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Single-shot antithrombin in human pancreas–kidney transplantation: reduction of reperfusion pancreatitis and prevention of graft thrombosis*

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Summary

Reperfusion pancreatitis and graft thrombosis often induce early graft loss in simultaneous pancreas–kidney (SPK) transplantation. Antithrombin (AT) is a coagulatory inhibitor with pleiotropic activities that reduces experimental ischemia/reperfusion injury. This study retrospectively analyses prophylactic high-dose AT application in patients with first SPK. In an university transplantation center, 53 consecutive patients with SPK were studied without randomization. In one group, 3000 IU of AT was given intravenously before pancreatic reperfusion (AT, $n = 24$). Patients receiving standard therapy including postoperative AT supplementation (controls, $n = 29$) served as controls. Daily blood sampling was performed as a part of the clinical routine during four postoperative days. There were no differences in demographic and laboratory parameters [donor/recipient age, ischemia time, perfusion solution, body weight, mismatches] between both groups. Baseline creatinine values were lower in the control group versus AT group ($P < 0.05$). Coagulatory parameters and bleeding incidence were not influenced by AT, while incidence of graft thrombosis was reduced (control: 7/29; AT: 4/24; relative reduction of risk: −33%; $P < 0.05$). Single-shot AT application during SPK modulated serum lipase activity on postoperative days 2 and 3, and minimized risk for graft thromboses without increasing perioperative bleeding. This new concept should deserve testing in a prospective clinical trial.

Introduction

During the last decades, simultaneous pancreas–kidney transplantation (SPK) has been established, and nowadays it plays a growing role in the treatment of insulin-dependent diabetes mellitus [1]. Despite technical refinements, improved perioperative adjunctive therapy protocols, and improvements in perfusion solutions during donor management and back table preparation [2], almost 32% of patients require relaparotomy due to postoperative complications [3]. These re-operations are related to increasing mortality (up to 9% and more) also in experienced centers [4]. Graft thrombosis is one of the most common causes of relaparotomy in up to 20% of patients [5]. Although some centers described preservation of pancreas function after anticoagulation, surgical thrombectomy, or even partial pancreatic resection [6–8], transplant thrombosis is often related to graft loss [8].

Early pancreatic graft loss may be caused by reperfusion pancreatitis and technical problems in the recipient, which may at least in part explain vascular thrombosis of the graft with subsequent graft necrosis

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[9]. Recently, pancreatic microcirculation and ischemia-induced tissue injury were correlated with the severity of graft pancreatitis in the postoperative course [10]. Another potential reason for early graft loss could be prothrombotic disorders (PTD). Under these circumstances ischemia reperfusion injury could induce coagulatory activation, graft pancreatitis and subsequent thrombosis [11].

Antithrombin (AT) constitutes one principle natural coagulatory inhibitor interfering with the clotting process at various sites [12]. In addition, AT has been shown to directly interact with the endothelial surface [13]. This interaction with the endothelium reduces microcirculatory disorders and tissue injury independently from anticoagulation [14] after renal [15], intestinal [16], and hepatic ischemia/reperfusion [17,18]. In addition, AT reduced rejection in renal xenotransplantation [19], and allogenic cardiac transplants [20].

Our group recently characterized significantly improved renal allograft reperfusion by single-shot AT application (4000 IU) during human kidney transplantation in a randomized controlled trial [21]. Thus far, however, prophylactic AT effects have not been investigated in patients with SPK. This study reports worldwide first results from a cohort of SPK patients who were pretreated with a single-dose AT in a nonrandomized fashion in a single pancreas transplant center.

Materials and methods

Patients

Before SPK transplantation all patients underwent a comprehensive medical evaluation by a multidisciplinary team of physicians to confirm the diagnosis of insulin-dependent diabetes mellitus and to establish the absence of any exclusion criteria for pancreas transplantation. In case of a positive history for PTDs (factor-V-Leiden mutation, prothrombin mutation, deficiency of protein C or protein S, antiphospholipid syndromes, and hereditary AT deficiency), patients were excluded from this evaluation. Only patients with end-stage diabetes and secondary diabetic complications and simultaneous deterioration of renal function were selected for the operative procedure.

All patients had confirmed type 1 diabetes as determined by low-serum C-peptide levels, and had undergone pre-operative cardiac stress testing or coronary angiography to exclude relevant coronary heart disease. All SPK transplant patients operated between January 2001 and June 2004 were consecutively included.

Study design

Antithrombin treatment included intraoperative single-dose application of 3000 IU of AT at the beginning of the renal vein anastomosis (Fig. 1). AT dose was reduced to 75% of the kidney transplant trial [21] to avoid bleeding in the pancreatic reperfusion period. The introduction of AT in the perioperative routine adjunctive treatment protocol was performed under the impression of positive findings in the kidney transplant trial [21]. We decided not to perform a prospective randomized single center trial because of enormous interindividual variabilities and specific patient histories (sometimes complicated clinical courses) in patients with SPK. Therefore, intraoperative AT application was introduced in the perioperative management prescriptions (volume therapy, intravenous (i.v.) heparin, i.v. prostacyclin application, aprotinine, fluconazole, imipenem, acetylcysteine, vitamin C, and vitamin E). Indeed two versions of perioperative protocols existed in parallel in our transplant unit database that could be applied during first SPK transplantations. Thus, inclusion in the different treatment groups remained a matter of chance (the influence of AT was not judged to be significant as a single factor at this time point). It is important to state that this procedure does not meet the criteria of a randomization process. The treatment protocol had to be finally approved by the operating surgeon on duty. Four surgeons performed transplantation procedures.

The prior perioperative management prescription in controls included AT supplementation in the later postoperative course. This low-dose AT application protocol included AT supplementation in cases where AT activity postoperatively decreased to <70%, but ≥60% with 1000 IU of AT. In case of lower AT activities (<60%), 2000 IU were routinely substituted as part of the prior standard protocol.

The present analysis only focuses on 53 patients with first SPK transplantation, and excludes 16 patients in the referring time period who were candidates for solitary pancreas transplantation [pancreas after kidney (PAK) transplantation]. For PAK patients lower graft survival rates are known that are not comparable with the simultaneously transplanted group [22].

Operative procedure

After mid-line incision, kidneys were implanted on the left common or external iliac vessels in an end-to-side technique. The pancreatic grafts were implanted intraperitoneally using the donor iliac artery construction of superior mesenteric artery with splenic artery or by implanting the splenic artery in the superior mesenteric artery with continuous suturing. A systemic venous drainage and enteric drainage of the exocrine secretions by side-to-side two-layer hand-sutured duodeno-jejunostomy was performed. Appendectomy was routinely performed to exclude potential subsequent appendicitis because in our center perforated appendicitis with peritonitis after

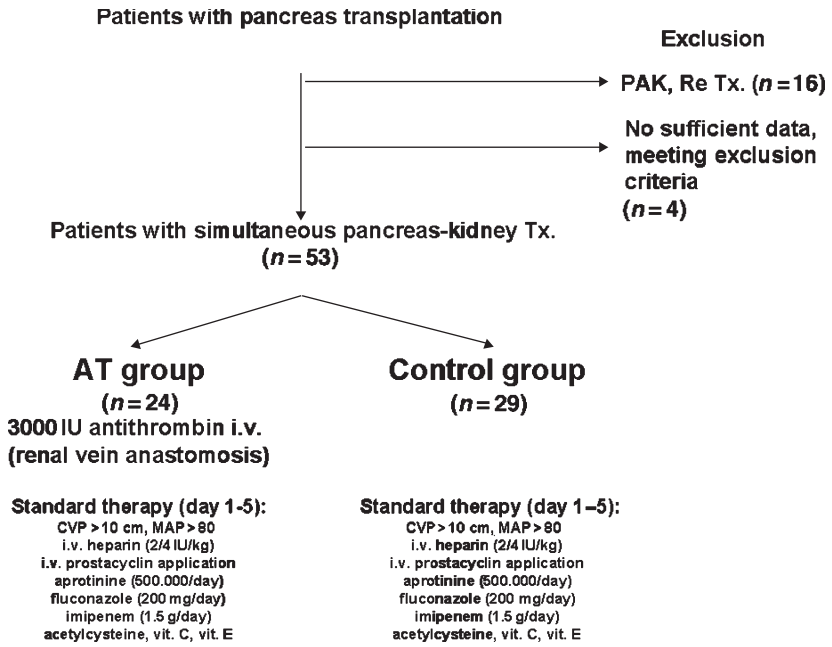

Figure 1 shows the design of the study. From a total of 73 patients, 53 patients met the inclusion criteria. Sixteen patients with pancreas after kidney transplantation and four patients not meeting study criteria were excluded. Antithrombin (AT) patients received a bolus infusion of AT (3000 IU) at the beginning of the renal vein anastomosis intraoperatively before graft reperfusion. Heparin administration was started using 2–4 IU/kg/h as early as 6 h after the operation according to coagulatory parameters and blood hemoglobin values. A central venous pressure (CVP) >10 cm and a mean arterial pressure (MAP) >80 mmHg were targeted by dopamine and crystalloid administration. Blood hemoglobin levels were kept constant around 10 g/dl by red blood cell concentrates. Additional standard perioperative therapy included i.v. heparin, i.v. prostacyclin, aprotinine, acetylcysteine, fluconazole, imipenem, vitamin C, and vitamin E, and was comparable between groups.

simultaneous transplantation occurred in two cases so far. However, appendectomy is not recommended generally.

Immunosuppression

A quadruple induction scheme was used with tacrolimus, mycophenolate mofetil (MMF), methylprednisolone and serum induction with antithymocyte globulin (ATG) or antilymphocyte globulin (ALG). Administration was started prior to graft reperfusion and continued postoperatively. Maintenance immunosuppression consisted of tacrolimus, MMF, rapamycin and prednisolone, which was tapered after 14 days.

Graft surveillance and therapy

Kidney biopsies were performed, when clinically indicated, in a ultrasound-guided percutaneous technique. Biopsies of the pancreas were not performed. Insulin was given when blood glucose exceeded 150 mg/dl. In case of increased glucose levels, fever, or relevant right lower quadrant abdominal pain, a conventional transplant angiography or magnetic resonance imaging angiography was performed. In case of complete thrombosis of the graft, emergency surgical thrombectomy was attempted but did not result in organ survival.

Laboratory analysis

Pre-operative and daily postoperative laboratory analysis was performed in blood specimens that were routinely taken as part of the routine monitoring. Blood samples for platelet, leukocyte count and hemoglobin analysis were collected in commercially available ethylenediaminetetraacetic acid tubes. Plasma samples were collected in citrate-treated tubes (Monovette, Sarstedt, Numbrecht, Germany). Samples were centrifuged for routine testing and analysis was performed in <1 h after sampling. Commercially available kits were used for all assays according to the manufacturers' instructions. Laboratory data were prospectively collected in the transplant database system. Also creatinine and urea concentrations were determined from serum specimens by routine laboratory methods.

Evaluation of AT side effects

During extensive clinical AT application in surgical patients with severe sepsis, we did not observe changes in

blood coagulatory parameters (partial thromboplastin time, thromboplastin time, and platelet count) and in the incidence of bleeding [23]. To exclude potential AT side effects, the records of all patients included in this analysis were retrospectively reviewed. All packed red blood cell units during the first 3 days were counted. Also use of fresh-frozen plasma, prothrombin complex, and platelet concentrate application was documented.

Data and statistical analysis

All parameters are given as mean ± SEM. To detect potential differences between patient groups, baseline parameters were compared with the unpaired *t*-test. If data were not normally distributed, the Wilcoxon test (Mann–Whitney *U*-test) for unpaired observations was used. Laboratory data were analyzed at the different time points postoperatively by using an unpaired *t*-test. Inner group comparisons were performed with paired *t*-test. A one-way analysis of variance was not used, since the maximum of changes over time was supposed at day 2 and 3. To compare the incidence of graft thrombosis, chi-squared test was used.

Results

Baseline parameters

Donor and patient demographics are presented in Table 1. Both treatment groups (control $n = 29$; AT $n = 24$) were well comparable in terms of recipient age, body weight, size, gender, and pre-operative urine output. Fifty donors received University of Wisconsin (UW) perfusion, whereas one donor (AT group) received histidine tryptophan-ketogluterate (HTK). In two donors (one AT and one control) the organ report form was not completed appropriately. Only UW solution was used for back table perfusion in both groups. Also other donor criteria (donor age, cold ischemia, type of perfusion solution, and number of human leukocyte antigen mismatches), dialysis modalities (data not shown) were comparable between groups. There was a tendency towards a higher urine output in control patients when compared with AT patients.

Table 1. Demographic parameters and baseline laboratory data in recipients of pancreas–kidney transplantation and respective organ donors.

	Control ($n = 29$)	Antithrombin ($n = 24$)	*P*-value
Recipient age	42.9 ± 1.6	44.4 ± 1.3	0.46
Body weight (kg)	68.0 ± 2.2	72.4 ± 2.5	0.19
Height (cm)	171.1 ± 1.5	171.0 ± 2.5	0.90
Diuresis (ml/per day)	994 ± 178	610 ± 135	0.09
Mismatches (*n*)	4.2 ± 0.2	4.5 ± 0.2	0.39
Cold ischemia time (h)	14.0 ± 0.7	12.6 ± 1.2	0.31
Donor age (years)	33.0 ± 2.6	32.1 ± 2.2	0.79
Blood glucose (mg/dl)	191 ± 17	175 ± 24	0.57
Hemoglobin (mg/dl)	11.2 ± 1.4	11.4 ± 1.5	0.91
Lipase (U/l)	103 ± 14	64 ± 27	0.20
Amylase (U/l)	90 ± 6	110 ± 20	0.23
Thromboplastin test (%)	89 ± 3	89 ± 3	0.93
Partial thromboplastin time (s)	35.1 ± 0.7	33.8 ± 0.7	0.18
Leukocytes (G/l)	8.5 ± 0.6	8.6 ± 0.5	0.37
Platelet count (G/l)	250 ± 15	272 ± 19	0.37
ALT (alanin amino transferase U/l)	12.5 ± 1.3	14.6 ± 3.3	0.38
Urea (mg/dl)	97 ± 7	108 ± 8	0.30
Creatinine (mg/dl)	6.4 ± 0.4	8.3 ± 0.5	0.01

Baseline laboratory parameters are also depicted in Table 1. There were no significant differences between both groups in pre-operative glucose levels and serum lipase, amylase, and serum liver enzyme activities. Also routine coagulatory parameters (thromboplastin time, partial thromboplastin time, and platelet count) were well comparable between groups. Although urea concentrations were comparable, creatinine analysis showed significantly lower values in control patients when compared with AT patients ($P = 0.01$). This observation corresponded with the higher urine output in controls.

In 24 AT patients, 72 000 IU of AT (3000 IU/patient) were given before reperfusion of the pancreas graft at the beginning of the renal vein anastomosis. In controls, AT was applicated in 16 from 29 patients with a mean dose of 1562 ± 157 IU at 5 ± 1 h postoperatively. There was no difference in serum lipase activities between control patients receiving postoperative AT supplementation and those control patients who did not (data not shown). This finding clearly indicates that only AT application before reperfusion can reduce serum lipase release.

Effect of AT application on serum lipase activities

Single-shot high-dosed intraoperative AT application (3000 IU) reduced postoperative serum lipase activity in AT patients when compared with controls (Fig. 2). Thus, AT-treated patients showed significantly lower lipase activities at postoperative days 2 and 3 with a tendency towards lower values also at days 1 and 4. There was a significant decrease in lipase activity over four postoperative days in both groups. Only AT-treated patients reached the normal range already after day 4. Amylase measurements were not performed routinely.

Effect of AT on standard coagulatory parameters

Despite a significant modulation of serum lipase activity, AT did not significantly influence standard coagulatory parameters (Table 2). Thus, prothrombin time and partial

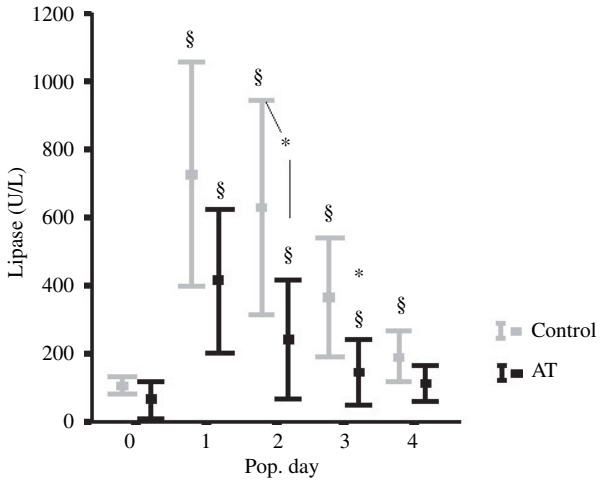

Figure 2 Effect of antithrombin (AT) bolus application (3000 IU) before reperfusion on serum lipase activity. AT represents patients ($n = 24$) who received 3000 IU of AT before graft reperfusion. Control represents patients ($n = 29$) in whom standard therapy without intraoperative AT application was performed. §Indicates significant ($P < 0.05$) inner group differences from baseline. *Indicates significant ($P < 0.05$) differences between AT patients and control patients at the respective time point. Data are the mean ± SEM.

Table 2. Laboratory data in recipients of pancreas–kidney transplantation at days 1–4.

	Day 1	Day 2	Day 3	Day 4
Blood glucose (mg/dl)				
Control	124 ± 10*	130 ± 7*	135 ± 9*	139 ± 9*
Antithrombin (AT)	121 ± 10*	125 ± 7*	134 ± 8*	118 ± 4*
Leukocytes (G/l)				
Control	11.0 ± 0.8*	11.8 ± 0.9*	10.3 ± 0.7*	9.3 ± 0.6
AT	10.6 ± 0.7*	10.7 ± 0.7*	10.1 ± 0.9	8.6 ± 0.7
Platelet count (G/l)				
Control	165 ± 11*	156 ± 10*	137 ± 10*	121 ± 9*
AT	177 ± 54*	184 ± 14*	159 ± 12*	147 ± 12*
Urea (mg/dl)				
Control	88 ± 6	111 ± 9	123 ± 11	137 ± 14
AT	83 ± 7*	96 ± 9*	117 ± 12	128 ± 14
Creatinine (mg/dl)				
Control	5.2 ± 0.3*	4.0 ± 0.5*	3.2 ± 0.4*	3.0 ± 0.5*
AT	5.1 ± 0.4*	3.8 ± 0.5*	3.7 ± 0.7*	3.5 ± 0.7*
Thromboplastin time (%)				
Control	75 ± 1*	74 ± 2*	79 ± 2*	78 ± 3*
AT	73 ± 1*	76 ± 2*	76 ± 2*	76 ± 2*
Partial thromboplastin time (s)				
Control	35 ± 1	38 ± 1	36 ± 1	36 ± 1
AT	35 ± 1	37 ± 2	33 ± 1	36 ± 1

*, Indicates statistically significant ($P < 0.05$) inner group changes from baseline.

thromboplastin time were not modified by AT application over time. Prothrombin time remained lower than baseline over the whole observation period.

Effect of AT on blood glucose, leukocyte count, platelet count, and serum urea and creatinine

Table 2 shows a clearly reduced glucose concentration as early as at day 1 after the operation during four postoperative days in both groups when compared with baseline values (Table 1).

The operative trauma induced increased systemic leukocyte counts in both groups at postoperative day 1–3 that were not significantly influenced by AT (Table 2). However, there was a tendency towards reduced leukocyte counts in AT patients. Leukocyte counts normalized as early as on postoperative day 4 in both groups.

In both groups, platelet counts were significantly decreased in the postoperative course when compared with baseline values Table 2. Decreasing platelet counts may be caused by blood loss and application of polyclonal antibodies for immunosuppressive induction therapy. There was a tendency towards higher platelet counts in AT-treated patients.

Urea concentrations were comparable between groups at baseline and decreased at postoperative day 1 independent from AT application. Urea concentrations increased at day 2–4 in both groups independent from AT application (Table 2).

Although serum creatinine concentrations were significantly higher in AT patients when compared with controls at the beginning of the study, both groups showed a comparable significant decrease in serum creatinine at day 1–4 that was related to functioning kidney grafts (Table 2).

Effect of AT on the incidence of graft thrombosis

Seven from 29 patients in the control group developed early graft thrombosis (24% of patients). In contrast, graft thrombosis was observed in four from 24 patients in the AT group (16%). The reduction of relative risk for graft thrombosis amounts 33% ($P < 0.05$).

Discussion

Total pancreas grafting has emerged as the standard operative procedure in most centers all over the world [24]. Despite standardization and improvements in operative techniques, vascular thrombosis of pancreatic grafts still remains to be an important cause of pancreatic graft loss [5,24–26]. The degree of ischemia reperfusion injury may represent one key mechanism for the development of graft dysfunction and postoperative graft thrombosis [24].

At baseline, both patient groups were well comparable in terms of known risk factors for pancreatic graft throm-

bosis. Due to a strict selection process during organ acceptance by the surgeon on duty, only selected donors were accepted without specific history of pancreatic disease (preconditions: no significant alcohol abuse, short cold ischemia time, no technical problems during organ procurement, ICU time <5 days). Only donors <50 years old were accepted. Thus, there were no significant differences in donor age, cold ischemia time, and number of antigen mismatches between patient groups. Also demographic recipient conditions (age and gender) were well comparable between groups. All surgeons used UW solution for back table perfusion of the pancreas. Moreover, there was no difference between the perfusion solutions used during organ grafting. Perfusion solutions have been experimentally shown to be critical during organ reperfusion [2]. As obesity is known to significantly influence complication rates and outcome during SPK transplantation [27], we also analyzed demographic data (height and body weight) from our recipients without finding differences between AT patients and controls. Thus, there was no evidence for any selection bias explaining better graft survival and less lipase release in AT-treated patients. Besides, in our center, PTDs were routinely excluded during initial evaluation for combined transplantation by the pre-operative measurement of AT activity, protein-C activity and factor-V Leiden mutations in case of a positive history for venous thrombosis according to Wullstein *et al.* [28].

The extent of ischemia reperfusion injury is known to contribute to graft pancreatitis, and becomes clinically evident by abdominal pain, and systemically and locally released pancreatic enzymes [24]. Postoperative elevation of pancreatic enzymes exceeding five days post-transplantation has been defined as an independent risk factor for graft thrombosis [22]. Interestingly, single-shot AT infusion significantly reduced serum lipase concentration on postoperative days 2 and 3, and lead to normalization of serum lipase at postoperative day 4. This significant reduction in serum lipase may indicate potential reduction of reperfusion pancreatitis resulting in less lipase liberation that may have been responsible for the lower incidence of vascular thrombosis. We focused our analysis on the first postoperative days after SPK, as vascular thrombosis and bland ischemic parenchymal necrosis are observed during the first postoperative days in almost 80% of patients [9]. Unfortunately, our routine monitoring for cost reasons was restricted to serial serum lipase measurements that were judged to be more specific for pancreatic injury than serum amylase levels.

There is increasing clinical and experimental evidence that also other proinflammatory processes producing coagulatory activation and clot formation can finally result in vascular thrombosis of the low-flow organ pancreas [5]. Besides, endothelial injury caused by surgical trauma may cause tissue factor release and subsequent coagulatory activation [29]. The local mismatch of pro- and anticoagulatory factors has been recently quantified by direct measurements of thrombin–AT complex concentrations and natural coagulatory inhibitor activity (protein C and AT activity) [30].

We did not observe AT-mediated changes on standard coagulatory tests (partial thromboplastin time, thromboplastin time, and platelet counts). This finding is in agreement with the literature where long-term AT application also did not influence these standard coagulation tests [23]. The missing effect on standard blood coagulation tests is in line with our observation that perioperative bleeding rates were not increased after single-shot AT application. Thus, we did not observe increased application of packed red blood cell concentrates in the AT group when compared with controls. This finding might have been due to the lower dose of AT (3000 IU) when compared with the allogenic kidney transplantation study performed in our center where we also could not detect an increased bleeding incidence (4000 IU) [21].

Perioperative management of patients with SPK transplantation remains a matter of debate. There are no standardized guidelines for perioperative anticoagulation in these patients, thus far, and many different anticoagulation policies are actually practiced in the respective transplantation centers. Some centers routinely use i.v. low-dose heparin to prevent graft thrombosis and subsequent aspirin prophylaxis [26]. Another group recommended the use of low molecular weight dextran followed by i.v. heparin and AT substitution in combination with long-term administration of acetylsalicylic acid [31]. Also dextran use followed by low-dose aspirin has been recommended [32]. Prophylactic application of high-dosed AT before pancreatic reperfusion has not been advocated by the literature, thus far, whereas postoperative supplementation as performed in our controls with the former perioperative treatment protocol has often been suggested.

Also other strategies which aim at a reduction of pancreatic ischemia reperfusion injury have been tested under experimental conditions. Thus, ischemic preconditioning which has been shown to reduce hepatic injury has been experimentally tested, however, without finding effects on the pancreatic microcirculation despite reducing apoptosis (33). On the other hand, nitric oxide (NO) application improved microcirculation to some extent (34), a fact that is also interesting under the view that AT has been shown to liberate NO by interaction with the endothelium [14].

In conclusion, it has to be stressed that, our study does not meet criteria for a prospective, randomized controlled clinical trial. As stated in the *Materials and methods*

section we did not assume that one single change in the perioperative protocol (AT application before versus after reperfusion and difference in dosage) would indeed change the rate of graft thromboses and the clinical outcome. Thus, the 'new perioperative treatment protocol' including AT application was introduced under the impression of a randomized kidney transplant trial [21]. However, data collection in patients with SPK was also performed in a prospective manner as a part of our routine perioperative procedure with our transplant database system.

Despite the potential shortcomings of our study, we feel that our experience with this new protocol may be important for the management of patients receiving SPK, and could save transplant organs, medical resources, and potentially patient lives that can be compromised by emergency pancreatectomy after graft thrombosis. Our promising concept should deserve clinical testing in a prospective, randomized clinical trial. To our knowledge, a protocol for a clinical phase III trial testing prereperfusion AT application in combination with acetylcysteine is currently in preparation.

First Authorship

Dr Fertmann and Dr Wimmer equally contributed to the performance of the study, and were equally involved in data management, presentation, and discussion. There are no sources of support to declare.

References

1. Tyden G, Bolinder J, Solders G, Brattstrom C, Tibell A, Groth CG. Improved survival in patients with insulin-dependent diabetes mellitus and end stage diabetic nephropathy after combined transplantation. *Transplantation* 1999; **67**: 645.
2. Troisi R, Meester D, Van Den Broecke C, *et al.* Functional and structural integrity of porcine pancreatic grafts subjected to a period of warm ischemia and cold preservation with histidine-tryptophan-ketoglutarate (custodiol) or University of Wisconsin solution. *Transplantation* 2003; **75**: 1793.
3. 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.
4. Gruessner RW, Sutherland DE, Troppmann C, Benedetti E, Hakim E, Dunn DL. The surgical risk of pancreas transplantation in the cyclosporine era: an overview. *J Am Coll Surg* 1997; **185**: 128.
5. Randhawa P. Allograft biopsies in management of pancreas transplant recipients. *J Postgrad Med* 2002; **48**: 55.
6. Ciancio G, Lo Monte A, Julian JF, Romano M, Miller J, Burke GW. Vascular complications following bladder drained, simultaneous pancreas–kidney transplantation: the University of Miami experience. *Transplant Int* 2000; **13**: S187.
7. Maraschio MA, Kayler RM, Merion RM, *et al.* Successful surgical salvage of partial pancreatic allograft thrombosis. *Transplant Proc* 2003; **35**: 1491.
8. Spiros D, Christos D, John B, Burke GW, Miller J, Ciancio G. Vascular complications of pancreas transplantation. *Pancreas* 2004; **28**: 413.
9. Drachenberg CB, Papadimitriou JC, Farney A, *et al.* Pancreas transplantation: the histological morphology of graft loss and clinical correlations. *Transplantation* 2001; **71**: 1784.
10. Schaser K-D, Puhl G, Vollmar B, *et al. In vivo* imaging of human pancreatic microcirculation and pancreatic tissue injury in clinical pancreas transplantation. *Am J Transpl* 2005; **5**: 341.
11. Wullstein C, Woeste G, Zapletal C, Trobisch H, Bechstein WO. Prothrombotic disorders in uremic type-1 diabetics undergoing simultaneous pancreas and kidney transplantation. *Transplantation* 2003; **76**: 1691.
12. Hoffmann JN, Mühlbayer D, Jochum M, Inthorn D. Effect of long-term and high-dose antithrombin supplementation on coagulation and fibrinolysis in patients with severe sepsis. *Crit Care Med* 2004; **32**(9): 1851.
13. Hoffmann JN, Vollmar B, Roemisch J, Inthorn D, Schildberg FW, Menger MD. Antithrombin effects on endotoxin-induced microcirculatory disorders are mainly mediated by its interaction with microvascular endothelium. *Crit Care Med* 2002; **30**: 218.
14. Roemisch J, Gray E, Hoffmann JN, Wiedermann CJ. Antithrombin: a new look at the actions of a serine protease inhibitor. *Blood Coagul Fibrinolysis* 2002; **13**: 1.
15. Özden A, Sarioglu A, Demirkan NC, Bilihan A, Düzcan E. Antithrombin III reduces renal ischemia-reperfusion injury in rats. *Res Exp Med* 2001; **200**: 195.
16. Ostrovsky L, Woodman R, Payne D, Teoh D, Kubes P. Antithrombin III prevents and rapidly reverses leukocyte recruitment in ischemia/reperfusion. *Circulation* 1997; **96**: 2302.
17. Harada N, Okajima K, Kushimoto S, Isobe H, Tanaka K. Antithrombin reduces ischemia/reperfusion injury of rat liver by increasing the hepatic level of prostacyclin. *Blood* 1999; **93**: 157.
18. Langley PG, Hughes RD, Forbes A, Keays R, Williams R. Controlled trial of antithrombin III supplementation in fulminant hepatic failure. *J Hepatol* 1993; **17**: 326.
19. Cowan PJ, Aminian A, Barlow H, *et al.* Protective effects of recombinant human antithrombin III in pig-to-primate renal xenotransplantation. *Am J Transpl* 2002; **2**: 520.
20. Arakami O, Takayama T, Yokoyama T, *et al.* High dose of AT induces indefinite survival of fully allogenic cardiac grafts and generates regulatory cells. *Transplantation* 2003; **75**: 217.

21. Hoffmann JN, Arbogast HP, Fertmann J, *et al.* High dose antithrombin therapy reduces ischemia/reperfusion injury during human allogenic kidney transplantation: first results of a randomized controlled clinical trial. *Transplantation* 2002; **74**, 394.
22. Troppmann C, Gruessner AC, Benedetti E, *et al.* Vascular graft thrombosis after pancreatic transplantation: univariate and multivariate operative and nonoperative risk factor analysis. *J Am Coll Surg* 1996; **182**: 362.
23. Hoffmann JN, Mühlbayer D, Jochum M, Inthorn D. Effect of long-term and high-dose antithrombin supplementation on coagulation and fibrinolysis in patients with severe sepsis. *Crit Care Med* 2004; **32**: 1851.
24. 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.
25. Rogers J, Chavin KD, Baliga PK, *et al.* Influence of mild obesity on outcome of simultaneous pancreas and kidney transplantation. *J Gastrointest Surg* 2003; **7**: 1096.
26. Humar A, Ramcharan T, Kandaswamy R, Gruessner RW, Gruessner AC, Sutherland DE. Technical failures after pancreas transplants: why grafts fail and the risk factors - a multivariate analysis. *Transplantation* 2004; **78**: 1188.
27. Menger MD, Vollmar B. Role of microcirculation in transplantation. *Microcirculation* 2000; **7**: 291.
28. Wullstein C, Woeste G, Zapletal C, Dette K, Bechstein WO. Simultaneous pancreas–kidney transplantation in patients with antiphospholipid syndrome. *Transplantation* 2003; **75**: 562.
29. Waydhas C, Nast Kolb D, Jochum M, *et al.* Inflammatory mediators, infection, sepsis, and multiple organ failure after severe trauma. *Arch Surg* 1992; **127**: 460.
30. Benz S, Busing M, Mayer JM, *et al.* Pancreas graft thrombosis: is there a role for trypsin. *Pancreas* 2004; **28**: 75.
31. Hopt UT, Busing M, Schareck W. Prevention of early postoperative graft thrombosis in pancreatic transplantation. *Transplant Proc* 1993; **34**: S24.
32. Tibell A, Brattstrom C, Kozlowski T. Management after clinical pancreatic transplantation with enteric exocrine drainage. *Transplant Proc* 1994; **26**: 1797.
33. Drognitz O, Liu X, Obermaier R, *et al.* Ischemic preconditioning fails to improve microcirculation but increases apoptotic cell death in experimental pancreas transplantation. *Transplant Int* 2004; **17**: 317.
34. Obermaier R, von Dobschuetz E, Muhs O, *et al.* Influence of nitric oxide on microcirculation in pancreatic ischemia/reperfusion injury: an intravital microscopic study. *Transplant Int* 2004; **17**: 208.

