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Article type : Case Report

Use of hyperglycemic clamp to assess pancreatectomy and islet cell autotransplant in patient with heterotaxy syndrome and dorsal pancreas agenesis leading to chronic pancreatitis

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/AJT.16084](#)

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Abbreviations: BW, Bodyweight; CT, Computed tomography; DPA, Dorsal pancreas agenesis; FBM, Functional beta cell mass; HS, Heterotaxia syndrome; IEQ, Islet equivalent; TPIAT, Total pancreatectomy and islet autotransplantation

Abstract

Patients with heterotaxy syndrome (HS) can present with an associated complete dorsal pancreas agenesis (DPA). They are considered to be at increased risk for developing diabetes due to a reduced functional beta cell mass (FBM) as well as for chronic pancreatitis leading to unmanageable pain. We report the case of a young female with chronic pancreatitis due to HS and associated DPA. She presented with a severe persisting upper abdominal pain refractory to non-surgical treatment. Unlike in previously reported cases, she had a high FBM (i.e. 150% of normoglycemic controls) as determined by hyperglycemic clamp. She underwent a total pancreatectomy followed within 24 hours by an intraportal autologous islet cell transplant containing 4×10^6 beta cells (4,700 IEQ)/kg bodyweight. After surgery, the pain resolved, eliminating the need for analgesics. The intraportal implant established an adequate FBM (72% of controls at posttransplant month 2) achieving glycemic control without need for insulin administration. A hyperglycemic clamp can assess utility and efficacy of an intraportal islet cell autotransplant following total pancreatectomy in patients with HS and complete DPA.

Introduction

Chronic pancreatitis is a progressive inflammatory condition, with pain as the most frequent and dominant symptom.¹ Treatment of chronic pain related to chronic pancreatitis is based on a step up approach, starting with conservative measurements and endoscopic procedures.² However, in a small part of chronic pancreatitis patients these therapies are not feasible or are unsuccessful. These patients are in general highly dependent on opioid analgesics and their quality of life is severely impaired. Total pancreatectomy followed by islet autotransplantation has been shown to be effective in reducing pain in these patients while preventing development of diabetes.³

Heterotaxy syndrome (HS) is a rare congenital disorder involving the normal left-right axis in the embryological development of the thoracoabdominal compartment. It is mostly sporadic and has an incidence of 1 in 6000 to 20000 live births. One of multiple anomalies that can be associated with this syndrome is dorsal pancreas agenesis (DPA).⁴ DPA is rare and its clinical presentation varies greatly. DPA most frequently presents with abdominal pain or diabetes mellitus if not diagnosed as an incidental finding. DPA is associated with acute, recurrent or chronic pancreatitis in up to 30% of cases.^{5,6}

We report the case of a patient with HS plus DPA with intractable pain caused by chronic pancreatitis, who was successfully treated with a total pancreatectomy followed by islet cell autotransplantation.

Case description

A 34 year-old, female patient, with a history of fibromyalgia and chronic fatigue syndrome, for which she was treated since 12 years, presented with chronic abdominal pain. Heterotaxy syndrome, a dorsal pancreas agenesis and polysplenia was diagnosed on abdominal computed tomography (CT) that was performed in the work-up of her fibromyalgia and chronic fatigue syndrome. There were no other associated medical conditions, including cardiac anomalies.

Five years earlier she had experienced two episodes of acute pancreatitis with typical pain syndrome and lipase elevation. Over time, the abdominal pain evolved to recurrent flares of acute pancreatitis with minimal or absent rises in serum amylase and lipase levels. Later on, the patient developed intractable pain, for which she was referred to our center. Other causes of chronic abdominal pain (such as vascular causes or Ladd's bands) were excluded on imaging. Endoscopic ultrasound suggested early chronic pancreatitis with minimal parenchymatic changes.

An intensive pain management trial of 6 months at our tertiary pain care center, including psychosomatic counselling, lidocaine infusions, and the use of pain modulating medications such as gabapentin were unsuccessful. Since the pancreatic duct was normal in size, without strictures or main pancreatic duct dilation on endoscopic ultrasonography, no endoscopic interventions were indicated. Also surgical interventions that aim at improving ductal drainage were not indicated for the same reasons.² The

patients FBM, which is a measure of the total number of beta cells and their functional capacity,⁷ was determined as 150% of normoglycemic controls by an hyperglycemic clamp test (Figure 1A).⁸ After discussion at our multidisciplinary board, a total pancreatectomy followed by autologous islet transplantation (TPIAT) was proposed. Surgical anatomy was evaluated by CT angiography with arterial and venous phase contrast-enhancement and conventional angiography. These examinations did not show any pancreatic branches originating from the coeliac trunk (Figure 2A); arterial supply to the pancreas was limited to an inferior pancreaticoduodenal artery originating from the superior mesenteric artery (Figure 2A & B). Venous drainage was through three veins directly draining into the portal vein that was found anterior to the duodenum (Figure 2C).

During pancreatectomy, warm ischemia to the endocrine tissue was limited by preserving pancreatic vascularisation until just before its removal.³ Immediately after en bloc removal of the pancreatic head and duodenum, the pancreas was topically cooled on ice and flushed with cold preservation solution (Institut Georges Lopez-1 solution, Institut Georges Lopez, Lissieu, France). During back table preparation, a hypoplastic pancreatic tail was seen as a nodule of pancreatic tissue separated from the pancreatic head and draining individually into the duodenum (Figure 2D). Both pancreatic ducts were separately cannulated and injected with a mixture of 50 ml cold preservation solution and alpha-1-antitrypsin at a concentration of 0.5 mg/ml.⁹ To avoid disruption of the pancreatic capsule, no tissue sampling for histopathological analysis was performed. The two pancreatic elements were cold stored and transported to our Beta Cell Bank. Biliary and alimentary continuity in the patient was reconstructed with a Roux-en-Y hepaticojejunostomy and gastrojejunostomy.

Both pancreatic parts were processed using a modification of the automated Ricordi method, followed by purification using a continuous gradient.¹⁰ Despite the low weight of the patient and the pancreas (48kg and 66g respectively), 191×10^6 beta cells [226,000 islet equivalents (IEQ)] were isolated at a beta cell purity of 26% and containing $18 \mu\text{g}$ insulin/ 10^6 beta cells (Table 1; graft characteristics determined after overnight culture as previously described¹⁰). The cells were cultured overnight in an Ham's F10 based serum free culture medium,⁸ and transplanted the following day as a cell pellet volume of 805 μl , in the portal vein using an ultrasound guided percutaneous technique.¹¹ When corrected for body weight of the patient, 4×10^6 beta cells (4,700 IEQ) per kilogram were transplanted.

Except for a prolonged postoperative ileus of 12 days necessitating nasogastric tube placement and total parental nutrition, postoperative course was uneventful. To optimize pain control, postoperative stay at the intensive care unit was prolonged to 9 days. Exogenous insulin (administered using a continuous intravenous drip of 15 to 30 units daily) was stopped 19 days after islet transplantation, and the patient was discharged 22 days after surgery. Postoperative evolution of non-fasting plasma C-peptide and blood glucose levels is shown in Figure 1B. From postoperative month 1, plasma C-peptide levels were above 0.5 nmol/l. At hospital discharge, pain was sufficiently managed using opioids, which could be gradually

reduced during outpatient visits. Two months after the TPIAT intervention, the patient was without pain, without opioid analgesics, insulin independent and with a stable bodyweight. At that moment, she exhibited a FBM of 72% of normoglycemic controls (Figure 1A), associated with a mean glucose of 96 mg/dl and 92% of glucose sensor values between 70 and 180 mg/dl (time in range, Figure 3).

Discussion

Total pancreatectomy followed by islet autotransplantation (TPIAT) is an established treatment option reserved for otherwise unmanageable pain in the setting of small duct chronic or recurrent acute pancreatitis.¹² This combination was shown to restore substantial islet function in most patients and to achieve insulin-independence in one-quarter of adults.³ The success of beta cell replacement will depend on the size of the functional beta cell mass in the retrieved pancreas and on that in the intraportal implant.⁷ We used the hyperglycemic clamp test⁸ to assess the functional beta cell mass (FBM) before and after TPIAT. In this test, C-peptide release is measured first at basal glycemia and then during sustained glucose stimulation (180mg/dl), taking the area under the curve as in vivo marker of FBM. These values are then expressed as % of normoglycemic controls.⁷ Intraportal islet cell transplants in C-peptide negative type 1 diabetes patients were thus shown to achieve insulin independency and substantially reduce glycemic variability when achieving a FBM of in average 25% of that in normoglycemic controls.⁸

In our multidisciplinary approach of TPIAT, preoperative FBM is taken into consideration as prospective marker to estimate the risk for diabetes mellitus after TPIAT. It is used in patient counselling when discussing potential benefits and risks of a TPIAT procedure and not as a threshold to deny it to patients. Our patient presented with a development disorder, heterotaxy syndrome with dorsal pancreas agenesis, polysplenia and vascular abnormalities as basis for a chronic pancreatitis and refractory abdominal pain. At diagnosis, she presented with a clamp-determined FBM that was 50% higher than the average in normoglycemic controls. This finding is at variance with previous cases of HS and DPA that were associated with diabetes.^{13,14} Also, in isolated DPA, more than half of patients have diabetes at the time of diagnosis.^{5,6} For these reasons, deceased donors with DPA are in general not considered suitable for whole organ pancreas transplantation and are also discouraged for islet isolation; one report did however mention a successful islet cell allotransplantation following isolation from of a deceased donor with partial DPA.¹⁵ At present, it is unclear whether the reported association with diabetes is due to the initial development defect and DPA itself or to the subsequent pancreatitis.^{5,6,13,14} It is conceivable that the reduced pancreas mass in DPA does not obstruct the development of a normal FBM,¹⁶ but that this FBM becomes threatened by recurrent bouts of pancreatitis with which DPA is associated,^{5,6} and which can impair both pancreatic endocrine and exocrine functions.¹⁷

In this case, the retrieved organ yielded an islet cell isolate that restored glucose control and insulin-independence following intraportal transplantation. After surgery our patient remained without pain

and was opioid free. At posttransplant month two, the FBM of the implant was 72% of normoglycemic controls. It was achieved with a donor preparation containing 4×10^6 beta cells (4,700 IEQ)/kg bodyweight (BW), a dose that met the minimal number needed to induce a functional beta cell implant in our allotransplant program (2×10^6 beta cells/ kg BW).⁸ The beta cell dose as given to this patient is often not sufficient to induce insulin-independence in non-uremic type 1 diabetes patients where auto- and/or allo-immune processes as well as immunosuppressive medication may impair the engraftment and function of the implants. A higher efficacy of islet cell autografts versus allografts has been previously observed.¹⁸

The method for reconstructing gastro-intestinal continuity may also have influenced the outcome of the implant. In obese type 2 diabetic patients, Roux-en-Y gastro-jejunostomy together with a gastric pouch creation is known to rapidly reduce blood glucose levels.¹⁹

In conclusion, this case shows that a glucose clamp test can assess utility and efficacy of an intraportal islet cell autotransplant following total pancreatectomy in patients with heterotaxia syndrome and complete agenesis of the dorsal pancreas.

Acknowledgments

The authors would like to thank Qing Ding, Tom Haex and Veerle De Punt for their continuous efforts in preparing high quality human beta cell grafts.

Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Jacques Pirenne declares unrelated disclosures: he holds named chairs at the KU Leuven from the Institut Georges Lopez and from the “Centrale Afdeling voor Fractionering” (DGF-CAF). The other authors have no conflicts of interest to disclose.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure Legends

Figure 1 (A) hyperglycemic clamp test in a patient before and after total pancreatectomy followed by an islet autotransplantation (TPIAT) for a heterotaxy syndrome with complete dorsal pancreas agenesis [C-peptide levels (connected ●)], compared to minimal and maximal values for normoglycemic controls (grey area). **(B)** evolution of non-fasting C-peptide (connected ●) and glycemia (-) before and after TPIAT. Time period when exogenous insulin was administered is marked in grey and the moment of insulin independence at posttransplant day 19 is marked with a full vertical line.

Figure 2 (A) Computed tomography angiography with 3D reconstruction and **(B)** conventional angiography demonstrating arterial supply to the pancreas limited to an inferior pancreaticoduodenal artery (PDI) originating from the superior mesenteric artery (SMA) and absence of pancreatic branches originating from the coeliac trunk (CT) **(C)** Computed tomography angiography in venous contrast-phase demonstrating three small venous branches (full circle) draining in the portal vein medial and anteriorly to the ventral pancreas (VP) and the D3 portion of the duodenum (D3) **(D)** Pancreas in situ with an anterior portal vein (PV) draining directly two smaller pancreatic veins (O) and preserved inferior pancreaticoduodenal artery (PDI). The hypoplastic pancreatic tail (DP) can be seen as a nodule of pancreatic tissue separated from the pancreatic head (VP) and draining individually into the duodenum (D1 & D3).

Figure 3 28-day summary tracing from a continuous glucose monitoring during postoperative month two, showing a mean 24 hour blood glucose of 96 mg/dl and 92% of glucose sensor values between 70 and 180 mg/dl (time in range).

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Table 1 patient and graft characteristics

Patient characteristics	
Age	34
Gender	Female
Weight	48
BMI	22
Graft characteristics	
Beta cell purity (%)	26
Insulin content ($\mu\text{g}/10^6$ beta cells)	18
Beta cells ($\times 10^6$)	191
Beta cells ($\times 10^6/\text{kg}$ body weight)	4
IEQ	226,000
IEQ ($/\text{kg}$ body weight)	4,700

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