Preservation of Beta Cell Function after Pancreatic Islet Autotransplantation: University of Chicago Experience

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The aim of the study was to assess the rate of insulin independence in patients after total pancreatectomy (TP) and islet autotransplantation in our center. TP followed by islet autotransplantation was performed in 10 patients. Severe unrelenting pain associated with chronic pancreatitis was the major indication for surgery. Islets were isolated using the modified Ricordi method and infused through the portal vein. Exogenous insulin therapy was implemented for at least two months posttransplant to support islet engraftment and was subsequently weaned off, if possible. Median follow-up was 26 months (range, 2 to 60 months). Median islet yield was 158,860 islet equivalents (IEQ) (range, 40,203 to 330,472 IEQ) with an average islet yield of 2,478 IEQ/g (range, 685 to 6,002 IEQ/g) of processed pancreas. One patient developed transient partial portal vein thrombosis, which resolved without sequela. Five (50%) patients are currently off insulin with excellent glucose control and HbA1c below 6. Patients who achieved and maintained insulin independence were transplanted with significantly more islets (median, 202,291 IEQ; range, 145,000 to 330,474 IEQ) than patients who required insulin support (64,348 IEQ; range, 40,203 to 260,476 IEQ; $P < 0.05$). Patient body mass index and time of chronic pancreatitis prior transplant procedure did not correlate with the outcome. The remaining five patients, who require insulin support, had present C-peptide in blood and experience good glucose control without incidence of severe hypoglycemic episodes. Islet autotransplantation efficiently preserved beta cell function in selected patients with chronic pancreatitis and the outcome correlated with transplanted islet mass.

CHRONIC PANCREATITIS IS a debilitating disease, which results in more than 122,000 outpatient visits and 56,000 hospitalizations annually in the United States.[1, 2] It is characterized by progressive inflammation that results in the loss of anatomical structure and the loss of exocrine and/or endocrine function. Moreover, it is associated with an increased risk of pancreatic adenocarcinoma.[3] Severe pain associated with chronic pancreatitis leads to narcotic dependence. Options for medical treatment of the pain of chronic pancreatitis include uncoated enzyme supplementation, antioxidant therapy, and escalating analgesic including opioids. Some patients may be treated by endoscopic intervention to remove stones or achieve duct compression.[4] Partial resection and/or drainage procedures are standard surgical options for patients who fail medical and endoscopic therapy and for those who are at risk of narcotic dependence.[5] The major objective of surgical intervention is the relief of pain with the secondary goal of preservation of endocrine and exocrine function. However, in as many as 50 per cent of patients, pain is incompletely alleviated or recurs after even surgical invention. Total pancreatectomy (TP) may be more effective in achieving durable pain relief because the entire organ is removed, although this option also induces complete postsurgical endocrine and exocrine insufficiency.[6–8] Complete loss of β-cell function and counterregulatory glucagon secretion from alpha cells after TP often results in a "brittle" form of diabetes with difficult to manage hyper- and hypoglycemia.[9]

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In 1977, Sutherland et al.[10] first demonstrated that autologous transplantation of the islets isolated from patients with chronic pancreatitis (CP) can restore normoglycemia and insulin independence. Transplantation of autologous islets in these patients was an attempt to alleviate the diabetic conditions associated with TP by preserving the insulin secretory capacity of the β-cells.

In this study, we report our up to 5-year observation of 10 patients with CP who underwent autologous islet transplantation at our institution. We demonstrate that transplantation of autologous islets isolated from the resected pancreas is able to restore normoglycemia and insulin independence in 50 per cent of the patients after TP. We further demonstrate that insulin independence correlates with transplantation of higher insulin mass in these patients. Insulin independence was observed in both younger patients (100%) treated with islet autologous transplant and in 37 per cent of the adults. These observations strongly suggest that autologous islet transplantation remains a viable treatment option in CP for patients who undergo TP.

Methods

The study was approved by the University of Chicago Institutional Review Board.

Patient Selection

Patients with CP and intractable pain resistant to medical, endoscopic therapy as well as to surgical drainage procedure in case of wide pancreatic duct were considered for TP with islet autotransplantation (TP/IAT). Those with active alcohol addiction or without good support to follow a complex postoperative regimen were excluded from this surgical intervention.

In addition, one patient with a benign, giant pseudopapillary tumor in the head of the pancreas compromising perfusion of the remaining gland was also qualified for TP/IAT. Another patient with a 2-cm ampullary adenocarcinoma and severe CP was treated with TP and simultaneous IAT to improve postoperative glucose control.

Total Pancreatectomy

Surgical resection of the pancreas in cases of CP was performed as described earlier.[11] During the operation, the pancreas was often atrophic, firm, and nodular. The pancreas was usually transected over the portal vein during the intraoperative dissection in the abdomen or on the back table after removal of the organ from the patient's body. Next, the gland was perfused with cold preservation solution, if possible through the splenic artery until the effluent from the splenic vein was clear. The pancreatic duct was cannulated with a 14- to 18-G Angiocath at the back table if feasible to facilitate digesting enzyme infusion during the pancreas processing and islet isolation in the laboratory (Fig. 1). The spleen was preserved only if blood supply through the short gastric blood vessels was not compromised. Splenectomy was required together with the pancreas and duodenum in four patients: in two patients with CP and in two with tumor. The pancreas was transported in cold preservation solution on ice to the University of Chicago cGMP laboratory for islet cell isolation. At the same time, the gastrointestinal tract was reconstructed with hepaticojejunostomy and gastrojejunostomy or duodenojejunostomy in an antecolic orientation. A jejunostomy tube was placed for postoperative feeding when necessary. The patient remained in the operating room intubated with an open abdomen awaiting islet infusion. In patients with tumor, tissue samples from the pancreas transection line were sent for intraoperative pathology. When the results were negative, an additional 1 cm of tissue was discarded before pancreas processing for islets.

Pancreatic Islet Isolation

Islet isolation was performed using modified Ricordi method based on the CIT protocols.[12] Briefly, the digestion solution containing Liberase MTF C/T GMP Grade (25 Wünsch U/g of pancreas weight of collagenase and 1000 U/kg of Thermolysinin final 350 mL Hanks' balanced salt solution with heparin 10 IU/mL) was injected into the pancreatic duct using a perfusion machine through previously placed angiocatheters. If necessary, additional enzyme was injected manually with a needle and syringe into poorly distended parts of the organ. Next, the pancreas was cut into multiple pieces and transferred into the Ricordi chamber with manual shaking for mechanical and enzymatic digestion. The time of digestion was 12 to 20 minutes, adjusted based on degree of tissue disintegration. If the digested pancreatic tissue volume was 20 mL or greater, islet purification was performed to reduce acinar content and tissue volume of the final preparation. The islets were purified with a continuous gradient using CIT Purification Density Gradients (CIT Purification Solution, Optiprep and Gradient Stock with densities of 1.113 to 1.060) and COBE 2991. Purified islets were washed and transferred to CIT Wash Medium. Before the release of the final islet product for infusion, islets were counted under a microscope and islet mass expressed as islet equivalents (IEQs). The preparation was also tested for islet viability (assessed using a fluorescent dye inclusion/exclusion assay), endotoxin levels by LAL assay (below 5 EU/kg), and Gram stain for sterility analysis. The final sample was also tested in microbiological culture assays (bacterial and fungal cultures).

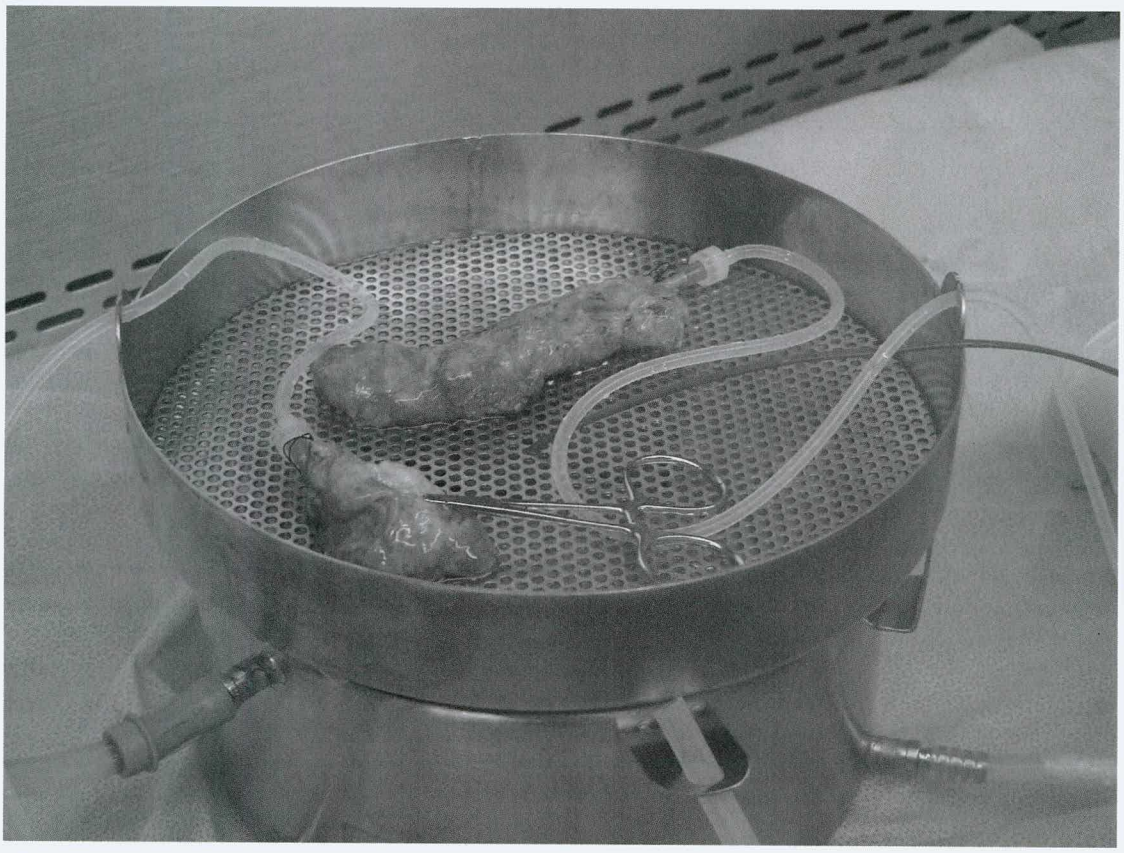

FIG. 1. Fibrotic pancreas resected from a patient with chronic pancreatitis. The pancreas was transected between head and tail. Next the pancreatic duct was cannulated with angiocatheters 14 G in the head and tail separately for the collagenase perfusion during the processing for the islet isolation.

Islet Transplantation

Isolated islets suspended in 250 mL of CIT Transplant Media containing 10 per cent human albumin and heparin (70 U/kg of patient's body weight) were placed in the Ricord islet infusion bag (Biorep, Miami FL) for infusion. On availability of the islets, a 14-G angiocatheter was inserted into the portal vein or a large tributary under direct vision and used to infuse the islets. Portal pressure was monitored at the start, in the middle, and at the end of islet infusion. The more purified islets were infused first followed by less pure fractions in separate infusion bags. Subcutaneous heparin was continued for one week after surgery for thromboembolic prophylaxis.

Follow-up

Patients were observed in the clinic for one to two weeks after surgery and had longer follow-ups if deemed necessary. Patients were subsequently released to care by their local primary care physician, gastroenterologist, and endocrinologist. Insulin requirements were established by local endocrinologists based on blood glucose control. Insulin independence was considered in patients who maintained fasting glucose below 110 mg%, postprandial below 180 mg%, and HgA1c less than 6 mg% without insulin therapy.

Statistical Analysis

Nonparametric Mann-Whitney U test was used for analysis. Statistical difference of $P < 0.05$ was considered significant.

Results

Patient Characteristics

There were 10 patients who underwent TP/IAT at The University of Chicago during a 5-year period starting February 2009. The patient characteristics are summarized in Table 1. Briefly, the median age of the patients was 34 years with a range of 11 to 53 years that included two teenagers. There were six females and four males. Eight of the patients (80%) had intractable abdominal pain as an indication for surgery, one patient had a benign tumor, and one patient had a small ampullary cancer with recurrent pancreatitis. The average body mass index (BMI) was 24.5 kg/m^2

TABLE 1. *Patient Characteristics*

Patient ID	1*	2	3	4	5	6	7	8	9	10
Age (years)	33	47	40	35	20	16	31	11	53	45
Gender	F	F	F	M	F	M	F	M	M	F
Height (m)	1.6	1.52	1.61	1.83	1.68	1.65	1.65	1.53	1.72	1.6
Weight (kg)	72	65.9	90	60.06	79.3	49.09	60.9	47.5	53	68
BMI (kg/m^2)	28.1	28.4	34.6	18	28.2	18	22.3	20.4	18	26.8
Indication for surgery	CP (PRSS1, CFTR)	CP	Begin tumor	CP (PRSS1, SPINK)	CP (SPINK)	CP (autoimmune)	CP (hereditary)	CP (hereditary)	Ampullary cancer	CP

*Patient 2 had a Puestow procedure several years before total pancreatectomy/islet autotransplantation.
BMI, body mass index; F, female; M, male; CP, chronic pancreatitis; PRSS1, protease, serine 1,(trypsyn 1); CFTR, cystic fibrosis transmembrane conductance regulator; SPINK, serine protease inhibitor, Kazal type 1.

with a range of 18 to 34 kg/m^2. The average duration of chronic pancreatitis was 7.5 years and ranged from two to 29 years. The islet infusions were well tolerated in these patients and there were no serious adverse events. We also observed no significant increase in the portal pressure or permanent liver dysfunction after islet infusion in these patients, although one patient developed transient partial portal vein thrombosis requiring heparin therapy. Also, transient elevation of liver enzymes was observed in all patients and resolved on its own within one week. None of the patients developed bleeding after the transplant.

Islet Isolation Characteristics

Islets were isolated from the excised pancreata by collagenase digestion using the modified Ricordi chamber at The University of Chicago cGMP facility. The average weight of the pancreas processed was 53 g (range, 26.8 to 95.4 g). Islet isolation yielded in median 2478 IEQs (range, 685 to 6002 IEQs) per gram of pancreas. Median total transplanted islet mass was 158,860 IEQs (range, 40,203 to 330,472 IEQs) and 2,340 IEQ/kg (range, 556 to 4,824 IEQ/kg) of the patient's body weight. The median viability was 92 per cent (range, 80 to 100%). Endotoxin levels in all preparations were negative (less than 5 U/kg).

Purification of Islets

In our second patient, infusion of a large amount of unpurified islet preparation led to increase in a portal pressure over 25 mmHg and cessation of infusion with subsequent intraperitoneal injection of the remaining tissue. To avoid such situations, in subsequent cases, if the pellet volume was greater than 20 mL, we performed islet density gradient purification. Three of the eight (37%) subsequent islet isolations yielded greater than 20 mL of tissue volume and required purification (Fig. 2D) and two of those three remained insulin-free.

Islet Graft Function

Patients were followed up for a period ranging from two months to five years (median, 26 months). TP/IAT resulted in five (50%) patients remaining insulin-free including both teenagers enrolled into the study (Fig. 2A). To determine the critical factors that correlate with achieving insulin independence, we analyzed the effect of total IEQ as well as IEQ/kg of patient body weight transplanted and the BMI of the patient on the endocrine function. Median follow-up for patients who were insulin-free was 24 months (range, two to 50 months) and did not differ significantly from the follow-up for patients who were insulin-dependent (30 months; range,

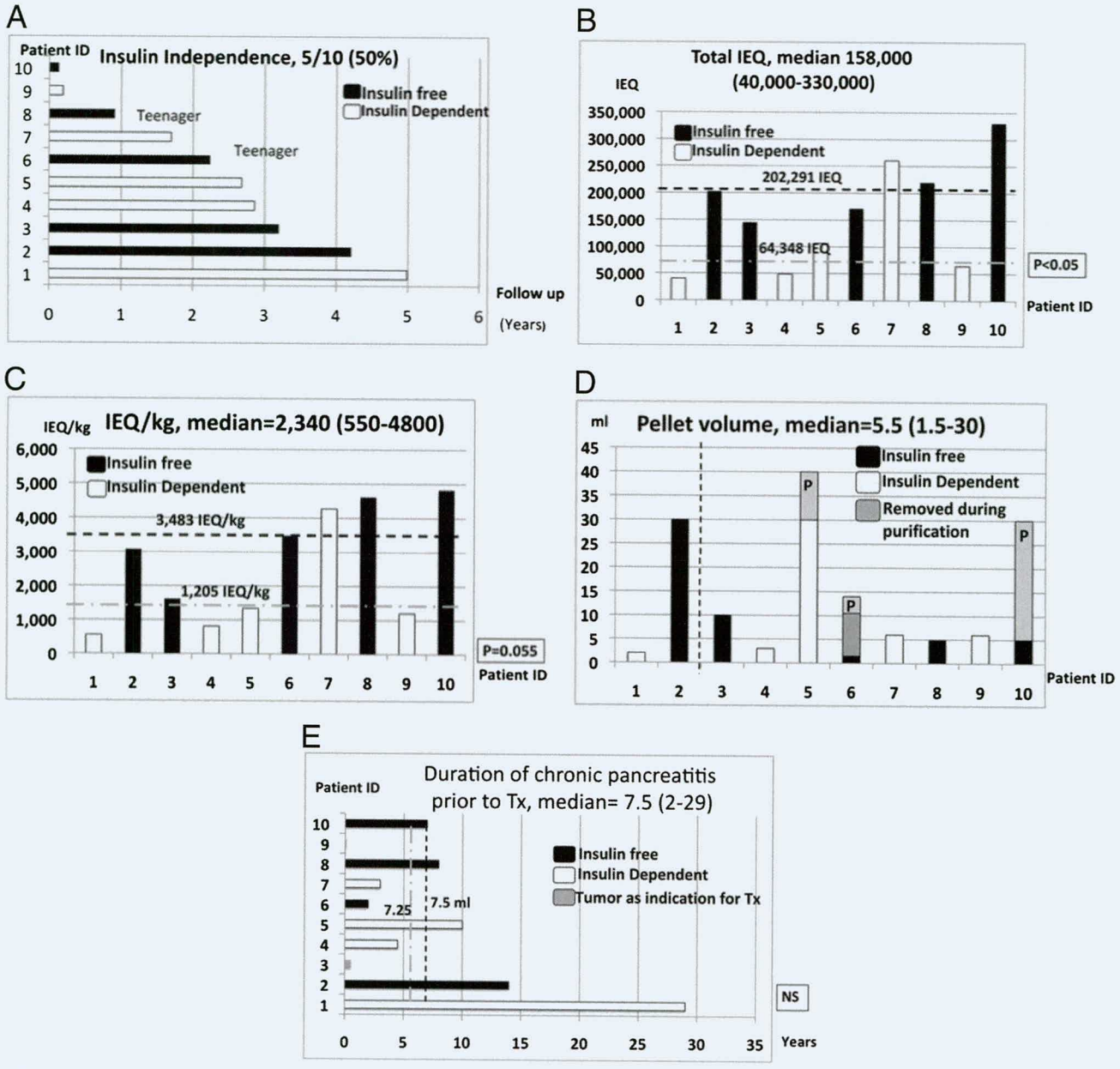

FIG. 2. Insulin independence after autologous islet transplantation in correlation with: follow- up (*A*), total islet mass (*B*), islet mass/kg patient body weight (*C*), pellet volume (*D*), and duration of chronic pancreatitis (*E*).

three to 60 months). Patients who achieved insulin independence received 202,291 IEQ (range, 145,000 to 330,474 IEQ), which was significantly more (threefold) than patients who currently still require insulin support (64,348 IEQ; range, 40,203 to 260,476 IEQ) ($P < 0.05$) (Fig. 2B). Similarly, nearly threefold higher islet dose per kilogram of patient's body weight was transplanted in insulin-free patients compared with insulin-dependent ones: 3483 IEQ/kg (range, 1611 to 4824 IEQ/kg) *versus* 1205 IEQ/kg (range, 556 to 4277 IEQ/kg), respectively, and the different approaches but does not reach statistical significance ($P = 0.055$) (Fig. 1C). Median BMI of patients before TP/IAT did not differ statistically between insulin-free and insulin-dependent patients (26 [range, 18 to 34] *vs* 22 [range, 18 to 28], respectively). Similarly, the average pellet volume transplanted and duration of CP did not differ significantly between insulin-free and insulin-dependent (median pellet, 5 mL [range, 1.5 to 30 mL] *vs* 6 mL [range, two to 30 mL]; Fig. 1C) and median duration of CP (7.5 years; range, two to 14 years *vs* 7.25 years; range, three to 29 years [Fig. 1D], respectively).

All of the patients had positive C-peptide at random time points after the transplant (median, 0.58; range, 0.04 to 1.86 pmol/mL). However, serial measurements of the C-peptide levels in the follow-up period are not

available for analysis in this cohort of patients. Nevertheless, all five patients who currently still require some insulin support enjoyed good glucose control without having severe hypoglycemic episodes.

Discussion

Total pancreatectomy is the last option for patients with CP with severe debilitating pain refractory to medical, endoscopic, and other types of surgical therapies. The main disadvantage is an extensive intra-abdominal surgical procedure leading inadvertently to postsurgical diabetes. Subsequent glucose control in these patients is especially challenging as a result of complete lack of beta and alpha cells resulting in a very "brittle" form of diabetes. Therefore, islet autotransplantation has been developed to prevent diabetes or at least to improve glucose control providing endocrine cell function after pancreatectomy.

Several studies in the literature have reported varying degrees of success in terms of preservation of beta cell function after TP/IAT. In this study, we report our observations from 10 subjects who underwent TP/IAT at The University of Chicago with up to 5-year follow-up.

We found that 50 per cent of the patients (five of 10) who underwent TP/IAT remained insulin-independent during the follow-up period. These results are similar to observations in other centers with longer experience and within larger patient population, where insulin independence rate varies from 18 to 41 per cent.[13–15] More significantly, both of the teenagers who underwent TP/IAT in our center remained insulin-free during the follow-up period (six and 21 months). Other studies also highlighted increased success of beta cell function preservation in a pediatric population indicating insulin independence rate as high as 29 per cent[16] to 60 per cent.[17] Those studies also demonstrated that higher islet mass transplanted correlated with better chance of favorable outcome and insulin independence. Our results also confirmed those observations. Insulin-independent patients received substantially more islets than patients who still require insulin therapy. Total islet mass per patient as well as islet mass per patient body weight was also over threefold higher in the insulin-independent patients when compared with those requiring insulin therapy. Nevertheless, one of our patients (No. 7) still requires insulin support despite receiving a relatively high number of islets. Of note, viability of the infused islets in this patient was approximately 80 per cent, which was lower than in remaining cases (greater than 90%) indicating that poor islet viability or quality might have had a significant impact on the outcome. Patient pretransplant BMI did not correlate with the endocrine outcome in our study. It is widely observed that progression of chronic inflammation of the pancreas has a detrimental effect on beta cell function before surgery, consequently on islet isolation yield and eventually on clinical outcome after the transplant.[1] In contrast, duration of CP did not affect the outcome based on our observations; however, the results might be biased by small sample size. Similarly, previous surgical procedures on the pancreas can compromise the final outcome of islet transplant as it can be observed in our study. Islet yield after a previous Puestow procedure in one of our patients was poor and postoperative glucose control required insulin support.[1]

Historically, and still today, in some islet-processing programs, islets have been infused along with acinar tissue without purification.[18] However, intraportal infusion of the tissue preparation as large as 30 to 50 mL might be challenging as a result of the elevation of the portal pressure and increased risk of portal vein thrombosis.[19, 20] In such cases, it is recommended that portal infusion be discontinued and the remaining islet preparation is either discarded or it may be injected into the peritoneal cavity with uncertain long-term function and at least a theoretical risk of intra-abdominal abscess. To avoid this situation, after our second case, we adopted an islet purification policy, when tissue volume was above 20 mL. In two of three subsequent isolations after islet purification was implemented, the patient remained insulin-independent, what reassured us that the purification procedure did not affect the outcome of the transplant. Advantages of islet purification have been also described by other centers.[1]

Those patients who required insulin support did not have major difficulties with blood glucose control. None experienced severe hypoglycemic episodes, consistent with the observation that they maintained at least partial islet function, as evidenced by a positive C-peptide after the procedure. Although we were not able to obtain serial serum C-peptide values, it was observed in other centers that C-peptide-positive patients with some residual islet function have adequate glycemic control and no episodes of hypoglycemia unawareness.[1, 21]

In our patient with a benign tumor, TP instead of a partial resection was performed as a result of compromised blood supply to the remaining part of the pancreatic gland, similar to other instances that have been reported.[22] The endocrine outcome depends on volume and quality of the gland subjected to islet isolation. This patient still remains insulin-free three years after the procedure. There have been only two other reported cases of ampullary cancer treated by TP with IAT.[23, 24] Although early observation did not show metastatic disease in the liver, long-term outcome for TP/IAT in the setting of malignancy remains undefined.[23, 24]

The two major limitations of our report are the small sample size and the lack of true metabolic tests during the follow-up period. Although we observed a significant correlation in the islet mass transplanted and achieving insulin independence during the follow-up period, the small sample size precludes us from identification of more of the variables that can predict the success of TP/IAT. Furthermore, the lack of true metabolic assessment of these patients impedes our ability to identify whether TP/IAT resulted in either better glycemic control or reduced hypoglycemia unawareness or improved the quality of life, especially in those patients who require some insulin support. Further studies are currently in progress to serially monitor patients at regular intervals to study their metabolic profile as well as assess their quality of life.

In summary, we have added our experience to the published literature demonstrating that TP/IAT is associated with the preservation of beta cell function, resulting in insulin independence in 50 per cent of this small series of patients.

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