive protein, pancreas amylase, and lipase were evaluated on the day of thrombosis diagnosis compared with the first week in event-free patients. **Results.** IL-10 and C5a were not detectable. Patients with no early complications (n = 34) demonstrated high IL-1ra, IL-6, IL-8, IP-10, and MIP-1β concentrations immediately after surgery, which decreased to steady low levels during the first 2 postoperative days (PODs). Patients with early graft thrombosis (n = 17) demonstrated elevated IL-6 (P = 0.003) concentrations from POD 1 and elevated IL-8 (P = 0.027) concentrations from POD 2 and throughout the first postoperative week compared with patients without complications. IL-6 (P < 0.001) and IL-8 (P = 0.003) were higher on the day of thrombosis diagnosis compared with patients without early complications. No differences between PTA (n = 35) and SPK (n = 32) recipients were detected. **Conclusions.** Local pancreas graft inflammation was increased in patients experiencing graft thrombosis, with elevated postoperative IL-6 and IL-8 concentrations, but did not differ between PTA and SPK recipients. Investigating the relationship between the local cytokine response and the formation of graft thrombosis warrants further research.

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Ischemia-reperfusion injury after solid organ transplantation triggers the immune system and leads to a thromboinflammatory response that is associated with acute organ damage and can affect long-term outcomes.[1-5] In pancreas transplantation, the ischemia-reperfusion injury is associated with an increased risk of pancreatitis and graft thrombosis.[6] Graft thrombosis is the most common cause of early pancreas graft loss, and complicates 5%–10% of pancreas transplants.[7,8] Pancreas graft thrombosis is also reported to be more frequent in pancreas transplant alone (PTA) recipients compared with simultaneous pancreas-kidney (SPK) recipients. The mechanisms are not clarified but are suggested to be related to differences in the immune response between patients with and without preoperative chronic renal failure.[8-12]

Thromboinflammation is a complex process including activated cells of the innate and adaptive immune systems, activation of the complement and coagulation cascades, and induction of inflammatory cytokines and chemokines.[2,13,14] It is thought that this is predominately a local process in the graft but can evolve into a systemic inflammatory response through the activation of inflammatory cells and mediators.[15] Improved understanding of these mechanisms could reveal diagnostic markers or identify potential targets for future therapies aimed at decreasing ischemia-reperfusion-induced organ damage.[5,13]

Previously, we have observed that systemic complement activation is associated with pancreas graft thrombosis, but we did not discover any association between increased systemic cytokine concentrations and pancreas graft thrombosis.[16] However, subtle local cytokine responses may get diluted in the systemic circulation and thus not detected by regular blood samples. In the same study, we reported a relative increase in postoperative systemic thromboinflammatory markers in PTA compared with SPK recipients, but whether thromboinflammation differs between these recipient types locally at the graft has not been investigated.

We have already used microdialysis catheters to investigate changes in local metabolites concerning early postoperative complications in pancreas-transplanted patients.[17] Microdialysis has also been used to investigate local tissue inflammation in several other organs.[18-20] In liver transplantation, patterns of local inflammatory mediators have distinguished grafts with early postoperative ischemia from those with rejections or no complications.[21,22]

Our main aim was to assess the local inflammatory pattern at the pancreas grafts in patients with no early complications compared with patients developing graft thrombosis during the first postoperative week and on the day of thrombosis diagnosis. Our secondary aim was to compare the local inflammatory response between PTA and SPK recipients.

MATERIALS AND METHODS

Study Design, Ethics, and Patients

The study is a substudy of the Norwegian pancreas transplantation study, which is a prospective, observational, single-center study (clinicaltrials.gov NCT01957696, South-Eastern Norwegian Regional Ethical Committee 2012/2278). Between April 2015 and March 2018, all PTA and SPK recipients at Oslo University Hospital, 18 y and older were eligible for study inclusion. Pancreas after kidney transplantation was rarely performed and therefore excluded from this study. All participants provided written informed consent. We report the study following the STROBE (strengthening the reporting of observational studies in epidemiology) initiative (**Figure S1, SDC**, http://links.lww.com/TXD/A599). The primary outcome was postoperative differences in organ-close inflammatory markers for patients with early venous pancreas graft thrombosis compared with those with no early complications during the first postoperative week. The secondary outcomes were differences in organ-close inflammatory markers and plasma measurements of C-reactive protein (CRP), pancreas amylase, and lipase on the day of thrombosis diagnosis compared with event-free patients during the first postoperative week, differences in inflammatory markers between PTA and SPK recipients during the first postoperative week, and the association between early graft thrombosis and 1-y graft survival. Graft survival was defined as no need for exogenous insulin therapy within 1 y after transplantation.

Patient Treatment: Surgery, Anticoagulation, and Immunosuppression

Patients were treated according to the established local clinical transplant protocol. In brief, surgery was performed as a midline laparotomy with enteric anastomosis to the native duodenum. The pancreatic graft artery was anastomosed to the right common iliac artery and the inferior vena cava was used for vein anastomosis. During the study period, the clinical protocol was changed regarding the anticoagulation strategy. The first 34 patients (16 PTA, 18 SPK) received intraoperative 30 IU/kg unfractionated heparin IV and 2500 IU low molecular weight heparin (LMWH) subcutaneously (SC) 6 h postoperatively. From postoperative day (POD) 1, SPK recipients received 2500–5000 IU LMWH SC and PTA recipients received 5000–7500 IU daily. All patients received oral acetylsalicylic acid (ASA) 75 mg starting from POD 7. For the remaining 33 patients (16 PTA/17 SPK) the treatment did not differ between PTA and SPK recipients. All patients received 30 IU/kg unfractionated heparin IV intraoperatively. In addition, 500 mL of dextran 40, 100 mg/mL was administered both intraoperatively and on POD 1. LMWH 5000 IU SC was administered 6 h postoperatively and thereafter once daily. Oral ASA 75 mg was introduced on POD 3.

Protocol immunosuppression consisted of perioperative methylprednisolone IV (250 mg, 350 mg if >90 kg), mycophenolate mofetil IV (1g BID) and anti-thymocyte globulin (ATG) IV (2 mg/kg). Depending on the T cell count during the first 10 PODs, additional ATG (1 mg/kg) was administered not exceeding a maximum total dose of 6.5 mg/kg. Maintenance immunosuppression was tacrolimus per orally (PO) (trough concentrations of 10–12 ng/L the first 8 wk and thereafter 6–10 ng/L), mycophenolate PO (1g BID), and a daily dose of prednisolone tapered from 20 mg on POD 1 to 7.5 mg once daily on week 8, and 5 mg once daily 6 mo postoperatively.

Clinical Postoperative Evaluations

The pancreatic grafts were routinely evaluated with Doppler ultrasound 4 h after surgery and on POD 5 together with computed tomography, both according to the clinical protocol. Additional imaging was performed on clinical indications. All venous thrombi found on imaging, from peripheral to central occlusive thrombosis, and regardless of any further intervention or treatment, were recorded.

Microdialysis

Two microdialysis catheters (MDialysis-65, outer diameter of 0.6 mm, 30 mm membrane with 100-kDa pore size, shaft

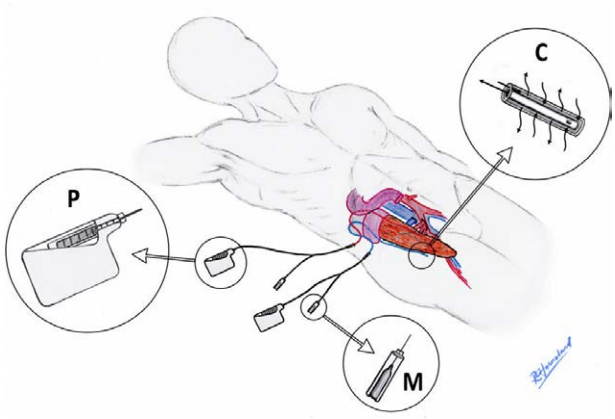

FIGURE 1. Microdialysis setup. Microdialysis catheters were placed anterior and posterior to the pancreas graft at the end of the transplantation. C, catheter tip with a semipermeable membrane (30 mm length, molecular weight cutoff 100 kDa) sampled the extracellular fluid; M, microvial holder where microvials were inserted to collect the microdialysate; P, portable battery-driven pumps perfused the catheters with 6% hydroxyethyl starch at a velocity of 1 µL/min. Picture from: Kjøsen G, Rydenfelt K, Horneland R, et al. Early detection of complications in pancreas transplants by microdialysis catheters, an observational feasibility study. *PLoS One*. 2021;16(3):e0247615.

length of 310 mm, MDialysis AB, Solna, Sweden) were placed adjacent to the anterior and posterior surface of the pancreas. The catheters were loosely sutured to connective tissue on the graft and not directly in the pancreas tissue, as described previously (Figure 1).[17] A battery-driven syringe pump (107 Microdialysis pump; MDialysis AB, Solna, Sweden) continuously perfused the microdialysis catheters with 6% hydroxyethyl starch (Voluven 60 mg/mL; Fresenius Kabi AS, Halden, Norway) at a velocity of 1 µL/min. Microdialysate was collected in microvials at 2- to 3-h intervals as long as the catheters were functioning or until the patient was discharged from the surgical transplantation department, and the catheters were removed. Samples from the catheter that functioned for the longest period were used for analyses of inflammatory markers. Microdialysate was first frozen at –20 °C at the ward, and within 14 d transferred to –80 °C until analysis.

Analyses of Inflammatory Mediators

Microdialysate was analyzed for cytokines interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8, interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein 1β (MIP-1β), IL-10, and the complement activation product complement activation product 5a (C5a). These markers were chosen upon previous experience regarding recovery through the microdialysis membrane.[21,23] Cytokines were measured using a 6-plex cytokine assay (Bio-Plex Pro Human Cytokine plex, BIO-RAD Laboratories, Inc, Hercules, CA) and C5a by ELISA kit (Hycult Biotech Inc, Uden, The Netherlands), all according to manufacturer instructions. To ascertain enough liquid for analysis, microdialysate from 2 microvials collected at adjacent time points was pooled. Microdialysate collected in the morning and evening every day up to 7 PODs were included in the analyses.

Plasma Samples

Blood samples were obtained preoperatively (CRP and pancreas amylase) and daily on POD 1-7 (CRP, pancreas amylase, and lipase). The Division of Laboratory Medicine at Oslo University Hospital analyzed the blood samples as part of the clinic routine.

Statistics

Baseline characteristics were compared between patients with early graft thrombosis and patients with no early complications (event-free) with student's *t* test for normally distributed and Mann-Whitney *U* test for non-normally distributed continuous variables. Chi-square test was used for categorical baseline variables. Normal distribution was determined with histograms and Shapiro-Wilk test. All cytokines were non-normally distributed. Log10-transformed data were used in mixed model analyses to investigate the effect on the cytokine concentrations of the group and time during the first postoperative week. Group (thrombosis/event-free, PTA/SPK) and time variables were fixed effects, and the patient number was random effect. A significant contribution to the model of each explanatory variable (time/group) was evaluated by the Wald Chi-square test. For the cytokines with a significant group effect in the mixed model analysis, we performed a postestimation analysis in addition. In this analysis, estimated marginal means with 95% confidence intervals were compared between groups for every sample time point. Mann-Whitney *U* test was used to compare cytokine concentrations and plasma markers in patients with thrombosis on the day of diagnosis with cytokine concentrations over the whole first postoperative week of nonevent patients. To investigate any association between thrombosis diagnosis during the first postoperative week and graft failure during the first postoperative year Fisher exact test was used. A 2-sided $P < 0.05$ was considered statistically significant.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.r-project.org/, and StataCorp 2019, Stata Statistical Software, Release 16 (StataCorp LLC, College Station, TX) were used for analyses and graphical presentation.

RESULTS

Study Cohort and Clinical Outcome

Sixty-seven consecutive pancreas transplant recipients (32 PTA and 35 SPK) were included in the study. One patient was unable to give consent because of language difficulties and was not included (Figure 2). Venous graft thrombosis was more common in PTA than in SPK recipients ($P = 0.007$, Table 1). Seventeen patients developed a venous graft thrombosis (7 nonocclusive and 10 occlusive), whereas 34 patients were event-free, and 16 had other complications (Figure 2). Arterial pancreas graft thrombosis occurred in 8 patients (3 PTA and 5 SPK) with concurrent venous graft thrombosis and were all deemed clinically insignificant.

Donor and recipient characteristics for patients with venous graft thrombosis and patients with no early complications (event-free) did not differ but for higher preoperative hemoglobin concentrations ($P = 0.025$) in patients with thrombosis, and longer cold ischemia times ($P = 0.01$) in event-free patients (Table 1). Of the 17 venous graft thrombi, 4 were treated with graftectomy and 7 were treated with thrombectomy combined with increased LMWH doses, whereas 4 patients were treated only with increased doses of LMWH and 2 patients received no treatment. During the first year,

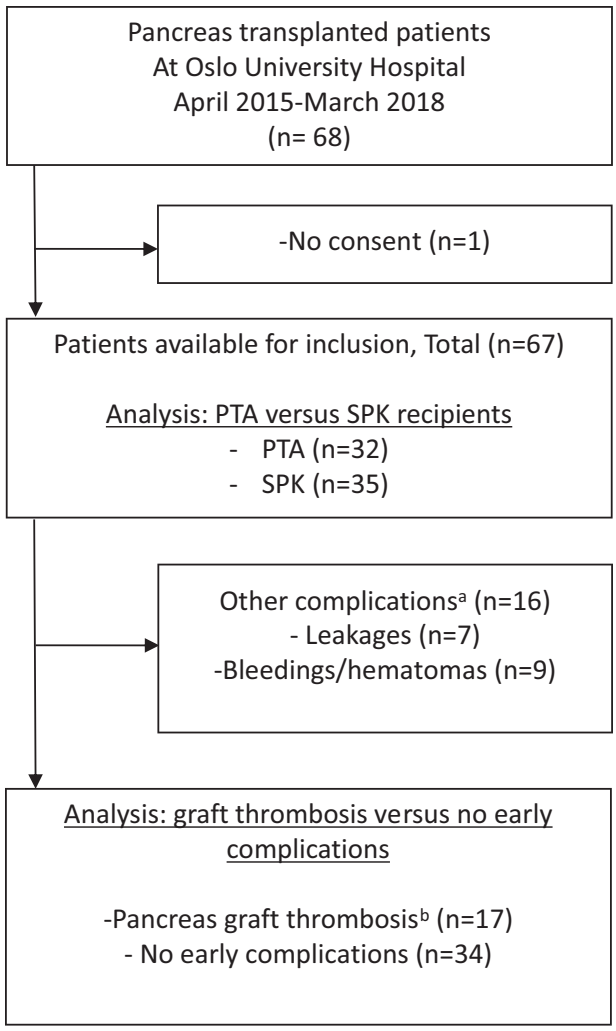

FIGURE 2. Flowchart of study inclusion and exclusion to the analyses of pancreas transplant alone (PTA) vs simultaneous pancreas-kidney (SPK) transplantation, and patients with pancreas graft thrombosis vs patients with no early complications. [a]Included all reported leakages and bleedings/hematomas regardless of need for intervention or not. [b]Partial or complete pancreatic graft vein thrombosis.

a total of 13 grafts lost their endocrine function. One-year graft losses were more common in patients with an early graft thrombosis (n = 8/17) compared with patients with no early complications (n = 1/34) ($P < 0.001$). Of the 8 graft losses with an early diagnosed thrombosis, 4 grafts were explanted within the first 2 postoperative weeks and the other 4 were lost within the postoperative months 4–12.

Microdialysis Samples

In total, we gathered 957 microdialysate samples, of which 710 samples (74%) were successfully analyzed, whereas 247 samples (26%) contained too little sample volume for analysis. We started sampling either on the date of the operation or on the date of the first POD, depending on the time of the day of the surgery. Therefore only 16 patients had samples from the morning and 44 from the evening of the operation day. From PODs 1 to 7, 51 samples were not obtained because of patient-related factors (Figure 3). No adverse effects were noted related to the microdialysis catheters.

Inflammatory Response in Patients With Graft Thrombosis and in Event-free Patients

IL-10 and C5a were not detected in any samples and were not included in further analyses. IL-6, IL-8, IL-1ra, MIP-1β, and IP-10 demonstrated the highest concentrations directly postoperatively and decreased during the first 2 PODs (Figure 4), all with significant time-dependent variations ($P < 0.001$). During the first postoperative week, IL-6 ($P = 0.003$) and IL-8 ($P = 0.027$) were significantly higher in patients with a pancreas graft thrombosis compared with event-free patients (Table 2). Immediately after surgery, IL-6 and IL-8 had similar concentrations in patients with thrombosis and event-free patients ($P > 0.9$ for both). However, IL-6 was significantly higher in patients with pancreas graft thrombosis from POD 1, and IL-8 from POD 2, with the most pronounced differences compared with event-free patients found on POD 7 ($P < 0.001$ for both) (Figure 4A and B, **Table S1, SDC,** http://links.lww.com/TXD/A599). IL-6 and IL-8 were significantly higher on the day of thrombosis diagnosis when compared with all samples obtained from event-free patients ($P < 0.001$ and $P = 0.003$, respectively, Figure 5). IL-1ra, IP-10, and MIP-1β did not differ between patients with graft thrombosis and event-free patients neither when viewed over the whole period, nor at the time of the diagnosis of graft thrombosis (Table 2, **Figure S2, SDC,** http://links.lww.com/TXD/A599). Plasma markers CRP, pancreas amylase, and pancreas lipase on the day of thrombosis diagnosis did not significantly differ from event-free patients during the first postoperative week (**Figure S3, SDC,** http://links.lww.com/TXD/A599).

IL-6 ($P = 0.73$) and IL-8 ($P = 0.91$) did not differ when compared to patients before and after the change in anticoagulation regimen.

Inflammatory Response in PTA and SPK Recipients

Postoperative cytokine concentration did not differ between PTA and SPK recipients during the first 7 PODs (Table 2, **Figure S4, SDC,** http://links.lww.com/TXD/A599).

DISCUSSION

This study compared the postoperative inflammatory response locally at the graft in solid organ pancreas transplantation. We demonstrated increased IL-6 and IL-8 concentrations locally at the pancreas graft in patients with early pancreas graft thrombosis compared with patients with no early complications during the first postoperative week. Early graft thrombosis was more common in PTA compared with SPK recipients, but the local postoperative inflammatory markers investigated did not differ between PTA and SPK recipients.

The total observed thrombosis frequency of 25% is comparable to other studies where both partial and complete thrombi were reported, whereas we had a higher frequency of complete thrombi 15% compared with the reported 5%–8%.[24-26] The predominance of early graft thrombosis in PTA recipients confirmed earlier observations.[10] We demonstrated an increased 1-y pancreas graft failure in patients diagnosed with graft thrombosis during the first postoperative week, which agrees with the previously observed association between pancreas graft thrombosis and graft loss.[8]

In all patients, a local inflammatory reaction at the graft was observed immediately postoperatively, which most

TABLE 1.

Patient characteristics of recipients and donors, and perioperative data

	All (n = 67)	Event-free (n = 34)[a]	Pancreas graft thrombosis (n = 17)[b]	*P*, graft thrombosis vs event-free
Recipient				
Age, y	41 (36–46)	41 (37–47)	43 (36–45)	0.77
Pancreas transplantation alone/simultaneous pancreas-kidney transplantation, n	35/32	23/11	4/13	**0.007**
Male/female, n	38/29	19/15	9/8	1
Body mass index, kg/m^2	24.5 (22.4–27.3)	24.0 (22.4–26.2)	28.4 (23.0–29.4)	0.095
Preoperative coagulation status				
Hemoglobin, g/dL	12.7 (11.3–14.5)	12.6 (10.6–13.5)	13.5 (12.6–14.9)	**0.025**
Thrombocytes, $\times 10^{12}$/L	273 (238–341)	273 (240–339)	333 (273–382)	0.056
International normalized ratio	1.0 (0.9–1.0)	1.0 (0.9–1.0)	1.0 (0.9–1.0)	0.85
Activated partial thromboplastin time, s	34 (31–36)	34 (31–36)	32 (31–35)	0.29
Fibrinogen,[c] g/L	3.7 (3.3–4.4)	3.6 (3.3–4.4)	3.8 (3.3–4.6)	0.79
Donor				
Age, y	23 (19–40)	22 (17–35)	36 (21–45)	0.069
Male/female, n	34/33	17/17	7/10	0.77
Body mass index, kg/m^2	23.5 (21.6–25.9)	22.8 (21.4–25.0)	23.5 (21.2–26.0)	0.75
Donation after brainstem death/donation after circulatory death, n	67/0			
Perioperative data				
Cold ischemia time pancreas	8:16 (6:45–10:44)	08:51 (07:39–11:40)	06:55 (06:13–08:03)	**0.01**
Pancreas artery flow, mL/min	200 (150–260)	215 (162–275)	168 (120–35)	0.073

Continuous variables are presented as median (25th–75th percentiles), categorical variables as numbers. Bold *P* values denote statistical significance at the $P \leq 0.05$ level.
[a]Event-free patients were defined as patients with no complications during the first postoperative week.
[b]Partial or complete pancreatic graft vein thrombosis.
[c]Fibrinogen not registered for the first 14 patients in the study.

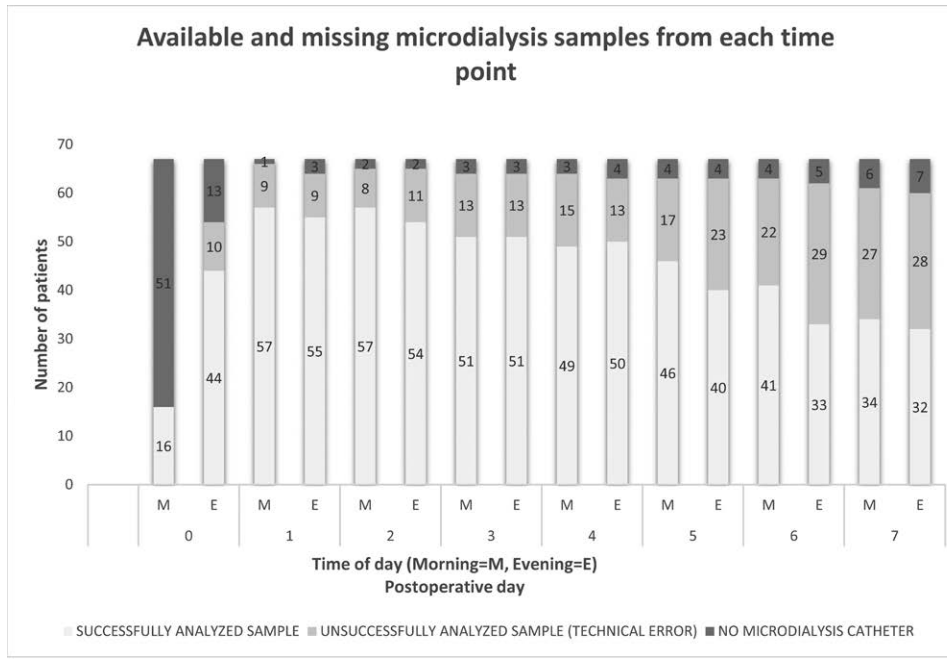

FIGURE 3. Analyzed and missing microdialysis samples from each postoperative sampling timepoint of 67 pancreas-transplanted patients. Sampling started either on the day of surgery (day 0) or on the first postoperative day depending on the time of day, on the day of the surgery. Therefore, not all patients have samples from day 0. Technical errors with the microdialysis were due to not enough sample volume, probably due to partially or totally occluded catheters. Reasons for no microdialysis catheter were patient-related: 1 patient with initial open abdomen and delayed placement of microdialysis catheters, 2 graft extirpations, 4 patients discharged from the surgical ward before postoperative day 7, and 1 patient accidentally discontinued the microdialysis.

probably was due to the ischemia-reperfusion injury[14] in combination with the surgical trauma.[27] In the event-free patients, the concentrations of the detected cytokines; IL-6, IL-8, IL-1ra, IP-10, and MIP-1β, were highest at the first measurements posttransplantation, and then gradually decreased to level out approximately on the second POD.

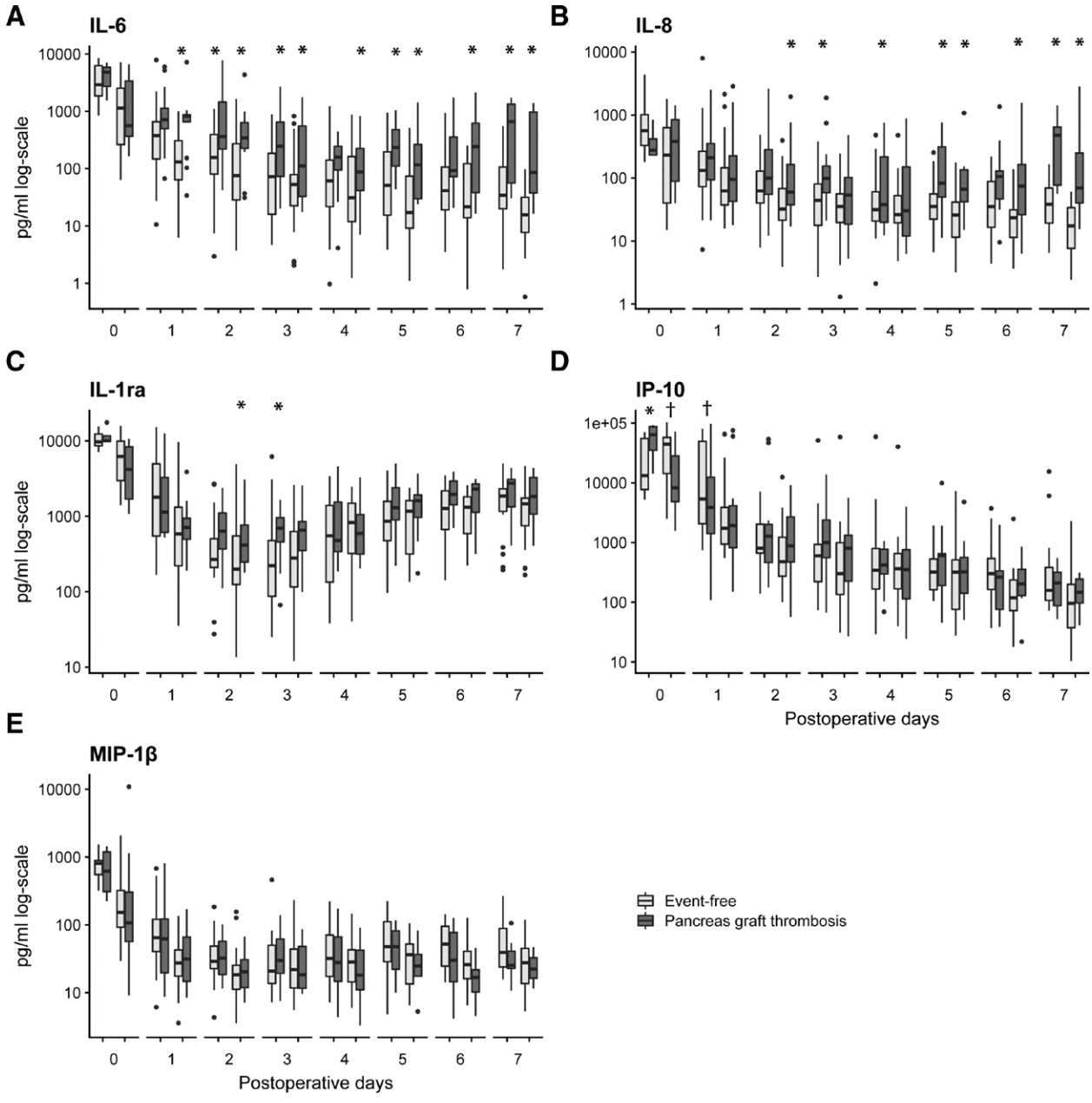

FIGURE 4. Locally measured cytokine concentrations over the first postoperative week for pancreas transplant recipients with an early pancreas graft thrombosis (n = 17) compared with those with no early complications (event-free) (n = 34). Samples obtained with microdialysis catheters adjacent to the pancreas graft were analyzed at 2 timepoints per day during the first postoperative week. Interleukin (IL)-6 ($P = 0.003$) and IL-8 ($P = 0.027$) were significantly higher in patients with graft thrombosis compared with those with an event-free course over the first postoperative week (mixed model analysis). Significant differences in cytokine concentrations, $P < 0.05$, of pairwise comparisons between graft thrombosis and event-free patients for every timepoint is marked with asterisks (graft thrombosis patients with higher cytokine concentrations) and daggers (event-free patients with higher concentrations). Exact *P* values for IL-6 and IL-8 are presented in **Table S1, SDC** (http://links.lww.com/TXD/A599) (postestimation of mixed model analysis with pairwise comparisons of estimated marginal means). Day 0 is the day of surgery. Data are presented as boxplots (*line*, median; *box*, interquartile range) with whiskers (25th and 75th percentiles ±1.5 × interquartile range). IL-1ra, interleukin-1 receptor antagonist; IP-10, interferon γ-induced protein 10; MIP, macrophage inflammatory protein.

This time pattern agrees with previous results of systemic cytokines in pancreas transplantation, which peak early postoperatively and then return to baseline within the first PODs.[16,28]

Patients with early pancreas graft thrombosis had significantly increased IL-6 and IL-8 locally at the graft during the first postoperative week compared with patients with no complications. This corresponds with the known pathophysiology of inflammation and thrombosis. IL-6 promotes coagulation and hemostasis[29] and is involved in several inflammatory and immunomodulatory pathways,[30] whereas the chemokine IL-8 is a potent chemoattractant of neutrophils[31] suggested to be both prothrombotic and involved in thrombus resolution.[32] Both IL-6 and IL-8 have been associated with venous thromboembolism (VTE)[33] and with postoperative ischemia/thrombosis in liver grafts.[21]

TABLE 2.

Overall effects of time and group on the cytokine time course during the first postoperative week[a]

	Time effect		Graft thrombosis vs event-free patients (group effect)		Simultaneous pancreas-kidney vs pancreas transplantation alone patients (group effect)	
Cytokine	**Wald χ^2**	***P***	**Wald χ^2**	***P***	**Wald χ^2**	***P***
Interleukin-1 receptor antagonist	471.84	**<0.001**	2.67	0.445	0	0.99
IL-6	269.47	**<0.001**	20.9	**0.003**	0.009	0.77
IL-8	129.58	**<0.001**	12.1	**0.027**	0.02	0.90
Interferon γ-induced protein 10	1095.66	**<0.001**	1.08	0.881	0.87	0.35
Macrophage inflammatory protein-1β	334.33	**<0.001**	5.91	0.795	0.85	0.36

Bold *P* denote statistical significance at the $P \leq 0.05$ level.

[a]Linear mixed model analyses on log-transformed data with time or group (thrombus/no event, SPK/PTA) as fixed effects, and individual as random effect. Overall effects were determined with Wald χ^2 test.

IL, interleukin; PTA, pancreas transplant alone; SPK, simultaneous pancreas-kidney.

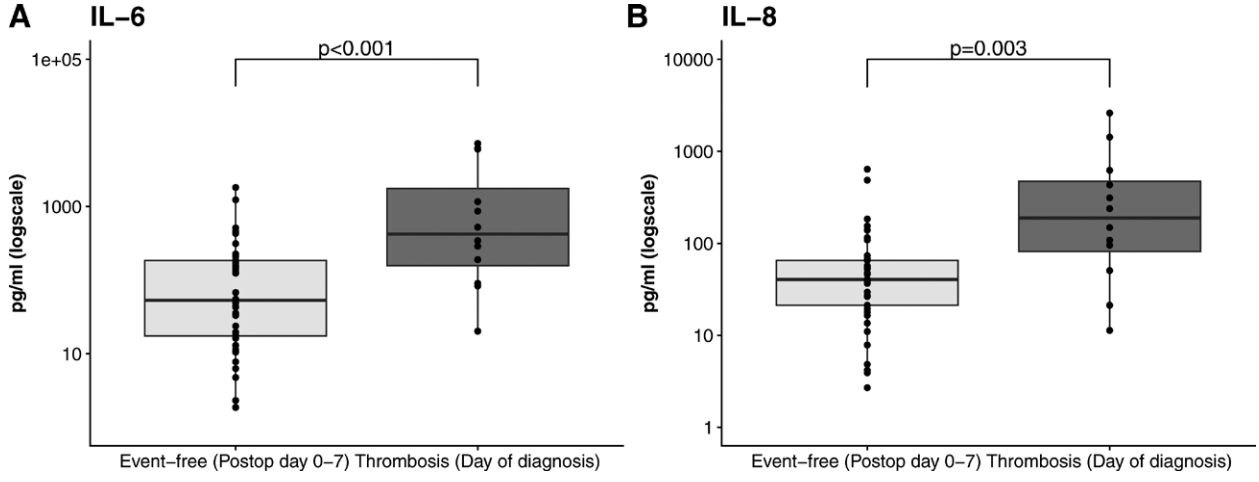

FIGURE 5. Patients with graft thrombosis had significantly higher interleukin (IL)-6 and IL-8 on the day of diagnosis compared with all samples from the first postoperative week of event-free patients. Group comparisons with Mann-Whitney *U* test, *P* values presented in the boxplots.

Our previous systemic cytokine measurements in pancreas transplantation demonstrated that IL-6, but not IL-8, was increased in patients with graft thrombosis, but only on the first POD.[16] This difference between systemic and locally assessed inflammation suggests the added value of higher measurement sensitivity obtained by organ-close monitoring.

Immediately after reperfusion, IL-6 and IL-8 concentrations were comparable between patients with graft thrombosis and event-free patients, but did not decrease to the same extent, and remained elevated in patients with graft thrombosis throughout the first postoperative week. This might suggest that the process of thrombosis generation is time-dependent and accompanied by the induction of proinflammatory cytokines. However, the observational study design excludes a description of a causal relationship between thrombosis and inflammation. In studies of VTE, proinflammatory cytokines were normal before a diagnosis of deep venous thrombosis but remained elevated for a long time after initiating anticoagulation treatment.[34] Nevertheless, once induced, proinflammatory cytokines may play a role in both exacerbating and maintaining the thromboinflammatory process and thereby serve both as markers of injury severity and mediators of thrombosis.[34] Future studies should consider the potential effects of cytokine inhibition as treatment/prevention of graft thrombosis after ischemia-reperfusion injury. Interestingly, the IL-6 receptor blocker, tocilizumab, has shown potential in decreasing the degree of tissue damage after ischemia-reperfusion injury in patients with myocardial infarction.[35]

On the day of diagnosis in patients with pancreas graft thrombosis, commonly used plasma markers of inflammation and pancreas damage: CRP, pancreas amylase, and lipase did not significantly differ from patients with no early complications. On the contrary, IL-6 and IL-8 concentrations were higher when compared with all measurements during the first postoperative week in event-free patients. Hence, local IL-6 and IL-8 could be considered diagnostic markers in postoperative graft monitoring. However, IL-6 and IL-8 could also increase from inflammation of other causes. In addition, these analyses are both time- and cost-consuming with significant limitations related to the microdialysis method itself. Thus, before monitoring inflammatory markers with microdialysis could be suited as a diagnostic tool in routine clinical practice, automation of the microdialysis method and point-of-care analysis of cytokines should be available.

Although we recently have seen differences in the postoperative systemic thromboinflammatory response between PTA and SPK recipients,[16] cytokine concentrations measured locally at the pancreas graft were comparable between the

recipient groups in this study. Hence, it is possible that differences in the thromboinflammatory response between the recipient types are primarily on a systemic level. Other factors in the local inflammatory response, such as complement activation or antibody response that were not assessed in our study could also be of importance for differences in outcome between these groups.

Even if C5a and IL-10 previously have been detected with microdialysis,[21] these markers were not detected in our setup. We had anticipated a local complement activation due to ischemia-reperfusion injury or associated with thrombosis formation because increased C5a has been associated with ischemia/thrombosis in liver grafts.[21,36] A possible explanation as to why we were unable to detect C5a may be due to its rapid receptor binding and degradation by proteases,[37] or due to inactivation enzymes present in the peritoneal fluid.[38] Sampling directly from the pancreas tissue would probably have been necessary to detect a local complement activation in the pancreas graft.

The limitations of this study are mainly related to the microdialysis technique. In fear of inflicting damage by penetrating the pancreas capsule, we opted to affix the microdialysis catheters to structures adjacent to the pancreas graft rather than inserting these into the pancreas parenchyma. Thus, the distances between the membrane on the tips of the microdialysis catheters and the pancreas surface might have varied between patients and could have affected cytokine concentrations. In addition, recovery rates of cytokines in microdialysate from extracellular fluid around the pancreas are not known and may vary. The recovery of cytokines included in this study has previously been evaluated in plasma to lie between 9% and 43%.[23] Metabolites measured on the organ surface correlate to levels measured in the parenchyma reasonably well,[39,40] but inflammatory markers are larger molecules, and concentrations measured on the organ surface may not completely reflect parenchymal concentrations, that is, parenchymal concentrations could be higher. However, our longitudinal approach ensures that trends over time reflect inflammation in or close to the pancreas graft.

Another limitation was that patient groups were not entirely comparable. Patients with graft thrombosis and patients without early complications had similar baseline donor and recipient characteristics apart from higher preoperative hemoglobin in patients with graft thrombosis, and longer ischemia times in nonevent patients. Higher hemoglobin in patients with thrombosis can be explained by the fact that most of these patients were represented by PTA recipients with normal kidney function. A higher hemoglobin concentration has not been reported as a risk factor for pancreas graft thrombosis but is associated with an increase in VTE in the general population, and this observation warrants further investigation.[41] A prolonged cold ischemia time is a known risk factor for graft thrombosis.[9] However, cold ischemia time was longer in event-free patients, and considerably shorter than 12 h in both groups, making it unlikely that cold ischemia time is a cofactor for graft thrombosis in this study. During the study period, the anticoagulation strategy was modified, which could potentially affect the thromboinflammatory response.[5] However, there were no observed clinical differences, with equal numbers of pancreas graft thrombosis before and after the change of anticoagulation. Second, despite the potential anti-inflammatory effects of heparin and its derivatives,[42,43] cytokine levels in the more extensively anticoagulated patients affected with graft thrombosis were higher compared with the less anticoagulated patients without complications. Furthermore, no differences were seen between PTA and SPK recipients although PTA recipients received higher doses of LMWH.

CONCLUSIONS

In conclusion, local postoperative inflammation occurs at the pancreas graft and resolves during the first PODs in patients with no early complications. Patients with an early pancreas graft thrombosis have significantly higher levels of local IL-6 and IL-8 as a sign of increased local thromboinflammation. If and how cytokines contribute to and/or aggravate thrombosis formation must be further investigated in interventional studies. Inflammatory components could be considered potential targets for cytokine inhibition therapy in future studies. The local inflammatory response investigated in this study did not differ between PTA and SPK recipients, highlighting that the immunogenic differences between these recipient groups may not be dependent on pancreas graft-associated inflammation.

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