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THESIS

Implementing Best Practice Guidelines in Autologous Islet Transplantation through an International Survey

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ABBREVIATIONS

ADA, American Diabetes Association

AP, Acute Pancreatitis

ASA, Acetylsalicylic Acid

AUC, Area Under the Curve

Auto-Itx, Autologous Islet Transplant

AVM, Arteriovenous Malformation

BMI, Body Mass Index

CF, Cystic Fibrosis

CITR, Collaborative Islet Transplant Registry

CP, Chronic Pancreatitis

CS, Celsior

CT, Computed Tomography

ΔPP, Change in Portal Pressure

DM, Diabetes Mellitus

DIC, Disseminated Intravascular Coagulation

DP, Distal Pancreatectomy

DPP-IV, Dipeptidyl Peptidase IV

DTZ, Dithizone

DVT, Deep Vein Thrombosis

DXA, Dual-Energy X-Ray Absorptiometry

EPITA, European Pancreas and Islet Transplant Association

ERCP, Endoscopic Retrograde Cholangiopancreatography

EUS, Endoscopic Ultrasound

FBG, Fasting Blood Glucose

F-FDG, Fluorodeoxyglucose

GAD65, Glutamic Acid Decarboxylase 65

GLP-1, Glucagon-like peptide-1

GIP, Gastric Inhibitor Peptide

GST, Glucagon Stimulation Test

HbA1c, Glycated Hemoglobin

HOMA, Homeostasis Model Assessment

HRQOL, Health Related Quality of Life

HTK, Histidine-Tryptophan-Ketoglutarate

IA-2, Islet Antigen-2

IAT, Islet Autotransplantation

IBMIR, Immediate Blood-Mediated Inflammatory Reaction

IEQ, Islet Equivalent

IGL-1, Institut Georges Lopez-1

IL-6, Interleukin-6

IL-8, Interleukin-8

INSindex, Insulinogenic Index

IPITA, International Pancreas and Islet Transplant Association

IPMN, Intraductal Papillary Mucinous Neoplasm

Ips, Islet Particles

iPRDM, Immediate Post-Resection Diabetes Mellitus

ISI, Islet Size Index

IU, International Unit

LMWD, Low-Molecular Weight Dextran

LMWH, Low-Molecular Weight Heparin

LUMC, Leiden University Medical Center

MMTT, Mixed Meal Tolerance Test

MRCP, Magnetic Resonance Cholangiopancreatography

MRI, Magnetic Resonance Imaging

NODM, New-Onset Diabetes Mellitus

OGTT, Oral Glucose Tolerance Test

OIH, Opioid-Induced Hyperalgesia

OR, Operating Room

PCR, Polymerase Chain Reaction

PD, Pancreaticoduodenectomy

PDAC, Pancreatic Ductal Adenocarcinoma

P-DM, Pancreatogenic Diabetes Mellitus

PEI, Pancreatic Exocrine Insufficiency

PERT, Pancreatic Enzyme Replacement Therapy

PET, Positron-Emission Tomography

PNET, Pancreatic Neuroendocrine Tumour

POPF, Postoperative Pancreatic Fistula

PP, Partial Pancreatectomy

PPy, Pancreatic Polypeptide

PPPD, Pylorus-Preserving Pancreaticoduodenectomy

POST, Prospective Observational Study of TP-IAT

PVT, Portal Vein Thrombosis

QC, Quality Control

QoL, Quality of Life

RAP, Recurrent Acute Pancreatitis

RNA, Ribonucleic Acid

RT, Reactive Thrombocytosis

RYGB, Roux-en-Y Gastric Bypass

SF-36, 36-Item Short Form

SPTP, Spleen-Preserving Total Pancreatectomy

SPTP-IAT, Spleen-Preserving Total Pancreatectomy-Islet Autotransplantation

T1DM, Type 1 Diabetes Mellitus

T2DM, Type 2 Diabetes Mellitus

T3cDM, Type 3c Diabetes Mellitus

TNF-α, Tumor Necrosis Factor-α

TP, Total Pancreatectomy

TP-IAT, Total Pancreatectomy and Islet Autotransplantation

TV, Tissue Volume

UFH, Unfractionated Heparin

US, Ultrasound

USA, United States of America

UPenn, University of Pennsylvania

UW, University of Wisconsin

UZ, Universitair Ziekenhuis

ABSTRACT

Background Islet Autotransplantation (IAT) following pancreatectomy serves as a mitigation strategy against postoperative diabetes. IAT has proven to be a promising technique with good functional outcomes. Subsequently, it is anticipated that indications for and use of IAT will increase in the coming years. Historically, the only indication for IAT was chronic pancreatitis (CP). A recent proposal has advocated that the criteria should now be expanded to include patients with high-risk pancreatic stump, extended parenchymal resections for benign diseases, and for the management of severe postoperative complications (i.e., pancreatic fistula). Currently, there appears to be significant variations in islet isolation techniques and transplantation strategies among expert centers, and consensus on standard of care policies are lacking.

Aim To evaluate current use and practice of IAT across international expert centers. The results will be analyzed to define the current indications for IAT procedure, the technical information about the pancreas processing, the location where the process of islet isolation takes place, when and how islet infusion procedure is carried out.

Methods A standard of care survey will be disseminated worldwide among IAT expert centers. Current indications for IAT, details regarding processing of the pancreas, islet purification and of islet infusion protocols will be assessed.

Discussion IAT is a promising procedure to prevent or alleviate new onset pancreatogenic diabetes mellitus and its complications. However, consensus regarding the IAT procedure is currently lacking among expert centers. The results of this international survey will be examined with the aim to suggest best-practice guidelines for IAT.

INTRODUCTION

1. Islet Autotransplantation Definition

Islet Autotransplantation (IAT) is a procedure aimed at preserving pancreatic islet mass and its endocrine function. IAT is performed following total or subtotal pancreatectomy.(1) It was first performed in 1977 at the University of Minnesota for the management of chronic pancreatitis (CP) refractory to medical and endoscopic treatments, in combination with total pancreatectomy (TP).(2) Nowadays, the use of total pancreatectomy and islet autotransplantation (TP-IAT) has shown an increasing trend.(3) TP involves the complete removal of the pancreatic parenchyma, resulting in lifelong pancreatic exocrine and endocrine insufficiency. The treatment of pancreatic exocrine insufficiency (PEI) consists of oral supplementation with pancreatic enzymes, while the management of endocrine insufficiency requires therapy with insulin or IAT.(1)

During IAT, patient's own islet cells are isolated from the pancreas and infused back within the organism without the need for immunosuppression. Insulin-secreting pancreatic β-cells play the central role in glucose homeostasis, they are organized with other endocrine cells in functional unit named the islet of Langerhans.(4) The complementary endocrine components consist in α- and δ-cells, as well as a smaller proportion of pancreatic polypeptide (PPy) and ε cells.(5) IAT prevents or mitigates the development of pancreatogenic diabetes, even called type 3c diabetes mellitus (T3cDM), and the associated morbidity. Following pancreatic gland removal, its main benefit lies in the preservation of the endocrine pancreatic function. Endogenous β-cell function (C-peptide positivity) is preserved in 90% of patients, thereby preventing brittle diabetes in the majority of TP-IAT recipients.(6) Post-pancreatectomy IAT offers to eligible patients a chance for insulin independence. However, the current literature regarding glycemic outcomes, mortality, and long-term follow-up remains limited and heterogenous. Additional data are essential to better define the risks and benefits of IAT procedure.(7)

The primary goals of TP-IAT are to improve of the quality of life (QoL) and to relieve pain in otherwise-refractory-CP patients, while preserving meaningful islet function. TP-IAT is an effective method for managing the disabling complications of CP and for reducing the risk of pancreatic cancer in very high-risk patients.[8] Pancreatectomy alleviates pain by removing the source of pancreatic inflammation, while IAT reduces the severity or likelihood of iatrogenic diabetes.[9] Though complete insulin independence following IAT is uncommon, its success is more accurately measured by improvements in QoL rather than insulin requirements alone.[10]

Despite the increase in IAT procedures over the past decade, many questions remain regarding the standardization of the approach. As noted in the paper by Melena D. Bellin et al., *Total Pancreatectomy With Islet Autotransplantation: Summary of an NIDDK Workshop,* there is a pressing need to standardize care before, during, and after surgery. This includes developing clinical guidelines aimed at reducing post-surgical diabetes risk and optimizing TP-IAT outcomes.[2]

1.1 Preoperative Patient Assessment

Prior to TP-IAT in case of CP, patients must undergo a rigorous preoperative protocol to confirm pancreatitis as a primary diagnosis, screen for diabetes, assess β-cell mass, evaluate the patency of the portal venous system, determine the presence of liver disease, and review the immunization status.[8] Finally, a comprehensive psychiatric and psychological evaluation to assess patient suitability for the procedure is necessary.

The diagnosis of diabetes is defined according to the criteria of the American Diabetes Association (ADA): fasting glucose ≥126 mg/dL or glycated hemoglobin (HbA1c) ≥6.5%. Intermediate values, fasting glucose between 100-125 mg/dL or HbA1c between 5.7-6.4%, should be further evaluated using a standard 75 g oral glucose tolerance test (OGTT).[8]

Preoperative glycemic function is assessed through HbA1c (mmol/mol), fasting C-peptide (in nmol/L), and insulin requirements defined as total amount of insulin units per day (IU/day).[10]

TP-IAT is often coupled with splenectomy, thus, patients should undergo standard pre-splenectomy vaccinations, because the risk of infection with encapsulated bacteria is high.[8]

Liver disease and portal vein patency are assessed using serum liver function tests and imaging modalities such as ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI).

TP-IAT should be performed before pancreatic islet functionality is compromised to maximize its success. The most informative test on β-cell function is the glucose-potentiated arginine stimulation test, which assesses insulin and C-peptide secretion. This test is preferred over intravenous glucose testing, which has limited utility when fasting glucose exceeds 100 mg/dL, and it becomes ineffective above 115 mg/dL. Notably, results from glucose-potentiated arginine stimulation correlate with the numbers of islets ultimately transplanted intrahepatically, making it a valuable predictor of both preoperative β-cell function and post-transplant islet mass.[2]

A related study[11] identified the arginine stimulation test as a valuable possibility to estimate native islets availability in patients with CP prior to TP-IAT. Functional β-cell mass can be estimated from serum C-peptide levels determined during OGTT or mixed-meal tolerance test (MMTT), although intravenous glucose tolerance, arginine stimulation, or glucose-potentiated arginine testing are more sensitive methods.

On the other hand, α-cell function is evaluated using hypoglycemic and hyperinsulinemic clamp studies. Glucagon, the principal counter-regulatory hormone to insulin, promotes hepatic glycogenolysis during hypoglycemia. Unfortunately, glucagon responses are largely suppressed in islets transplanted

intrahepatically. However, these islets respond better to intravenous arginine, suggesting that the defect in glucagon secretion is specific to hypoglycemia and not due to a reduced islet mass. Interestingly, when a portion of islets is transplanted into the peritoneal cavity, particularly in younger recipients, glucagon responses and hypoglycemia awareness improve, supporting the hypothesis of a site-specific defect in α-cell function.[2] Furthermore, the liver as an engraftment site is associated with a deficiency in PPy release, which may contribute to hepatic insulin resistance.[2]

1.2 Predictors of IAT Success and Glycemic Outcomes Post-Pancreatectomy

The primary aim of IAT is preventing or reducing the incidence of iatrogenic diabetes following pancreatectomy. To optimize its benefits, predictive factors for favorable outcomes must be assessed preoperatively.

Glycemic control and the maintenance of normal HbA1c levels after pancreatectomy are more easily achieved in the presence of endogenous β-cell function. Multiple variables influence islet yield, including histopathology, islet viability, β-cell functional capacity, diffuse calcifications in the pancreas[12] and tissue fibrosis from prior pancreatic surgery,[13] recipient characteristics particularly gender, insulin sensitivity, and pre-transplantation body mass index (BMI).[6] While the Islet Equivalent Quantity per kilogram (IEQ/kg) transplanted is theoretically central to predict the outcomes, findings across the literature remain inconsistent. Elements that determine the quantity of islet yield per gram of pancreas are the dose of collagenase, gland fibrosis, patient's age, perfusion time, ductal pressure, and temperature.[2] Further studies are necessary to confirm these associations.[7] Higher islet yields per gram of pancreas are associated with prolonged diabetes-free survival.[14] However, criteria for the minimum islet mass required for infusion vary across institutions. Some studies recommend a minimum of 5000 IEQ/Kg as the threshold to support metabolic function,[15,16] while others suggest that transplantation can proceed with at least 1800 IEQ/Kg.[17] Low islet mass infusion

is the most consistent predictor of graft failure.[18] Emerging evidence suggests that the mass of islets transplanted, often expressed as IEQ or IEQ/kg, is the predominant factor minimizing the effect of iatrogenic diabetes following pancreatectomies.[19] Estimation of pre-operative islet mass is challenging but it is of supreme importance to support physicians with some evidence-based guidance in advising patients before pancreatectomy and IAT. An elaborative assessment of glycemic control and functional β-cell mass is necessary and it should include HbA1c, blood glucose and C-peptide levels in responses to MMTT or OGTT.[20] Fasting plasma glucose <100 mg/dL and stimulated plasma C-peptide ≥4 ng/mL are independently associated with higher islet yield, typically ≥2500 IEQ/kg.[19] Conversely, elevated HbA1c and glucose area under the curve (AUC) values are negatively correlated with islet mass.

Higher preoperative stimulated C-peptide levels correlate with improved islet yield, whereas lower values often reflect progressive β-cell failure typical of CP.[6] The noninvasive glycemic homeostatic model assessment for β-cell function (HOMA-β) predicts postoperative insulin independence when in normal range (60%-100%). HOMA-β stands for Homeostatic Model Assessment-Beta Cell Function (%), it is a marker of β-Cell function measured through fasting glucose and insulin or C-peptide.[21] OGTT results, particularly in 1-hour and 2-hour glucose levels, are strong predictors of postoperative glycemic outcomes, especially when combined with HOMA-β indices.[22]

The islet particle number per kilogram (Ips/kg) and the islet size index (ISI), defined as IEQ/IP in a given preparation, also contribute to metabolic outcome prediction. An ISI <1 indicates a mean islet diameter <150 μm and it is associated with better glycemic outcomes, likely due to improved oxygen and nutrient diffusion early post-transplant (<10-14 days).[23]

Preliminary data suggest that ductal epithelial and glucagon-positive ductal cells may enhance long-term graft function. Their infusion, along with acinar tissue, may improve metabolic outcomes.[24]

T3cDM is the cardinal complication following pancreatic resection, numerous studies have been conducted to identity predictive factors that could help in performing risk assessment and stratification of surgical patients prior to pancreatic resection, in particular, Marvi Tariq et al. identified OGTT >130 mg/dL and HbA1c >6.0% as predictors for pancreatogenic diabetes mellitus (P-DM) development.[25]

As IAT protocols have been constantly evolved, the transplantation of larger volumes of islets in a controlled setting is possible with minimum side effects.[19]

Metabolic outcomes and diabetes development vary according to the extent of pancreatic resection, recognized as an independent predictor.[26] Islet yield is compromised by prior surgery due to pancreatic tissue fibrosis, resection area, and ductal architecture.[13] Patients undergoing partial pancreatectomy (PP) often receive fewer islets, yet they demonstrate exceptional metabolic outcomes, thanks to preserved endogenous insulin production. Conversely, TP recipients face higher risks of graft failure and reduced β-cell function over time.[27]

Recently, it has been demonstrated that high body mass index (BMI) is associated with increased islet yield and tissue volume; however, this also raises portal pressure during infusion and increases the risk of complications, making BMI a double-edged factor in IAT.[28] In concordance to the beforementioned information, the study[29] reveals by multivariate analysis, three factors associated with higher possibility of insulin independence after TP-IAT in the pediatric population: (1) male sex, (2) lower body surface area, and (3) higher total IEQ per kilogram body weight. Among them, total IEQ represents the most significant determinant; in conclusion, β-cell function depends on islet yield.[1]

Postoperative β-cell function and glycemic control are forecasted by preoperative body weight and dual energy x-ray absorptiometry (DXA) derived parameters, while they did not predict insulin dependence,[30] which instead is associated with sarcopenia.[31]

Finally, pre-existing insulin resistance and prediabetes are confounding variables that significantly increase the risk of developing diabetes within 12 months post-pancreatectomy.[14]

2. Pancreatogenic Diabetes (Type 3c Diabetes Mellitus)

P-DM, also referred to as T3cDM, is defined by the ADA as diabetes mellitus (DM) that is acquired within 5 years after pancreatic resection, and it is classified as 'other specific type of DM'. P-DM occurs due to inherited or acquired pancreatic disease or resection. Underlying causes include acute pancreatitis (AP) and CP, pancreatic neoplasms, cystic fibrosis (CF), pancreatic trauma, hemochromatosis, fibrocalculous pancreatopathy, pancreatic agenesis, and pancreatic resection.[32]

The consequence of pancreatic parenchyma loss is impaired glucose metabolism secondary to hormone deficiencies and altered end-organ response to pancreatic hormones.[33] P-DM has a unique pattern of hormonal and metabolic profile.

P-DM negatively impacts QoL, and it is characterized by a more challenging glycemic control than for patients with type 2 diabetes mellitus (T2DM) due to the combination of reduced circulating insulin and glucagon concentration and concurrent PEI. Affected individuals suffer from frequent episodes of iatrogenic hypoglycemia, referred to as 'brittle' diabetes.[33] It poses a significant challenge for patients undergoing pancreatic surgery.[27]

There is lack of awareness about T3cDM, and it is often misdiagnosed as T2DM. The incidence of P-DM increases as the follow-up period after surgery becomes longer; it ranges from 5% to 50%. The development of P-DM depends on the type of surgery, the extent of resection, the progression of the underlying pancreatic disease, and the indication for surgery. TP always results in pancreatogenic diabetes. Because of its high frequency, patients should be made aware of and counselled on this potential morbidity.[33]

A specific subcategory of P-DM is the immediate post-resection diabetes mellitus (iPRDM) which is defined as diabetes requiring pharmacological treatment within 30 days post-operatively. iPRDM occurs in 4% of all patients undergoing pancreaticoduodenectomy (PD).[34]

CP accounts for approximately 75% of T3cDM cases and it is associated with an elevated risk of pancreatic carcinoma.[32] Given its high prevalence and clinical implications, patients undergoing pancreatic surgery should be thoroughly informed and counselled regarding the risk of developing P-DM.

2.1 Hormonal Pathophysiology

Insulin is secreted from β-cells distributed throughout the pancreas. They decrease the serum glucose concentration by downregulating hepatic gluconeogenesis and glycogenolysis, while facilitating hepatic glycogen synthesis. Nondiabetic patients have lower fasting serum insulin and reduced C-peptide secretion after pancreatic resection. P-DM is characterized by an increase in peripheral sensitivity to insulin and in the insulin-binding capacity of red blood cells.

In addition to insulin, pancreatectomy reduces fasting glucagon concentration due to the loss of α-cells, which are predominantly located in the pancreatic body and tail. During fasting in a healthy population, glucagon maintains adequate glucose production by hepatocytes through stimulation of glycogenolysis and gluconeogenesis that together function as counter-regulatory mechanisms to control hypoglycemia. The absence of glucagon-secreting ability in T3cDM poses the risk for severe hypoglycemia due to loss of counter-regulatory mechanism.

PPy, a third key islet hormone, is secreted by PPy cells concentrated predominantly in the ventral head and uncinate process of the pancreas.[5] PPy deficiency is characteristic of P-DM and it is related to hyperglycemia resulting from unsuppressed glucose production. PPy deficiency is associated with glucose intolerance and hepatic insulin resistance. These findings show the potential reversibility of PPy deficiency in the abnormal glucose metabolism following pancreatic resection.[35] The awareness of the role of PPy deficiency in the pathogenesis of P-DM suggested the conservation of the head and uncinate process of the pancreas as method for better outcomes after the surgical treatment;

duodenum- and/or pancreas-sparing techniques have been associated with a lower incidence of postoperative diabetes.[32]

The pathophysiology of P-DM is related to pancreatic hormone deficiency and altered responses of the liver and peripheral organs to reduced hormone levels.[35] The etiology of endocrine insufficiency in the post-resection period is multifactorial; the contributing factors are the loss of pancreatic parenchyma, the alteration of neuro-hormonal responses, recurrence or progression of underlying pancreatic disease, progressive gland atrophy, and additional adjuvant therapy.[34] Notably, the clinical phenotype of P-DM differs from T2DM. Ketoacidosis and severe hyperglycemia are uncommon in P-DM. Hyperglycemia develops when the amount of insulin produced or administered is insufficient because of unsuppressed hepatic glucose production secondary to a deficient in PPy. In contrast, hypoglycemia occurs when insulin is excessive secondary to enhanced peripheral insulin sensitivity and glucagon deficiency.[35] Due to the peculiar pathophysiology of P-DM, patients have a more difficult glucose control than patients with T2DM, HbA1c is usually quite high, neuropathy, nephropathy, and retinopathy can develop as a result of long-term inappropriate therapy. Glycemic variability is further influenced by nutritional state, pancreatic exocrine function, and gastrointestinal absorption capacity.

2.2 Risk Factors and Predictors by Type of Pancreatectomy

A study involving 4255 patients who underwent PD or distal pancreatectomy (DP) revealed that the incidence of PD-M was 32.3% after a median follow-up of 3 years.[33] Several factors have been associated with increased risk of P-DM development including male sex, DP, undergoing resection for malignancy, family history of diabetes, being classified as prediabetic in the preoperative period. On the other hand, race, age, preoperative obesity, and complexity score were not linked to an increased risk of P-DM.[33] Consistent with these findings, Ferrara et al. identified predictors such as male sex, preoperative glucose intolerance, increased creatinine, higher BMI, elevated preoperative glucose, prolonged operative time, tumour size, and operative length as contributors to the development

of P-DM.[34] Furthermore, Maxwell and colleagues proposed a predictive tool, the Post-Pancreatectomy Diabetes Index, highlighting advanced age, BMI, and elevated preoperative HbA1c as independent risk factors.[33]

Similarly to what has been stated above, Michel et al.,[34] identified three factors predictive of iPRDM: pre-operative glucose intolerance, fasting blood glucose (FBG) ≥126 mg/dL, and increasing specimen length. The most relevant finding from the study is that the incidence of iPRDM in patients with a normal pre-operative FBG is only 1%. The identified risk factors associated with the development of T3cDM are supported also by the systemic review conducted by Linda Wu and colleagues,[36] they identified the type of pancreatic resection, lower remnant pancreatic volume, and higher preoperative HbA1c (> 5.7%) as the most indicative predictive elements.

Data on T3cDM by type of resection show a significant correlation between the type of surgery and the incidence of T3cDM. The fact that TP causes T3cDM is clearly established. Among partial resection category, the highest incidence of P-DM and the worst deterioration of glucose regulation are associated with DP (21%) because it consists in the resection of the greatest pancreatic volume.[33] Secondary PD (16%), while central pancreatectomy (6%) is associated with the lowest incidence of new-onset diabetes mellitus (NODM).[36] Central pancreatectomy carriers a lower risk of P-DM but it is associated with a notable greater incidence of postoperative pancreatic fistula (POPF).[37] The incidence of NODM varies with the type of surgery; at 18% to 27% after PD, and 5% to 42% after DP. Moreover, healthy patients without DM at baseline have a higher risk of P-DM development when DP is performed rather than PD; in parallel, it is possible to notice that the clinical presentation and glucose regulation of patients with preexisting DM worsen when DP is performed compared with the PD group.

Regional differences of density in islet distribution can explain the higher risk of P-DM after DP since β-cells concentration is more than 2-fold higher in the tail region while it is quite similar between the head, neck, and body regions.[4] Moreover, physiological and anatomical changes after PD affect the metabolic

outcome after PD. PD reconstruction is similar to Roux-en-Y gastric bypass (RYGB) surgery which is noted to improve the metabolic profiles and reduce body weight through increased incretin hormones (e.g., glucagon-like peptide-1 (GLP-1)). The enhanced incretin value is the possible etiology of the improvement in glycemic control in PD patients, however further studies are necessary to clarify GLP-1 role.[38]

Patients with CP have a 25-50% risk of developing DM shortly after DP, and the late-onset diabetes tend to be related to the clinical course of the disease. It is of paramount importance to monitor the development of late-onset diabetes in patients undergoing TP-IAT.[35] A possible explanation for the higher occurrence of P-DM in patients with CP undergoing DP has been postulated by Kirstin M.J. De Bruijn et al., the most probable reason is the preceding destruction of the pancreatic parenchyma and inflammatory β-cell dysfunction, along with ongoing damage to remaining parenchyma after DP. In contrast, in patients with other benign or malignant disease, pancreatic function often remains intact, thus these patients carrier a lower risk of P-DM development.[37]

Together with proximal DP, splenectomy has been identified as a risk of glucose dysregulation because the spleen is a source of adult multipotent stem cells, which may represent the progenitors of pancreatic islet secretory cells.[38]

2.3 Clinical Presentation

P-DM results in PEI and endocrine dysfunction. The amount of insulin that pancreas produces is variable among patients. PEI occurs when the pancreas is unable to produce digestive enzymes (amylase and lipase) secondary to pancreas lesions.

The clinical presentation of T3cDM includes classic diabetes symptoms such as dry mouth, polydipsia and polyuria, blurred vision, hypoglycemia, and fatigue. Patients may also experience numbness or tingling in the extremities and frequent skin or vaginal yeast infections. Symptoms of PEI include stomach pain, diarrhea

and constipation, frequent passage of gas and bloating, fatty or oily stools, and unexplained weight loss. P-DM is characterized by disabling episodes of hyperglycemia and hypoglycemia, increased morbidity and mortality, and target organ damage, the reason behind the complex clinical presentation is the absence of insulin and of insulin counterregulatory hormone: glucagon; particularly in cases of TP or extensive pancreatic resection that removes the endocrine tissue entirely. The relative deficiency in insulin, glucagon, and PPy causes brittle fluctuations of hyperglycemia and hypoglycemia, making the control of blood glucose levels extremely difficult. P-DM ranges in severity from mild to extreme. One of the cardinal features of T3cDM is the occurrence of pancreatic exocrine dysfunction, it should be regularly screened through the fecal elastase test. Protein and fat malabsorption occur when more than 90% of the pancreatic exocrine function is lost. Steatorrhea is one of the principal signs, moreover, the absorption of fat-soluble vitamins (A, D, E, and K) may be impaired, leading to the development of metabolic bone diseases.

The degree of endocrine deficiency correlates to the resected region of the pancreas because of the anatomical variation in islet cells distribution. DP is more likely to result in glucagon deficiency and severe hypoglycemia since glucagon-producing α-cells are located mainly in the pancreatic body and tail. Approximately 70% of β-cell mass also resides in this region. Additionally, pylorus and/or duodenum resection influences incretin action and glycemic control; patients show an increased in GLP-1 secretion, reduced gastric inhibitor peptide (GIP) levels, reduced insulin and PPy secretion.[36][25]

2.4 Complications and Prognosis

Poorly controlled P-DM can lead to serious complications comparable to those observed in type 1 diabetes mellitus (T1DM) and T2DM, such as ischemic heart disease, stroke, renal insufficiency, blindness, peripheral artery disease.[1]

Hypoglycemic attacks are a characterizing feature of P-DM because of increased peripheral sensitivity to insulin, decreased glucagon secretion, and irregular

intestinal glucose absorption; exogenous insulin administration is frequently the underlying cause. Iatrogenic hypoglycemia occasionally leads to hospitalization, irreversible damage to the central nervous system, or death.[35]

Glycemic control markers, such as HbA1c and C-peptide, are predictive of long-term outcomes. Studies have shown that lower Hb1Ac levels and preserved C-peptide secretion are associated with delayed onset and reduced severity of diabetic complications, such as nephropathy and neuropathy.[7] Furthermore, maintenance of C-peptide secretion is correlated with a more manageable and less "brittle" form of diabetes characterized by fewer episodes of hyper- and hypoglycemia episodes, and a better QoL.[39]

In addition to metabolic complications, epidemiological evidence links diabetes, including T3cDM, as a risk factor for the development of various cancers, notably of the pancreas, liver, kidneys, esophagus, and colon. This association underscores the importance of close monitoring and comprehensive management strategies for patients with P-DM.

2.5 Diagnosis

The diagnosis of T3cDM requires the confirmation of pancreas damage and the exclusion of other types of diabetes. Diagnosis should begin with confirmation of structural or functional pancreatic damage. This includes identifying underlying pancreatic pathology such as CP, pancreatic neoplasms, CF, or previous pancreatic resection.

A comprehensive diagnostic workup typically includes:[35]

- FBG test: diabetes is diagnosed if FBG ≥126 mg/dL.
- HbA1c test: HbA1c ≥6.5% supports diagnosis of diabetes.
- Pancreatic imaging: CT scan, MRI, endoscopic ultrasound (EUS) may reveal structural abnormalities or lesions in the pancreas.
- Pancreatic function tests: fecal elastase levels and blood tests for amylase and lipase to assess exocrine function.

- Pancreatic hormone levels: insulin, C-peptide, glucagon, and PPy measurements help to evaluate endocrine insufficiency and insulin sensitivity.
- Autoantibody testing: diabetes autoantibody panel, including glutamic acid decarboxylase 65 (GAD65) autoantibody, islet antigen-2 (IA-2) autoantibody, and insulin autoantibodies, is necessary to exclude autoimmune T1DM.

Diagnosis of T3cDM is established when diabetes criteria are met in conjugation with evidence of PEI and/or structural damage, and in the absence of autoimmunity.

2.6 Treatment

There are no specific recommendations for the treatment of T3cDM in the current *Standards of Medical Care in Diabetes*. Therefore, the treatment for T3cDM typically follows the algorithms for the management of T2DM. The main objective of the therapy is the reduction in HbA1c levels below 7%. The therapeutic opportunities are extremely variable; they depend on the underlying cause and on the degree of pancreas damage.

Lifestyle improvements represent the first therapeutic strategy, they include diet modification, daily exercise, abstinence from alcohol, and smoking cessation. Furthermore, medical treatment may be necessary in some patients.

The dichotomy of insulin versus non-insulin therapy for the initial treatment of T3cDM depends on the clinical presentation; the use of insulin and insulin secretagogues in the management of this form of diabetes requires extreme caution because of the increased risk of pancreatic neoplasia development. For the majority of T3cDM patients, oral therapy can be initiated with periodic retesting of HbA1c level. The therapeutic possibilities are oral diabetes medications: insulin, insulin secretagogues, agents that increase insulin sensitivity by their actions on liver, skeletal muscle or adipose tissue, and agents that affect glucose absorption; sulfonylureas are the most widely prescribed drugs for treating hyperglycemia,[32]

metformin, and/or insulin in the form of injections or pump to manage blood sugar levels. Oral diabetic medication is good when the disease burden is moderate. The treatment may change over time as the underlying clinical condition worsens, a strict follow-up is necessary. Many patients with T3cDM require insulin at an earlier stage compared to people with T2DM to manage blood sugar levels. Metformin is the preferred initial oral therapy because of its insulin-lowering effects on glucose metabolism, and its anti-neoplastic actions on cellular mediators of replication and protein synthesis. Moreover, there is the possibility to combine medications from different class of drugs (e.g., thiazolidinedione or alpha-glucosidase inhibitor); while sulfonylureas, GLP-1 analogues, and dipeptidyl peptidase IV (DPP-IV) inhibitors should be avoided or used with caution. In patients with marked hyperglycemia, typically defined as fasting glucose over 180 mg/dL (10 mmol/L), and HbA1c levels above 8.5%, and symptoms glycosuria and weight loss, insulin therapy is usually necessary. The minimum insulin dose necessary to maintain the glycemic control in T3cDM is significantly less than in other insulin-dependent patients. Metformin, or other oral agents, should be continued despite the addition of insulin as it has been shown to reduce the daily insulin administration and the risk of pancreatic carcinoma.[32]

Standard pharmacological therapy experience difficulties in controlling glycemic balance after TP due to absence of glucagon-dependent counter-regulation; thus, new technologies have been developed through the years such as IAT, PPy replacement and artificial endocrine pancreas. A safe and efficient therapeutic opportunity is the artificial endocrine pancreas, which controls blood glucose concentrations in hospitalized patients. The instrument continuously monitors blood glucose levels by withdrawing blood from a peripheral vein, and it administers insulin or glucose to maintain the target glucose concentration. Together with insulin replacement therapy, PPy administration alleviates the difficulties associated with controlling glucose levels. PPy administration upregulates hepatic sensitivity to insulin improving the clinical outcome. The benefit of PPy infusion correlates with the degree of PPy deficiency. IAT is a promising procedure which aims at decreasing the morbidity related to DM after

extensive pancreatic resection. It allows to achieve better glycemic control and the prevention of diabetic complications; however, long-term insulin independence is not a usual outcome. The effects of IAT and oral antidiabetic drugs, metformin with or without vildagliptin, after DP have been analyzed [40] and the results show that IAT maintains insulin secretory function and glycemic variability, conversely oral antidiabetic drug improves insulin resistance but insulin secretion and glucose tolerance are not restored. Microencapsulated islet transplantation is a field under investigation which may help to overcome the disadvantages typical of IAT.[35]

Finally, PEI treatment consists in oral pancreatic enzyme replacement therapy (PERT) consisting on initial dose of 1000 lipase units/kg/meal and a goal dose of 1500 lipase units/kg/meal and fat-soluble vitamin supplement.[29] PERT ameliorates or reverses the difficulty in digesting protein and lipid products in patients, in addition the optimization of exocrine insufficiency improves incretin secretion and glycemic control.[36]

3. IAT Indications

IAT is appropriate in a highly selected group of patients. Historically IAT has been performed only in case of CP characterized by incapacitating pain refractory to any other medical and endoscopic therapy, and secondarily for recurrent acute pancreatitis (RAP).[41] TP-IAT is effective in treating debilitating CP both in the adult and in the pediatric populations, especially when performed in the preadolescent period probably because of less prior surgical intervention and less lesions to the pancreas, concomitantly to the protective effect of a less-insulin-resistant metabolic environment.[9,29,42] Recently advances in imaging technology (CT scan, MRI) have allowed for earlier detection of premalignant, benign, and indeterminate pancreatic lesions; the improvement of diagnostic imaging techniques and the easier access to them has led to an increased number of pancreatic surgeries augmenting the interest for IAT procedure.

Over the past decades, clinical indications have been rising, including: high-risk conditions for Pancreatic Ductal Adenocarcinoma (PDAC) such as hereditary pancreatitis associated with mutations in PRSS1, SPINK1 genes, intraductal papillary mucinous neoplasm (IPMN), neuroendocrine neoplasm, increased anastomotic risk associated with partial pancreatectomy (e.g., “high-risk pancreatic stump”), extensive parenchymal resections for benign conditions, borderline neoplasms of the pancreatic body-neck regions, management of severe postoperative complications like grade C POPF, pancreatic trauma, completion pancreatectomies, and rare pancreatic diseases such as pancreatic arteriovenous malformation (AVM) and CF.[1] The expansion of IAT indications has been significantly shaped by the Milan Protocol, which has played a central role in extending the use of IAT to a broader population, including those with malignant and non-malignant pancreatic diseases.[17]

3.1 Chronic Pancreatitis

CP is an irreversible morphological and functional damage to the pancreas resulting from long-standing pancreatic inflammation and fibrosis. It has a multifactorial origin: alcohol and cigarette smoking give the greatest contribution to its development; other etiologies include CF, hereditary pancreatitis (PRSS1 gene mutation), hypertriglyceridemia, autoimmune pancreatitis, tropical (fibrocalcific) pancreatitis, and idiopathic conditions. Hereditary pancreatitis is the principal cause of CP among children and adolescents.[43]

3.1.1 Clinical presentation

The hallmarks of CP are pancreatic calcifications, pain syndromes, pancreatic insufficiency and its related symptoms, and increased risk of PDAC.[44] Endocrine and exocrine insufficiencies develop in the later stages of CP secondary to decreased secretions of enzymes and bicarbonate produced by acinar cells and ductal cells, while excruciating pain mitigates as the disease progresses because the gland atrophies.[42] Pain in CP can be devastating requiring the administration of narcotic and impairing severely the QoL. Evidence suggests that duct obstruction resulting in raised intraductal pressure, pancreatic ischemia, neuronal injury, and neuroimmune interaction are the determinants of pain.[45] The cornerstone of the surgical treatment of CP is to relieve pain while preserving as much pancreatic function as possible. Lastly, this patient population presents certain factors at plays influencing the coagulation system. CP course is characterized by a pathological response involving inflammation, coagulation, and endothelial dysfunction which pose the correct elements for the development of venous thromboembolism.[46]

3.1.2 Treatment

Treatment options for CP include pharmacological therapy, endoscopic interventions or surgical drainage and resection. The choice of intervention mostly relies on pancreatic morphology, including the size of the main pancreatic duct and pancreatic head.[6] In select cases, celiac axis nerve blocks may also be considered

for pain control. Patients with large-duct disease may respond to surgical or endoscopic decompression, while those with small-duct disease or failure endoscopic decompression can benefit from coeliac plexus block or splanchnicectomy.[45] In the presence of refractory pain, TP represents the last and the only possible intervention to completely remove the root cause of pain, since the pain is intrinsic to the residual pancreas that remains after partial surgical procedures.[47] TP is the antithesis of the beforementioned principle unless combined with IAT to preserve the majority of β-cell mass and insulin-secretory capacity.

The main aim of TP-IAT in patients affected by CP is the relief from intractable pain while preventing or reducing the severity of iatrogenic diabetes. Importantly, a systemic review has demonstrated that TP-IAT reduces opiate use while enabling a large percentage of patients to remain insulin-independent.[45]

Many patients who undergo TP have a history of narcotic use and may suffer from opioid-induced hyperalgesia (OIH), specifically morphine-induced hyperalgesia characterized by reduced cold-induced pain tolerance. Secondary to the long duration of pain, some patients may develop a neurological syndrome known as central sensitization resulting in persistent pain.

3.1.3 Pancreatectomy and IAT indications

Selection criteria for patient with CP for TP-IAT has evolved over the years. Currently, candidates must experience abdominal pain lasting at least 6 months associated with impaired QoL (e.g., inability to work or attend school, frequent hospitalizations), or persistent narcotics need, coupled with failure to respond to maximal medical treatment or endoscopic pancreatic duct drainage procedures. These indications must be coupled with at least one of the following:

1. Evidence of pancreatic calcifications on CT scan, abnormal endoscopic retrograde cholangiopancreatography (ERCP), or >=6/9 criteria on EUS.
2. Two of following three findings: (a) ductal or parenchymal abnormalities on secretin stimulated magnetic resonance cholangiopancreatography

(MRCP), (b) EUS with 4/9 criteria positive, or (c) abnormal endoscopic pancreatic function testing with peak bicarbonate <80 mmol/L).

3. Histopathologic confirmed diagnosis of CP from previous operations.
4. Diagnosis of hereditary pancreatitis (e.g., PRSS1 gene mutation) with a compatible clinical history.
5. History of RAP with more than 3 episodes of pain associated with imaging diagnostic of AP and/or elevated serum amylase or lipase 3 times normal.

Nowadays, a paradigm is observed: TP-IAT is offered to most patients after failure of endoscopic duct drainage or to patients in whom endoscopic approach failure is predictable.

An important consideration is about the timing of TP-IAT: operating earlier is recommended since it facilitates narcotic withdrawal, it avoids opioid induced hyperalgesia, central pain sensitization, and narcotic bowel syndrome. Furthermore, it is associated with a larger β-cell mass retrieval. The optimal timing of TP-IAT is defined by severity, frequency and duration of pain, narcotic drugs requirement, decreased QoL, residual pancreatic islet function, rate of disease progression, and patient's age.[1] Simultaneously, the timing of the procedure can be defined by pre-procedural glucose metabolism. When CP characterized by severe intractable pain is the only indication for TP-IAT, metabolic outcome is better in non-diabetic patients; however, patients affected by diabetes with positive C-peptide can still benefit, although in a minor fashion, from TP-IAT, the outcomes are like those of pre-diabetic recipients comprising pain relief and long-term functionality of infused islets.[48] Studies are needed to determine the optimal timing of TP-IAT for PDAC prevention.[8] The Prospective Observational Study of TP-IAT (POST) is a multicenter research study with the objective to define recipients selection and timing for TP-IAT to optimize clinical outcomes in patients affected by CP.[49]

3.2 Recent IAT Indications

Advancements in diagnostic imaging and deeper understanding of pancreatic neoplasm pathogenesis have led to an increased frequency of pancreatectomies for

benign, low-grade, and malignant tumors. Surgical resection should take into consideration both radiological parameters and neoplasia biology.[50]

Extended left pancreatectomy, defined as resection of more than 50% of otherwise healthy pancreatic parenchyma, for lesions in the pancreatic neck, whether benign, borderline or malignant is associated with NODM in 9% to 31% of the patients within a few months post-surgery. The trend increases up to 20% to 50% during the follow-up. This complication represents the attractive reason to conduct studies for the implementation of the new indications of IAT procedure.[51]

While CP remains the primary indication for IAT, its potential application in other clinical settings remains controversial. The Milan group has published initial data regarding the feasibility to extend IAT to patients with known malignancy having either (i) completion pancreatectomy for severe pancreatic fistulas, (ii) extension DP for neoplasms of the pancreatic neck, or (iii) TP for increased anastomotic leak.[17,26] The Milan Protocol represents a significant advancement in the field of IAT, aiming to extend its application to a wider patient population with both malignant and non-malignant pancreatic diseases, since until now, the fear of infusing occult carcinoma cells inside the islet preparation has limited the use of this procedure. The Milan Protocol study analyzed the potential dissemination of occult carcinoma cells, and the long-term metabolic follow-up (modified Igls criteria, MMTT, and sustained C-peptide secretion) necessary to assess the durability of IAT effects on glycemic control and the preservation of endocrine function.[27] Despite these efforts, results are divergent and inconclusive, and the role of TP-IAT in pancreatic and peripancreatic malignant neoplasms is debated. While a few case reports have been narrated, currently, IAT does not represent the standard of care.[1] Although additional data are required to eliminate the risk of neoplastic cells spread, the study conducted by Balzano and colleagues suggests that IAT indications can be extended to selected patients with neoplasm; their study emphasizes that a diagnosis of malignancy alone should not be an absolute contraindication to IAT. However, the risk of metastatic liver disease is a serious concern which requires a strict follow-up for the early detection of this complication.[17]

In addition, simultaneous IAT following TP should be offered also in case of pancreatic trauma in case of rescue completion pancreatectomy because of the enormous metabolic advantages and enhancement of QoL.[52]

In conclusion, emerging evidence supports a broader scope for IAT beyond CP. While further data are needed to definitely establish IAT role in malignancy, current findings support its feasibility and safety in well-selected patient populations under rigorous monitoring protocols.[26,53]

3.2.1 Cystic neoplasm of the pancreas

Cystic neoplasm of the pancreas is the example of a new entity for which IAT could become the standard of care. Cystic neoplasms of the pancreas are an in increasingly diagnosed pathology because of improvements in radiologic technology. These lesions have the potential to become malignant; thus, the preferred approach is aggressive resection of the pancreatic parenchyma. IAT is a promising approach to prevent P-DM after a major pancreatectomy, however consensus about its use in the context of cystic lesions is under development. The Korean study conducted by Byung-Wan Lee and colleagues tried to answer the main following question: “Does distal pancreatectomy coupled with IAT implant malignant cells into the portal vein?”[53] To exclude any possibility of malignancy, after pancreatic resection, it is necessary to perform a histopathological examination of the pancreatic segment containing the tumor and incubation for at least 24 to 48 hours at 37°C in culture medium for the decision of the patient candidacy for islet transplantation. The study results reveal that during the follow-up after IAT, no evidence of recurrence has been identified by clinical or radiological findings by tumor markers. IAT after major pancreatectomy performed for benign focal diseases or pancreatic injury is a safe procedure, and it offers glycemic control benefits and preservation of physiological glucose regulation.

3.2.2 High-risk pancreatic resection

The study[54] demonstrates the practicability and efficacy of preemptive TP-IAT in patient candidates for PD at high risk of POPF, validating the extension of IAT indication beyond CP. In the light of the latest evidence, TP-IAT is a feasible alternative to PD as it reduces severity, complications, and hospital length of stay. It is necessary to select patients in whom pancreatic anastomosis is associated with high risk of dehiscence according to the presence of narrow main pancreatic duct and soft and/or frail pancreatic texture, because these patients benefit from TP rather than DP.[1,55] Surgical treatment options for lesions in pancreatic head include pylorus-resecting (Whipple procedure) and pylorus-preserving pancreaticoduodenectomy (PPPD). These surgical procedures are complex, and postoperative complications can arise in 10-30% of interventions. POPF is caused by leakage of pancreatic-enteric anastomosis. It is of paramount importance to stratify patients for risk of POPF development. Risk assessment is primarily based on intraoperative factors like pancreatic duct diameter (≤3 mm), parenchymal texture, and blood loss. However, an early risk estimation, ideally based on routinely available preoperative data, plays a key role in the pre-surgical decision-making leading to a better perioperative management of the patients. Retrospective studies have identified CT-based radiomic features to be associated with POPF,[55] including mesh-based volumes of annotated intra- and peripancreatic structures and preoperative clinical data as relevant risk signatures with the aim to develop risk models for the prediction of POPF after pancreatic head resection. Radiomic features have a good prognostic power. In summary, small pancreatic duct size and large pancreatic remnant volume, obtained from CT images, are associated with the risk of POPF development after pancreatic head resection. Pre-operative POPF risk assessment helps to identify high-risk patients who benefits from a different approach rather than Whipple procedure or PPPD. Alternative treatment options include different anastomotic techniques, somatostatins applications, drain management, strict follow-up, and TP coupled with concurrent IAT.[55]

3.2.3 Hereditary pancreatitis

Among the various recent indications for IAT, hereditary pancreatitis with *PRSS1* gene mutation is present, because it is endowed with a higher risk of adenocarcinoma development later in life. The risk is multifactorial, smoking and diabetes play the most significant role. Furthermore, CP causes pancreatic parenchyma distortion, making imaging-based surveillance for early cancer complicated. However, no post-TP-IAT cases of cancer of pancreatic origin have been reported in the liver up to now.[8]

3.2.4 Neuroendocrine neoplasm and IPMN

Recently, pancreatic neuroendocrine tumors (PNET) have been proposed as indication for TP-IAT. The studied clinical case describes an incidentally discovered PNET during pancreatic surgery for CP. In this scenario is necessary to perform an intraoperative biopsy to investigate the unexpected malignancy.[56] IAT technology can be efficiently applied after extensive distal pancreatic resection of benign and borderline lesions located in the neck of the pancreas, as it has been demonstrated by the study, however, lesions >3 cm or IPMN were not considered.[57] It is necessary to be cautious regarding the risk of unrecognized malignant tumor dissemination to the liver. In the study case the risk has been avoided by performing extemporaneous frozen section histological examination of the lesion with a safety margin longer than 3 mm.

In addition, the study shown that the numbers of islets recovered from segments of pancreata resected for benign lesions are twice as high as those from organs with CP, and even four times higher when normalized per gram of pancreas.[57]

3.3 Exclusion Criteria

Contraindications to IAT include pre-existing insulin-dependent diabetes (e.g., C-peptide negative diabetes and T1DM) since IAT requires functional β-cells to be infused in the patient, steatohepatitis or other liver disease, portal vein thrombosis

(PVT), portal hypertension, cardiopulmonary disease, malignant or pre-malignant pancreatic lesions, and any medical condition that, in surgeon opinion, might have a negative influence on procedure safety. PVT, portal hypertension, and severe liver disease are contraindications to general pancreatic surgeries, in parallel, they are selective contraindications to cells infusion within the liver; ongoing studies are investigating the efficiencies of alternative infusion sites, intraperitoneal or sub-serosa. Furthermore, poor candidates are considered patients suffering from visceral hyperalgesia and "functional" pain symptoms.[39]

Similarly to clinical conditions, there are psychosocial features which make a patient unsuitable for receiving IAT, including poor social support, active alcoholism, active illicit substance use, drug-seeking behaviors, uncontrolled or untreated psychiatric illness. The reason behind the exclusion of these patients is the presumptive inability to adhere the complex medical follow-up which is necessary for the management of a such complicated procedure.[8]

In general, patients presenting with malignant or pre-malignant pancreatic lesions are excluded from IAT program because of the risk of seeding occult cancer cells into the liver, considering that some acinar and ductal tissue is co-transplanted with the islets. Nevertheless, some islet autotransplants have been reported in patients with localized pancreatic cancer, but currently it is not a standard.[39] Furthermore, the 15-Y Study of the Milan Protocol demonstrated absence of progression of baseline neoplasia diseases following TP-IAT; the recurrence pattern did not show significant differences between patients receiving IAT and those who did not. The results of the study uphold that IAT does not increase the risk of disease recurrence or metastatic in patients with malignant neoplasms.[27]

The contraindications for IAT according to the Milan Protocol include the presence of any multifocal pancreatic neoplasm at preoperative imaging or intraoperative evaluation, diagnosis of multifocal IPMN, involvement of the pathologic pancreatic transection margin, including any degree of dysplasia, diagnosis of multiple endocrine neoplasm, any medical condition that may interfere with IAT safety. Interestingly, pancreatic malignancy per se is not an absolute

contraindication, and as defined previously IAT indications are expanding comprehending patients affected by pancreatic cancer, in particular focal IPMN.[17,26]

3.4 Inclusion Criteria

Indications for IAT according to the Milan protocol are categorized in (i) clinical indication, and (ii) general indications.[17,26] Among the clinical indications for IAT there are (1) CP and AP, (2) high-risk pancreatic stump, (3) extensive DP for benign/borderline neoplasm of pancreas body neck, and (4) severe complications after pancreatic surgery (e.g., grade C pancreatic fistula). General indications for IAT comprehend (1) being older than 18 years of age, (2) fasting glycemia level lower than 126 mg/dL without the use of glucose-lowering medications, (3) ability to provide written informed consent, (4) mental stability and ability to comply with study procedures.[26]

Pancreatic surgery and IAT are major surgical procedures with potential serious complications thus patients must be precisely selected. Furthermore, it must be taken into consideration that the operation is irreversible, there is risk of serious complications, pain relief is not always experienced, protection from diabetes is often incomplete and of variable durability, all patients require life-long PERT, post-surgical gastrointestinal mobility dysfunction may occur. Additionally, once TP-IAT has been performed, it is not possible to benefit from any future medical approaches for pancreatic disorders.[8] A multidisciplinary team discussion is a necessary component of the preoperative, perioperative and post-operative phases of IAT to understand the benefits and the contraindications of the procedure.

4. IAT Procedure

Islet isolation technique and transplantation strategies have changed over the years, and they are variable across different expert centers in Europe, this aspect influences the outcomes of IAT. The backbone approach to perform IAT starts with the intraoperative collection of the pancreas for transplantation through open or laparoscopic surgery, the spleen can be removed or preserved. Subsequently, in the sterile setting of a reference laboratory the islets must be isolated and possibly purified. Eventually the cells are reinfused back in the patient through portal infusion, or in other infusion sites, the same day or in a different moment according to the hospital guidelines.

4.1 Pancreatectomy

IAT procedure is dependent on pancreatic resection. The extent of the resection is variable: TP, extensive left pancreatectomy, completion pancreatectomy, PD, or DP. Historically, there was reluctance to refer patients for TP because of the complete exocrine insufficiency and brittle diabetes that result from it, however with the advancement of IAT, treatment of brittle diabetes with insulin may become less challenging or not even necessary. Pancreas procurement can be performed by open laparotomy or minimally invasive techniques.

PD indications include lesions located at the pancreas head, distal common bile duct, ampulla of Vater, or duodenum. PD consists in the removal of the head of the pancreas, common bile duct, gallbladder, duodenum, and sometimes the distal of the stomach (antrum and pylorus), unless pylorus-preserving Whipple procedure is performed. Generally, about 40% of the pancreatic parenchyma is resected. The pancreas is cut off at the level of the neck, which overlies the superior mesenteric vein. Finally, the proximal jejunum is anastomosed to the remaining pancreas (pancreatojejunostomy), the bile duct (choledochojejunostomy), and the residual stomach (gastrojejunostomy or duodenojejunostomy if pylorus-preserving).[38]

DP is indicated for lesions at the body or tail of the pancreas; and the transection lines vary according to the lesion pathology and location. When pancreatic cancer is present, wide resection margin and lymph node dissection are required, the transection line is at the level of the neck of the pancreas and the removal of up to 80% of the organ is pursued.[38]

TP consists of two stages: first, resection of the body and tail (DP), and subsequently, the pancreas head and the duodenum are removed (PD). The cardinal principle of TP-IAT is the preservation of the blood supply as long as possible. The blood supply to the gland is interrupted just before the removal of it in order to minimize the warm ischemic time to the islet tissue, through the ligation of the gastroduodenal artery, the origin of the splenic artery and termination of the splenic vein.[6]

Once pancreatectomy has been completed, gastrointestinal reconstruction is undertaken according to patient's characteristics. Roux-en-Y reconstruction is preferred to avoid bile reflux and afferent limb problems in patients with high prevalence of gastroparesis.

When pancreatic tumour is the indication for resection clear margin must be left, in the Balzano study 1 cm of the pancreatic remnant proximal to the pancreatic margin was resected and sent for frozen section examination to confirm margin negativity.[17]

Splenectomy is not a mandatory procedure; occasionally anatomic circumstances are favorable to preserve the spleen on the short gastric vessels.[39] The decision to retain or remove the spleen is based on individual patient factors, for instance when scarring and necrosis of the splenic vessels is present splenectomy is mandatory; however, spleen-preserving total pancreatectomy (SPTP), using the Warshaw technique, is a surgical option when the preservation of the blood apport to the spleen is achievable. TP-IAT can be safely performed either with spleen conservation or spleen removal.[58] Splenectomy is performed in 91% of adults and in 100% of children undergoing TP-IAT.

4.2 Pancreas Dissection and Decontamination

After pancreatic resection, the majority of the protocols include the flushing of the pancreatic tissue in the operating room (OR) with preservation solution, mainly University of Wisconsin (UW),[17] and the immersion of the organ in cold balanced electrolyte solutions, such as Hank's and UW,[45] for transportation to the islet processing facility. The separation of the spleen and duodenum can occur in the facility or directly in the OR along with the assessment of the pancreatic duct integrity and exsanguination of the organ by flushing the arterial vessels with cold preservation solution and opening the venous outflow.[1,39]

Once the pancreas is brought to the islet isolation laboratory, the next phases consist in an initial visual inspection of the pancreas for color, shape and obvious signs of damage; subsequently the excess fat, the duodenum and the spleen are trimmed off, if not removed previously, to prepare the organ for enzymatic perfusion. Great care is taken to avoid lesions to the pancreatic capsule which would cause reduction of enzyme efficacy and altered organ distension.[59] Finally, the organ undergoes decontamination step through antiseptic and lavage solutions of 1 min each.

4.3 Pancreas Perfusion

Once the organ has been weighted, the pancreatic ducts are cannulated with 14 20 H catheter. The organ gets perfused and distended by infusion of cold collagenase solution (Collagenase NB1 Premium Grade, Serva, Heidelberg, Germany). The protocol for collagenase use is not uniform, in the study conducted by JJ Wilhelm et al.,[19] a combination of collagenase with intact C1 and neutral protease from *Clostridium Histolyticum* is dosed on the basis of pancreas weight, in particular the collagenase:protease ratio and the unit dose may be adjusted based on individual pancreas factors (e.g., donor age and fibrosis severity). The enzyme mixture necessary for the procedure is critical; furthermore its concentration needs to be standardized.[60] Enzymatic digestion facilitates the successive separation of

the endocrine pancreatic portion from the exocrine one. This step causes the major trauma and stress to the islets affecting the quality,[60] thus studies to advance enzymatic combination, together with islet isolation techniques and delivery methods that may favor islet recovery are necessary.[2]

4.4 Islet Digestion

The primary objective of the islet digestion step is the enzymatic disruption of the pancreatic tissue with the attempt to release relatively pure islets, which represent only 1-2% of the total pancreatic volume.[16,39] Although modifications have been introduced over the years, the basic method of islet isolation has been the same over time: dispersion of the pancreas parenchyma in a stepwise fashion, firstly pancreas distension by intra-ductal injection of collagenase and protease solution to disrupt the exocrine pancreas, then gentle mechanical dispersion and digestion at 37 °C using the Ricordi chamber.

The filtration chamber, or Ricordi chamber, is the core of the Automated Method developed by Dr. Camillo Ricordi in 1988 to enhance the disassembling of the pancreatic tissue via combined enzymatic and mechanical digestion of the pancreatic parenchyma while preserving the endocrine cell cluster integrity.[59,61,62] Following the enzymatic digestion of the parenchyma, the automated method for the isolation of human pancreatic islets comes into play, it allows to perform a mechanically enhanced enzymatic digestion of the pancreas. The technique gathers some requirements: 1) minimal traumatic action of the cells, 2) continuous digestion in which the liberated islets can be promptly removed to avoid further enzymatic action and conservancy of cluster integrity, 3) less human intervention in digestion procedure, 4) high yield and purity of isolated islets.[63] Once pancreatic digestion is over, the pancreas is not uniform, part of it is over-digested and other portions are under-digested.

The Ricordi chamber is an isolator made of two stainless steel compartments separated by a stainless-steel screen with seven marbles or metal spheres (1 cm diameter) placed within it. The pancreas is loaded in the isolator intact or cut into

6-8 pieces and supplementary collagenase solution (2 mg/mL) is added. The dissociation chamber is manually shacked (320 oscillations/min), and a peristaltic pump is switched on with a flow rate of 40 mL/min. At this point, digestion is conducted in a close system in which Hanks' solution is aspirated through a filter located in the upper portion of the recirculation cylinder; and from it, the solution passes through the pump to a heating circuit and reaches the lower chamber of the isolator, gradually diluting the collagenase solution inside the chamber. The heating circuit allows to maintain 37°C temperature within the digestion chamber. From the chamber, the solution moves through the 280 μm screen to a cooling circuit that inactivates the enzymatic solution and then returns to the lower portion of the recirculation cylinder. Collagenase inactivation is reached by placing the cooling circuit and the recirculation cylinder in an ice bath (4 °C) to preserve the islets. The solution is drained from the top of the cylinder through the 94 μm filter while the islets remain steady at the bottom of the chamber. Every 2 minutes, samples of the preparation and drawn to monitor the percentage of free islets in respect to the acinar tissue through dithizone stain (DTZ). When it reaches 40-50%, the recirculation cylinder and the heating circuit are bypassed and the islet separation is conducted on an open system characterized by a progressive reduction in temperature, and the collagenase solution is diluted by fresh Hanks' solution.[63] Isolation procedure lasts about 30-60 min, at the end of it, islet preparation is collected in 2-L flasks, whereas in the Ricordi chamber fibrous network of ducts and vessels remain.[59]

The beginning of the digestion in the Ricordi chamber represent the end of cold ischemia, which starts at the *cross-clamp* time during the retrieval of the organ.

The Ricordi chamber facilitates the mechanical dispersion and liberation of the islets through the shaking of it. The current enzyme mixture consists of intact C1 collagease (Vitacyte) and neutral protease (SERVA).[6] In the Milan Protocol study, the pancreas was enzymatically digested using collagenase NB1 and neutral protease.[27] It underlies the individualization of each center in this procedure and the lack of a concordant guidelines.

The pellet containing acinar and endocrine tissue is collected and assessed for islet count, viability, purity, and endotoxin content, a sample is sent from Gram stain and microbiology culture.[39] An effective isolation process results in an optimal response to glucose stimulation after overnight culture at 37 °C, characterized by a fivefold increase from basal insulin secretion.[63]

The automated method to attempt islet isolation is not the only possibility, a manual method has been described mainly for research purposes. The latter consists in liberase injection into the pancreatic duct through a syringe, and disposal of the distended pancreas into a sterile container at 37 °C which is agitated to aid digestion. Samples of the digest are collected to monitor the digestion level and upon completion of the process, enzyme activity is concluded by cooling and adding bovine serum albumin to the compost and cold UW solution. Finally, centrifugation is performed and interface containing islet cells is removed and prepared for culture. The manual method is more economical, but it provides lower islet yield than the automated method. It can be considered for the retrieval of islets for research purposes when the pancreases are unsuitable or unlikely to yield great viable islets.[59]

The success of the islet isolation primarily relies on the efficient delivery of the collagenase enzyme within the pancreatic parenchyma; alterations in pancreatic ductal structure, calcifications, strictures, and fibrotic material can hinder enzyme perfusion.[2]

Islet cells are highly sensible to deviations of physiological variables, thus a strict monitoring of few determinant factors such as pH, pCO2, pO2, and temperature during islet isolation procedure and in vitro culture through a continuous multiparametric monitoring system allows to identify critical values and promptly correct them during the procedure. Constant checking is achievable through the TrendCare/Paratren 7+, an automated fiber optic probe system that controls in real time pH, pCO2, pO2, temperature, bicarbonate concentration, and base excess values. The final objective is an amelioration of the quality of transplanted islet cells and a better glycemic control.[64]

Interestingly, intraoperative islet isolation is a possibility when a reference islet facility is lacking. Its effectiveness, in term of metabolic outcomes, is comparable to islet isolation in the laboratory.[65] Intraoperative islet cell isolation requires islet equipment which is brought into the OR. The process starts with the cannulation of the pancreatic duct and the infusion of a warm enzymatic solution made of proteases, collagenase, and buffers. Secondarily, the pancreas is cut into small pieces and placed into the Ricordi chamber at 37 °C which is manually shaken. Once digestion process is over, the system is cooled down to 4 °C. The digest is gathered and mixed with 5% human serum albumin and then centrifugated. Purification process is never performed. Ultimately, the pellet is suspended with 5% human serum albumin, and 35 IU/kg heparin is added to the compound.[65]

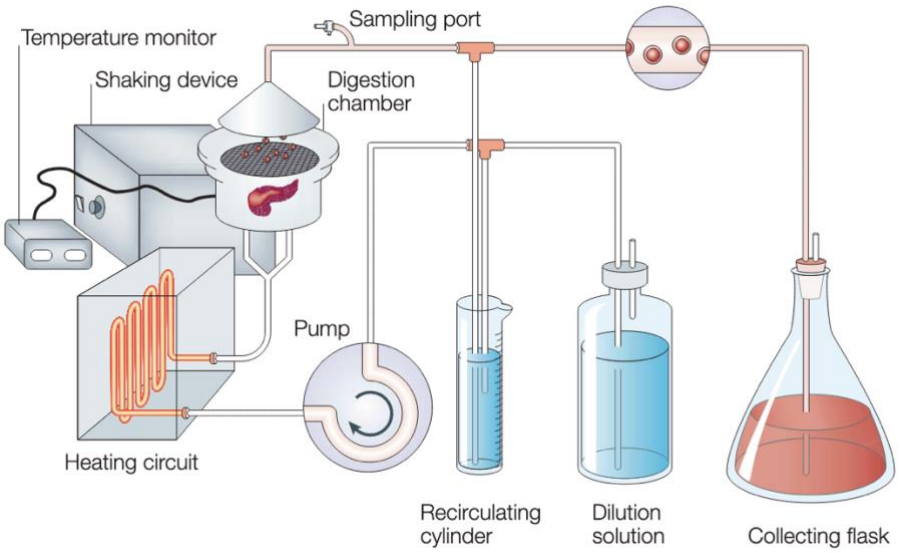

Figure 1 - Schematic representation of human islet cells digestion process [62]

4.5 Islet Purification

Islet digestion step can be followed by purification or partially-purification of the post-digested volume when it is large. Islet purification is not a compulsory step. The decision to purify depends mainly on the post-digestion tissue volume, and there is not an agreed techniques among studies.[45] Sutherland and co-workers reported that, since 2006, purification is performed when the digest tissue is over 20 cm^3,[45] while in the study conducted by Matsumoto and colleagues,[28]

purification is undertaken when tissue volume exceeds 15-20 mL. Currently a post-digest tissue volume greater than 0.25 cc/kg (mL/kg) is the indicative threshold to purification, or >15 mL as stated in the study.[28] Since there is a significant correlation between tissue volume and final portal pressure, large tissue volume infusion within the portal vein increases portal pressure and risk of IAT complications. The rationale behind pellet purification is to minimize the increase in portal pressure, the risk of portal thrombosis and bleeding that occur during islet embolization to the liver, intrahepatic microembolization and inflammatory reaction.[28] Post-infusion pressure is clearly a function of tissue volume. Islet purification effectively reduces tissue volume improving clinical outcomes when islet transplantation volume is large and it does not negatively affect islet characteristics,[28] making it an important step for the improvement of clinical outcomes in the presence of large tissue volumes.[1] However, islet purification is associated inevitably with at least 30% loss of the islet mass, particularly when the islet cells are surrounded by exocrine tissue or characterized by abnormal density, frequently observed in pancreases affected by CP or in younger patients. Purification should be weighed against wastage of islet cells, contrarily to allo-islet manufacturing protocols in which purification and tissue infusion limits are set standardly, in TP-IAT there are not guidelines.[19]

The pancreas is heavier among the population of patients undergoing purification process, while after purification, there is no noteworthy variance in tissue volume between the two classes of patients.[28]

In the vast majority of cases, islet purification is performed using density gradients separation of the islet, carried out through the COBE 2991 cell processor,[62] using a continuous HBSS-Ficoll (Biochrome, Berlin, Germany) gradient or using a density-adjusted iodixanol-based continuous density gradient.[28] Controlled density gradients with iodixanol improves islet recovery rate resulting in a higher rate of transplanted volume.[15] Purified cells are pooled in Final Wash ((mediatech Cellgro, VA) plus 1% Pen/Strep, 1% Glutamine (Lonza,

Basel, Switzerland), counted and expressed as islets normalized to a 150 μm diameter (islet equivalent [IEQ]).[17]

The study [63] describes a similar purification procedure, briefly 3 mL aliquots of pelleted tissue from islet preparation are loaded into 250 mL plastic syringe, furthermore the purified tissue is washed with Hanks' solution by centrifugation at 950 x g for 5 min at 4°C. At the end, islet pellets are combined and suspended in 200 mL of tissue culture medium containing 10% fetal calf serum, penicillin (100 U/mL), streptomycin sulphate (100 μg/mL), D-glucose (1 mg/mL), L-glutamine (2 mM), and HEPES (25 mM), pH 7.4.[63]

In the Milan Protocol study, the digested tissue underwent purification using a continuous gradient of Hanks's balanced salt solution-Ficoll on a cell separator. The resulting purified islet fractions were then combined and pooled in Connaught Medical Research Laboratories 1066 medium.[27]

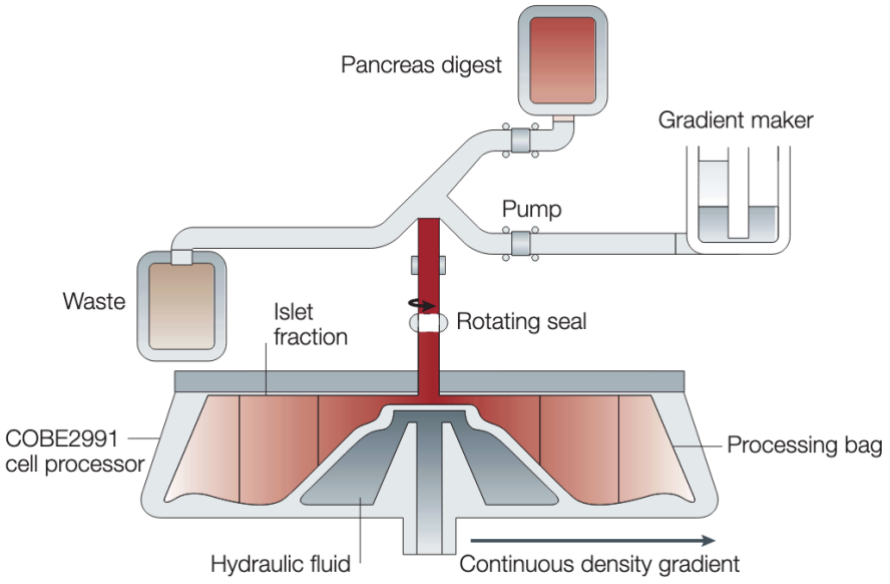

Figure 2 - Schematic representation of purification process of human islets by density-gradient separation[62]

4.6 Islet Culture

Furthermore, culture of islet preparation is an important phase, and culture time deserves further discussion. Islets can be placed in culture at 37°C for a maximum of 48 hours. Culturing islets lead to a better success rate, short-term pretransplant culture may induce a quicker recovery from stress-related changes and a reduction in the early posttransplant loss. Culture time between 1 hour and 14 hours at 37°C determines a favorable effect on the occurrence of insulin independence among islets recipients.[51]

4.7 Quality Control (QC)

During the entire process of islet isolation, samples are taken to perform quality control, with the aim of evaluate efficacy and safety of isolated islets. QC evaluates some parameters:[1,28]

- Purity and islet yield (islet count is expressed as IEQ), through DTZ staining.
- Viability, through fluorescein diacetate (10 mol/L) and propidium iodide (15 mol/L) staining.
- Sterility, through gram staining, bacterial and fungal cultures, and assessment of endotoxins.

The final islet tissue preparation is suspended in 500 mL of CMRI culture medium (Mediatech, Inc) with human albumin added to a concentration of 2.5%.[6]

4.8 Islet Infusion

Islet infusion step differs among centers accordingly to clinical and logistic conditions: transplantation can take place immediately after pancreas processing while the patient is still in the OR, principally when the islets are returned to the OR from 3½ to 6½ hours after the pancreatectomy without culture time,[39] or post-operatively within 48 hours by a percutaneous approach under ultrasonography

and/or fluoroscopic guidance. Combined fluoroscopic and ultrasonographic approach makes the procedure securer.[66] Islets are infused by gravity over a 15-60 min period after heparin administration. Pre-infusion or intraoperative heparin administration is of paramount importance to prevent islet aggregation, thrombotic complications, and to enhance islet engraftment. Noteworthy, protocols of heparin administration and dosage vary among expert centers, for example according to the protocol outlined by study group in the paper *"Diabetes-free survival after extended distal pancreatectomy and islet auto transplantation for benign or borderline/malignant lesions of the pancreas"*, 2000 IU of heparin are added to the islet preparation before transplantation.[51] In contrast, a protocol described in the study *"Proposed Thresholds for Pancreatic Tissue Volume for Safe Intraportal Islet Autotransplantation After Total Pancreatectomy"* suggests an anticoagulant scheme based on the weight of the patient and administered intravenously, it involves the infusion as a bolus of 70-100 IU/kg heparin in the patient.[19] Although practices vary, avoidance of heparinization increases the risk of portal vein clotting and disseminated intravascular coagulation (DIC).

Dextran 0.5 cc per kilogram to a maximum of 10 cc per hour continuous infusion is also administered to patients to inhibit the extrinsic pathway of coagulation.[29] Upon completion of the transplant, the infusion catheter is removed, and a thromboplastic paste (DSTAT or Avitene) is infused to obliterate the catheter opening.[16]

The final product gets diluted in a specific solution for the transplant and divided in 50 mL syringe, which are characterized by no more than 1 mL of pellet and by a defined purity gradient.

Islet infusion site is under debate, the portal system is the most common transplant site using a stump of the splenic vein, via direct puncture of the portal, or by cannulation of the umbilical vein.[1,19,39] Although intraportal islet transplantation is still the gold-standard site for islet infusion, there are some disadvantages including greater glucolipotoxicity and toxin exposure due to the direct contact with portal blood. Moreover, intraportal islets may elicit a rapid

endovascular mediated inflammatory reaction or immediate blood-mediated inflammatory reaction (IBMIR), which causes loss of approximately 50% of transplanted islets.[2] Furthermore, stressors that negatively influence the engraftment of the islets are the relatively hypoxic environment of the portal venous system, Kupfer cells and hyperglycemia.[2] To address this, alternative transplant sites for example bone marrow at the super-posterior iliac crest, renal subcapsule, testicle, thymus, omentum, small intestinal mucosa, gastric submucosa, and intramuscular injection describes by Rafael et al., as well as encapsulation technology are areas under investigation.[39,67]

The successful engraftment of islets in the bone morrow, and the absence of adverse effects related to the cells infusion in the iliac crest article have been studied by few articles.[68] Moreover, the bone marrow well-vascularized microenvironment support islet revascularization providing the necessary oxygen pressure, pH, toxic metabolites clearance, and nutrient access. Lastly, the benefit of bone marrow implantation site is the possibility to perform multiple biopsies, histological and immunohistochemical analyses, and real-time quantitative polymerase chain reaction (PCR) analyses of messenger ribonucleic acid (RNA).

The study[10] compares liver and skeletal muscles (i.e., brachioradialis muscle) as IAT-sites, skeletal muscles represent an easy access for graft monitoring following transplantation. It has been noticed that all patients require insulin administration post-operatively, in particular the mean insulin dose is lower in the liver-IAT recipients compared to the muscle-IAT recipients, vice-versa fasting C-peptide level is higher in the liver-IAT recipients, it defines the superiority of liver IAT-site in respect to brachioradialis muscle concerning effective islet engraftment and subsequent glycemic management.[10]

Although the numerous limitations, intraportal islets infusion still remains the best implantation site because its capacity to support long-term islet engraftment, C-peptide production, and insulin independence maintenance.[67]

During the islet infusion step, an important principle that must be followed: the larger the tissue volume, the slower the infusion rate in order to minimize portal pressure elevation; thus, portal pressure is closely monitored during the procedure. Significant increase in portal pressure can cause blood flow reduction leading to portal vein thrombosis. Consequently, if the pressure within the portal vein reaches 25 cmH_2O, or 30 mmHg,[2] the infusion is temporary stopped, and any remaining tissue is dispersed within the peritoneal cavity,[6] or injected into the leaves of the mesentery or omentum, bowel subserosa, gastric submucosa, or intramuscular space.[39]

Noteworthy, islet implantation site determinates glucagon response to hypoglycemia. Recipients of intrahepatic islets only, differently from patients receiving both intrahepatic and non-intrahepatic islets, do not mount sufficient α-cell response to respond to hypoglycemia. The reason behind the lack of response is that the intrahepatic environment is characterized by higher glucose concentration than in the systemic blood, which impairs the ability of islet to correctly perceive glucose level and adjust glucagon and insulin secretion during episodes of hypoglycemia.[69]

4.9 Remote Islet Processing

Remote islet processing is an area of interest to expand the number of facilities performing IAT. The explanted pancreas is transported to a separate location for isolation process, and the obtained islet cells are taken back to the hospital where the patient is to be infused. The biggest issue is cold ischemia; however, several studies[1,70] demonstrate that cold ischemia time does not influence the quantity of IEQ/kg, nor the post-operative change in C-peptide secretion when remote isolation is performed. According to this data, geographically remote islet isolation procedure is an effective and safe alternative.[54,70,71] Nevertheless, the results are controversial, because a study[1] found that the metabolic control is better is patients following the procedure with local islet isolation, the consideration of the

advantages of higher number of centers performing IAT and the disadvantages of worse metabolic performance is indispensable.

5. Complications

The 30-day mortality for IAT post-TP is 5% and it is comparable to the rate of TP without IAT.[7] Factors associated with increased risk of death following TP-IAT are: older age, higher basal transaminase levels, malignant neoplasia, lesions localized in the head of pancreas, and large extent pancreatectomy.[27] TP-IAT is considered a safe procedure with rate of postoperative morbidity ranging from 15% to 65% according to the centers where it is performed,[10] with obesity and fluid-electrolyte disturbances as the most significant risk factors of in-hospital sickness.[72] Although pancreatectomy per se is the most significant etiology for the arise of complications following IAT, additional risks are associated with the intraportal infusion of the islet preparation. The most relevant intraoperative complications secondary to intraportal infusion of pancreatic tissue are elevation of portal pressures, PVT, and bleeding. Islet volume and purity are the causative agents.[1]

Bleeding is a relevant complication, and an underling cause is high tissue volume infusion.[19]

Postoperative reactive thrombocytosis (RT) (platelets ≥ 500 K/μL) has been diagnosed in patients following TP-IAT, it is partially attributed to splenectomy. In the postoperative stage, RT can cause PVT, thus, heparin is infused to reduce the thrombotic complication risk.[1]

Minor complications linked to the surgical operation are wound infections requiring surgical debridement, intraabdominal infection requiring reoperation, anastomotic biliary leak, enteric leak, bowel obstruction, omental infarction, bowel ischemia, delayed reconstruction because of bowel edema, gastrointestinal tract perforation, liver abscess requiring long-term antibiotic treatment.[2] Moreover, nausea, vomiting, poor oral intake, and constipation are common problems present both in patients with CP and in patients after TP-IAT.[2] Regarding infrequent

complications, in 2015, Bellin et al. described a case of autoimmune-mediated β-cell failure following TP-IAT.[1,73]

The underlying disease process of CP and the extend of surgery contribute to the complications risk. The state of inflammation the patients are experiencing make them more susceptible to thrombosis upon islets infusion.[19]

Lastly, when the islet cells are directly exposed to plasma, a chain of events start which is called IBMIR. This condition activates the complement and the coagulation cascade, with an increased production of tissue factor. Studies demonstrated that IBMIR favors the occurrence of PVT, together with islets apoptosis and blunting of graft function.[46] The identification and characterization of the pathogenesis of IBMIR by doctors William Bennet, Carl G. Groth, Olle Korsgren and *Coll.* at the Karolinska institute conducted to the application of anti-inflammatory approaches to enhance islet engraftment and persistence, including the introduction of the anti-inflammatory treatment targeting CXCR1/2 by doctor Piemonti and *Coll.* at the San Raffaele Institute.[61,74]

5.1 Portal Vein Thrombosis

PVT incidence after TP-IAT is about 5%. In most cases, the left portal vein is involved. As it has been elucidated by the study conducted by JJ Wilhelm et al.[19] some factors lead to a greater increase in change in portal pressure (ΔPP), they include female gender, IEQ and tissue volume (TV) transplanted. Among them, the most significant independent predictor of ΔPP is the total tissue volume per kg body weight (TV/kg) infused intraportally. By contrast, decrease in ΔPP is related to size index (IEQ/IPN), height, weight, body surface area, liver volume, pancreas fibrosis severity, and islet concentration (IEQ/cc tissue).[19] TV/kg and ΔPP are linked to increase in complication rate, however, ΔPP is clinically more relevant. PVT occurrence increases above the suggested cut-point of 26 cmH_2O, which in turn depends on the age as well. The beforementioned ΔPP appears to be more likely when the total TV >0.25 cc/kg.[19] The multivariate regression modeling reveals

two factors independently associated with PVTs: increased portal pressure and patient age.[19]

In conclusion, based on the obtained data to prevent the occurrence of PVT, the recommended indications include the infusion of TV <0.25 cc/kg and the halt of intraportal infusion, at least temporarily, if the ΔPP> 25 cmH2O.[19] TV and ΔPP thresholds to aid in decision-making are not agreed worldwide, other islet transplant centers suggest cut-offs that range from 20 to 31 cmH_2O or 5-10 cc of TV.

Risk of PVT and bleeding are inversely correlated and greatly dependent on the coagulation management in the peri- and post-transplant periods. The difference of the aggressiveness of anticoagulation generates inter-center and inter-surgeon variation in the complication rates.

The pediatric population presents surgical challenges and theoretical higher risk of PVT because of smaller-caliber vasculature.[42]

5.1.1 Prophylaxis

PVT risk reduction is obtained by administering heparin to the patient prior and during the infusion of the islet preparation, and by providing postoperative pharmacologic prophylaxis at high dose.[46] The prophylaxis scheme is variable among centers and the medical options are enoxaparin 40 mg subcutaneously twice daily for 7 days, or unfractionated heparin (UFH) as continuous infusion (~5 units/kg/h) until postoperative days 3-5 followed by subcutaneous enoxaparin through day 7. It was proven that anticoagulation therapy did not influence PVT risk.[1] Enoxaparin, which is a low molecular weight heparin (LMWH) has a more predictable pharmacokinetic profile than UFH, and data show it is safer in oncology patients, whereas UFH is the choice for patients with renal insufficiency. Anticoagulation treatment option, UFH or enoxaparin, is not associated with a difference in PVT development, neither graft function does, suggesting that either strategy is optimal as prophylaxis after TP-IAT.[46]

In the context of non-PVT thrombotic complications, enoxaparin patients develop a greater number of thrombotic conditions including provoked line-associated deep vein thrombosis (DVT), nonocclusive femoral DVT, and pulmonary embolism; however, provoked DVTs are more likely to be dependent on the instrumentation rather than the prophylaxis regimen.[46]

When portal pressures exceed the threshold or the flow is severely reduced, infusion is stopped for 10-30 minutes, it can be restarted if pressure recovers, otherwise the remaining islets can be placed in the peritoneal cavity or submucosa.[19]

5.1.2 Treatment

Treatment of PVT typically is weight-based LMWH twice daily,[27] or UFH drip until Coumadin can be started,[46] or immediate coumadin administration.[19] Anticoagulation regimen is stopped once resolution is observable on US or at 3-6 months based on local protocols. Occlusive portal vein thromboses are removed via thrombectomy and intravascular tissue plasminogen activator.[46] The extremely reassuring consideration is that PVT is not associated with sequelae, evidence suggests that there are no differences in graft function at six months or one year between patients who had developed PVT and who had not.[46]

6. Monitoring and Follow-up

6.1 Perioperative Monitoring

Autologous transplantation inevitably causes loss of islet mass and subsequent decline in β-cell function, it has been estimated that 30% to 60% of the infused islets are lost. Infused islets are exposed to mechanical, osmotic, hyperglycemic, and hypoxic stress, leading to up-regulation of pro-apoptotic pathways, and full function resumption is delayed.[2] At the islet infusion time, the cells and surface tissue factor are exposed to the blood and it leads to a procoagulatory cascade through the increase in thrombin-antithrombin complexes and reduction in the number of platelets 3 hours after the procedure; followed by an increase in proinflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) in the first week after islet infusion[2] which accelerate injury to the cells.[69]

Islets necessity some time for engraftment, up to 6 or 12 months in some patients, and strict glucose control is necessary to prevent toxic hyperglycemia. Therefore, intensive exogenous insulin therapy starts immediately after transplantation and it is maintained for at least 3 months to relieve β-cell functional stress, initially by continuous infusion, followed by transition to subcutaneous administration.[1,39] An attempt to wean off insulin is done when blood glucose levels are in a target range (FBG <125 mg/dL; post-prandial <180 mg/dL; and HbA1c ≤ 6.5%); if these parameters are not met, the patients are maintained on insulin.[6] Post-operatively, Dextran 40 is maintained for 48 hours, after it is substituted with acetylsalicylic acid (ASA) 2 mg/kg.[29]

Additional patient monitoring is represented by serial assessment of portal vein patency through Doppler US at day 1, 15, and 30 when intraportal islet infusion is performed.[17]

An interesting area of research is the real-time visualization of early engraftment of islets by the use of positron-emission tomography (PET)-CT scan. The protocol

requires that a portion of the islet equivalents internalize fluorodeoxyglucose (F-FDG) immediately before transplantation. This technique would allow the simultaneous documentation of islets survival and distribution.[75]

6.2 β-Cell Function Following IAT

The assessment of the functional status of the transplanted islets varies among different protocols. The immediate postoperative islet function can be ascertained by detection of C-peptide level (defined as positive for levels ≥0.3 ng/mL), HbA1c, glycemia, average daily insulin requirements and basal (fasting) and 10-to-120-min time course of glucose, C-peptide and insulin levels during arginine test and/or MMTT, beta-score, and transplant estimated function.[17] The International Islet Transplant Registry defines Islet Cell Graft Failure according to C-peptide value, in particular when level is below 0.3 ng/mL.[76] Higher C-peptide levels denote greater probability of graft function and adequate metabolic control.[77] Furthermore, the likelihood of insulin independence correlates with post-transplant C-peptide to glucose ratio 1 month after the procedure.[7]

In the study[53] postoperative islet function is determined through OGTT and 1-mg glucagon stimulation test (GST) 1 week after IAT after overnight fasting and insulin administration cessation for at least 12 hours. Additional follow-up of islet function is performed by using 75-g OGTT and/or 1-mg GST at 1,3, 6, and 12 months, and annually since the transplantation. The metabolic parameters for the evaluation of postoperative glycemic index after IAT in the Korean study[53] are the HOMA-β and insulinogenic index (INSindex). HOMA-β assays approximate β-cell function and insulin resistance, while INSindex measures early-phase insulin secretion.[53]

The BETA-2 score is among the various β-cell function markers checked in patients following TP-IAT to control β-cell function and it represents the strongest positive correlation to β-cell measurements. The score is obtained from the patient's daily insulin requirements, HbA1c, fasting plasma glucose level, stimulated and

fasting C-peptide levels. When the score indicates 16 or above, it precisely predicts insulin independence post-IAT.[69] BETA-2 score and the dose of transplanted islets together predict insulin independence and HbA1c control after TP-IAT with 74.1% accuracy.[78]

According to some studies, β-cell function can deteriorate from postoperative inflammatory reaction; in agreement with this hypothesis, anti-inflammatory drugs should decrease the risk of this complication; a possible therapeutic approach can be the combination of etanercept and anakinra (ANA + ETA).[1] Etanercept is a tumor necrosis factor-α (TNF-α) inhibitor which can determine the short-circuit of the innate inflammatory reaction. Etanercept efficiently optimizes early islet engraftment, but long-term benefit is still ambiguous.[79] Currently the use of hydroxychloroquine is under investigation to improve β-cell functionality.[69] Post-transplant β-cell dysfunction can be recognized by a decline in proinsulin processing capacity, demonstrated by reductions in insulin-to-proinsulin ratios in patients with normal or near-normal glycemic control.[80]

6.3 Long-term Metabolic Outcome Assessment

The Leicester experience demonstrated that long-term islet graft function following TP-IAT is preserved in 10-year follow up. The unpredictable graft function is likely to be due to numerous factors including IEQ/kg, alcohol abuse, and CP duration; however, the research of consensus approach for the assessment of long-term metabolic function following TP-IAT has been unsuccessful; thus different centers utilize their own programs.[81]

Indicator of prolonged glycemic control is HbA1c, and in the long-term follow-up, insulin-independent patients show a lower median HbA1c value then insulin-dependent patients.[77]

Insulin-independence, defined as C-peptide positivity, and goal glycemic control are more consistently achieved in those recipients with a moderate to high islet mass transplanted. However, even when <2500 IEQ/Kg have been transplanted, 71% of

patients maintained a mean HbA1c <7%. Islets autograft are more durable than islet allografts, and twice as many IEQ/kg are needed for an allograft to induce insulin-independence or C-peptide positivity than for an autograft.[7]

1 year after IAT, approximately 30-40% of patients can withdraw entirely from insulin therapy; however, diabetes lifelong monitoring is required.[8]

Basal insulin requirement for 72 hours after TP-IAT is a predictive value for long-term metabolic outcomes, this parameter is useful to identify patients who need aggressive DM education and strict metabolic follow-up.[82]

The prolonged efficacy and durability of TP-IAT is achievable both using an on-site and an off-site islet isolation laboratory, it allows to widen the numbers of centers performing TP-IAT.[77]

In the Milan Protocol study, the long-term metabolic outcome has been evaluated through the modified Igls criteria validated through the arginine test and MMTT. The arginine test includes the administration of 30 g of arginine hydrochloride for 30 min under fasting conditions, following overnight withdrawal of insulin therapy. Subsequently, blood samples are collected to measure insulin, glucose, and C-peptide concentrations at the baseline, and at 5, 10, 20, 30, 40, 50, 60, 90, and 120 min. MMTT was performed using a test meal of 250 kcal. Intravenous arginine induces simultaneous insulin, C-peptide, and glucagon responses, which are surrogate markers of islets mass in IAT recipients since arginine test is a predictor of β-cell mass.[11]

Arginine test and MMTT provide esteemed standardized factors for assessing IAT success, including measurements of stimulated insulin and C-peptide secretion.[27]

6.3.1 Modified Igls criteria

Igls criteria provide a consensus functional and clinical definition for β-cell replacement therapy outcomes for diabetes treatment. These criteria have been developed in occasion of the workshop held in Igls (Austria) in January 2017 by the *International Pancreas and Islet Transplant Association* (IPITA) and the *European Pancreas and Islet Transplant Association* (EPITA). The criteria allow comparison among variable β-cell replacement techniques according to the clinical outcome and on individual bases for assessment of graft function over time. The Igls criteria are relevant for the process of standardization of the outcomes of β-cell replacement therapy.(83)

Piemonti et al. adopted the modified Igls criteria to evaluate the long-term metabolic outcomes of IAT procedures performed in accordance with the Milan protocol.(27) Specifically, revised Igls criteria categorize β-cell graft function into 4 groups: optimal, good, marginal, and failed, based on HbA1c, severe hypoglycemia events, insulin requirements, and C-peptide level. Based on fasting C-peptide level, the threshold between good and marginal function has been set at 0.5 ng/mL, between marginal function and failure at 0.3 ng/mL. Over time a gradual decline of the β-cell function has been documented.(27)

Table I - Modified Igls criteria(27)

Modified Igls criteria for metabolic classification of IAT

	HbA1c	SHE (per year)[a]	Insulin dose	Fasting C-peptide[b]
Optimal	<6.5%	None	0 U/kg/d	>0.5 ng/mL
Good	<7%	None	<0.5 U/kg/d	>0.5 ng/mL
Marginal	≥7%	≥1	≥0.5 U/kg/d	>0.3 ng/mL
Failed	–	–	–	≤0.3 ng/m

HbA1c, glycated hemoglobin; IAT, islet autotransplantation; SHE, severe hypoglycemia event.
[a]Any occurrence in the past year of hypoglycemia resulting in loss of consciousness or seizure.
[b]For the assessment of fasting C-peptide, we adapted the original Igls criterion by considering the threshold for stimulated C-peptide.

6.4 Follow-up

Lifelong monitoring for DM and IAT complications, mainly hyperglycemia and hypoglycemia, must be performed at least annually, it includes self-monitored blood sugar and measurement of FBG, and HbA1c through stimulatory tests to

control islet function over time. Endocrine insufficiency monitoring is coupled with nutritional supervising which includes the assessment of steatorrhea, weight maintenance, and fat-soluble vitamin deficiencies, since life-long PERT is required.[8] Postoperative screening for T3cDM is challenging, HbA1c does not reflect the acute changes in glycemic control postpancreatectomy, while insulin and C-peptide have been defined as indicators of insulin deficiency by multiple studies.[36] Follow-up appointments are scheduled at 1, 3, 6, and 12 months, and then annually after the index surgery.[27,57]

When pancreatic resections are performed because of the presence of a malignancy, CT scan and blood tumor markers are checked every 3 or 6 months.[26]

7. IAT Outcomes

Regarding endocrine function after transplantation, the most recent systematic review reports an insulin independence rate of approximately 30% in the adult population and 50% in the pediatric population. Furthermore, the studies underlies that great improvement in glycemic control is achieved by the majority of insulin-dependent patients, as defined by lower HbA1c levels in the postoperative period.[84]

The Milan Protocol classified different possible outcomes: insulin independence, partial graft function, and graft loss. Insulin independence is defined as no need for exogenous insulin and adequate glycemic control (i.e., HbA1c < 7%, fasting glucose levels <140 mg/dL >3 times per week, and 2-h postprandial levels <180 mg/dL >4 times per week). Partial graft function is characterized by fasting C-peptide level ≥0.2 ng/mL, need for exogenous insulin, or inadequate glycemic control (i.e. HbA1c ≥ 7%, fasting glucose levels ≥140 mg/dL >3 times per week, and 2-h postprandial levels ≥180 mg/dL >4 times per week). Graft loss includes "primary nonfunction" (i.e. fasting C-peptide level <0.2 ng/mL after islet infusion) and "complete graft loss" (initial increase in fasting C-peptide levels followed by a decrease to <0.2 ng/mL).[26]

In the Minnesota series, at 3-year follow-up, one-third of patients achieved insulin independence, one-third have partial graft function defined by positive blood C-peptide. Insulin supplementation is necessary in those who achieved only partial graft function; however, the overall glucose control is improved.[39]

The paper[6] found 18 years as the longest documented duration of insulin independence, while partial function, after being insulin-independent for 16 years, is 22 years.

The 36-item Short Form (SF-36) Integrated Survey shows a relevant improvement in every health-related quality of life (HRQOL) scale measured after TP-IAT. The failure of narcotic withdrawal after the procedure is not equal to

operation failure since QoL improves in those patients as well. QoL improves regardless of whether the patients were insulin-independent or insulin-dependent, although it was more significant in insulin-independent patients, demonstrating the value of preserving β-cell mass.[6] A relevant point is that although IAT post-TP does not prevent the occurrence of DM, it may contribute to fairly stable glycemic control and the prevention of DM-associated complications;[7] the accomplishment of TP-IAT does not equal inescapably to insulin independence, but to the improvement in QoL, and QoL recovers regardless the need for insulin supplement after the procedure.[10,85] In summary, offering IAT after TP is successful in improving QoL better than TP and exogenous insulin administration.[86]

AIM OF THE STUDY

The primary aim of the present study is to evaluate the current practices and utilization of IAT across international expert centers. The findings will be used to develop the first international standard of care practice for IAT. This standard of care will encompass indications for IAT, technical aspects of pancreas processing, islet isolation and purification, as well as the location and timing of islet infusion.

Despite nearly five decades of clinical practice, consensus guidelines for IAT remain lacking because of its limited application. Nevertheless, IAT is a promising procedure in the field of pancreatic pathology, offering numerous potential benefits. It could represent a paradigm shift in pancreatic surgery if standardized protocol was established among expert centers, and if the number of centers performing the procedure were expanded.

MATERIALS AND METHODS

A research group from the University of Padua, in collaboration with the Leiden University Medical Center (LUMC), has developed a standard of care survey, which has been distributed among IAT expert centers worldwide. The survey is endorsed by the EPITA. The survey consists of two sections. The first section, coordinated by the University of Padua group, investigates general variations in IAT practices with the objective of identifying best-practice guidelines. The second section addresses specifically the management of pancreatic calcifications during IAT. The findings from each section will be analyzed and presented in separate research publications. In Europe, EPITA facilitated the dissemination of the survey, while the study group from Leiden University facilitated outreach worldwide through their network of contacts. The questionnaire was created using Microsoft Forms and distributed via email. The survey was open for response from April 30th, 2025 to May 25th, 2025. Subsequently, the analysis of the collected results has been conducted.

The survey includes questions aimed at evaluating current IAT indications, pancreas processing methods, islet purification technique, and protocols for islet infusion. A comprehensive literature review on IAT procedure was also conducted, which revealed significant variability and uncertainty in both indications and technical aspects among specialized centers. This study seeks to promote consistency in IAT process and to propose standardized best-practice guidelines.

1. Survey Questions

This thesis focuses on the first section of the survey, with the objective of gaining a deeper understanding of the current protocols and standards of care in IAT across various islet centers. The section of the survey of our interest can be split into two main sub-sections. The first portion gathers general information about the respondents and the institutions at which they practice, including:

1. Name of the respondent.
2. Name of the institution.
3. Country the institution is based.
4. Function of the respondent.
5. Is IAT performed after pancreatic surgery in your center?
    a. Yes
    b. No
6. Is the program funded by your government?
    a. Yes
    b. No
    c. Partly
    d. I do not know
7. In which year did the institution start performing IAT?
8. Is IAT performed in pediatric patients (<18 years), in adult patients (≥18 years), or both?
    a. Pediatric only
    b. Adult only
    c. Both
    d. I do not know
9. Average annual number of IAT procedures at the institution during the last year (2024), specify the number pediatric and adult cases.

The second portion investigates on the specific indications and procedure of IAT. The questions include:

1. IAT indications, the possible answers are:
    a. CP
    b. Hereditary pancreatitis with gene mutations
    c. Benign/borderline pancreatic tumours localized in the pancreas head (e.g., intraductal papillary mucinous neoplasms, neuroendocrine neoplasms)
    d. High-risk pancreatic resections with a high risk of post-operative anastomotic or stump leak

   e. Postoperative completion pancreatectomy
   f. Pancreatic trauma
   g. Other, specify
2. After the retrieval of the pancreas, is the pancreas processed in an on-site isolation islet laboratory or is the pancreas transferred to a reference specialized laboratory (remote processing)?
   a. On-site laboratory
   b. Reference specialized laboratory
   c. I do not know
3. Is a vascular flush performed prior to islet isolation?
   a. Yes
   b. No
   c. I do not know
4. Which vascular flush do you use?
5. On average, what is the mean ischemia time (in minutes) per IAT procedure?
6. Is the pancreas perfused with collagenase in the operating room or in specialized laboratory?
   a. Operating room
   b. Reference specialized laboratory
   c. I do not know
7. Does the local protocol include islets purification for IAT?
   a. Yes
   b. No
   c. Both
   d. I do not know
8. When are the islets infused?
   a. During the primary surgery in the operating room
   b. After primary surgery (i.e., postoperative percutaneous approach the following day)
   c. I do not know
   d. Other (specify)

9. Is there a minimum and a maximum volume of islets infused in a single procedure?
    a. Yes
    b. No
    c. I do not know
10. What is the minimum volume of islets infused (in mL)?
11. What is the maximum volume of islets infused (in mL)?
12. Is heparin used during the process of islet infusion?
    a. Yes
    b. No
    c. I do not know
13. How do you calculate the dosage of heparin?
14. How is heparin delivered?
    a. In the islet infusion bag, added when preparing the transplantation product
    b. In the islet infusion bag, added right before the transplantation
    c. An intravenous bolus into the patient
    d. A combination
    e. I do not know
    f. Other
15. Please elaborate how heparin is delivered.
16. Is portal pressure monitored during the process of islet infusion?
    a. Yes
    b. No
    c. I do not know
17. What is the threshold of the portal pressure to stop the infusion (in mmHg)?
18. Is anticoagulation medication administered after the surgery?
    a. Yes
    b. No
    c. I do not know
19. Specify which type of anticoagulation therapy.

20. At your institution is spleen preserving TP-IAT performed, or is TP-IAT with splenectomy routinely performed?
    a. Spleen preserving TP-IAT
    b. TP-IAT with splenectomy
    c. Both
    d. I do not know
21. If splenectomy is performed, is any treatment provided for thrombocytosis after surgery? Specify which kind of treatment.

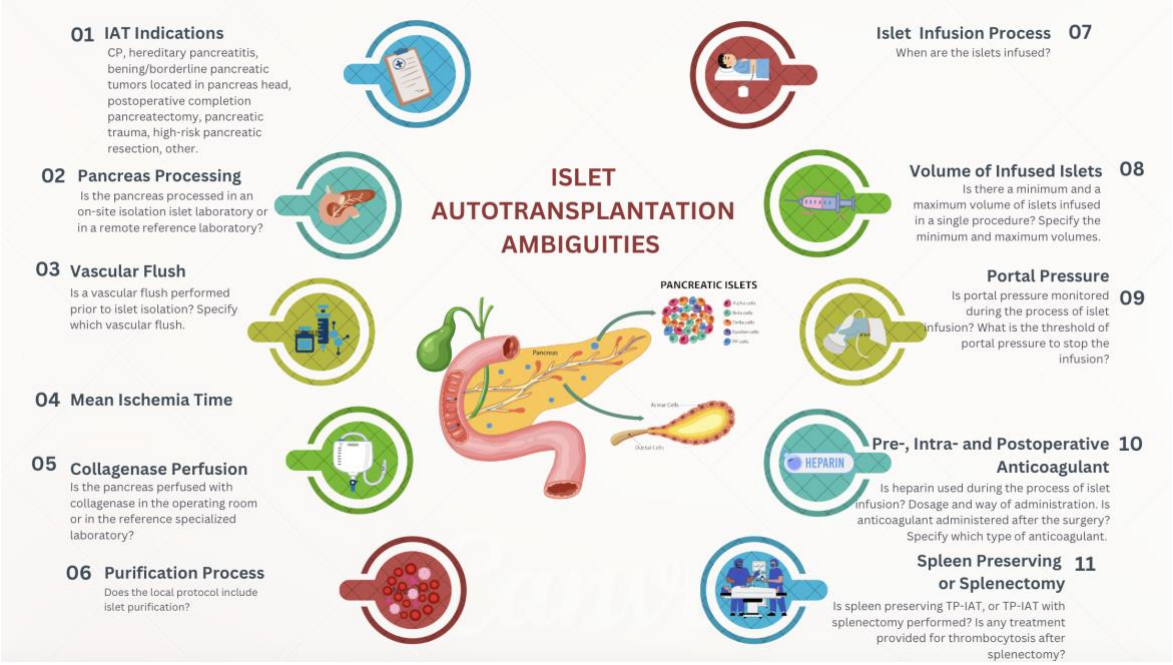

Figure 3 - Graphical representation of the ambiguities in IAT practice

RESULTS

In the present survey, the first of the two sub-sections collects general information about the respondents and the institution they represent, while the second one explores specific aspects of IAT procedure. The principal arguments we wanted to investigate regard the indications and the technical steps required to perform IAT procedure, with the aim of promoting a homogenous way of processing across different centers. The results highlight both areas of agreement and disagreement among respondents regarding various aspects of the procedure.

Between April 30th, 2025 and May 25th, 2025 fifteen islet transplantation centers completed the survey distributed via email.

The most active countries in terms of participation were the United States of America (USA) with five responses and Belgium with three responses; a graphic representation, *Figure 4,* of the data distribution can be found below, together with the complete list of participating countries and institutions. Among the fifteen responses received, one hospital declares that the IAT program following pancreatic surgery is not active, thus for the remaining questions, fourteen respondents are considered instead of fifteen.

Complete list of participating countries and their corresponding institutions:

- USA: five centers, University of Minnesota, University of Louisville/Norton Healthcare, University of Pennsylvania (UPenn), University of Chicago, and Hospital of the University of Pennsylvania
- Belgium: three centers, Vrije Universiteit Brussel, Universitair Ziekenhuis (UZ) Brussel, and LBCB Brussel.
- Netherlands: one center, LUMC
- Spain: one center, Barcelona Hospital Clinic
- Germany: one center, University Hospital “Carl Gustav Carus” Dresden

- Norway: one center, Oslo University Hospital
- Sweden: once center, NNCIT
- France: one center, University of Lille
- Italy: one center, University of Padua

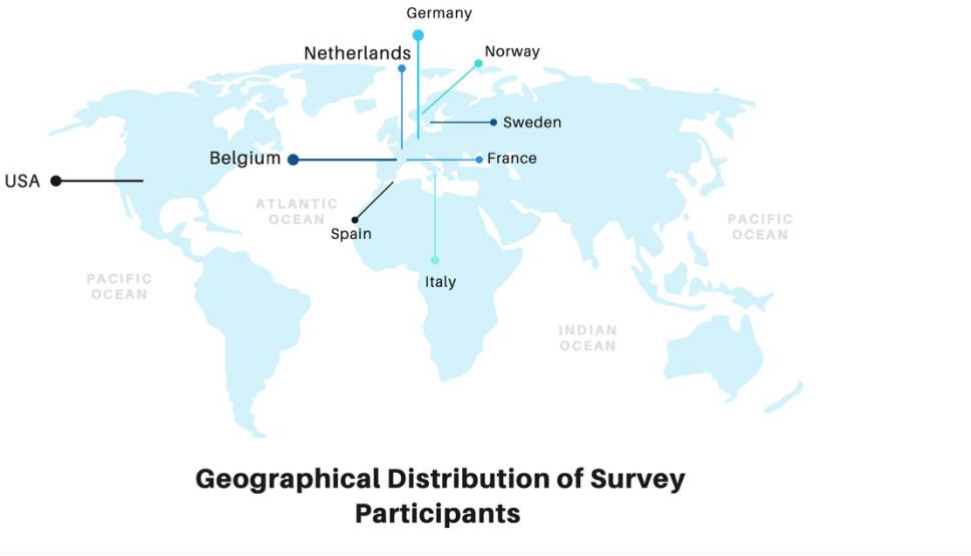

Figure 4 - Graphical representation of survey respondents

Professionals with varying roles completed the questionnaire, as illustrated in *Figure 5*. These comprehend transplant surgeons (n = 4), directors of the islet transplant program (n = 7), technicians of the islet facility (n = 2), and one clinical PhD researcher (n = 1).

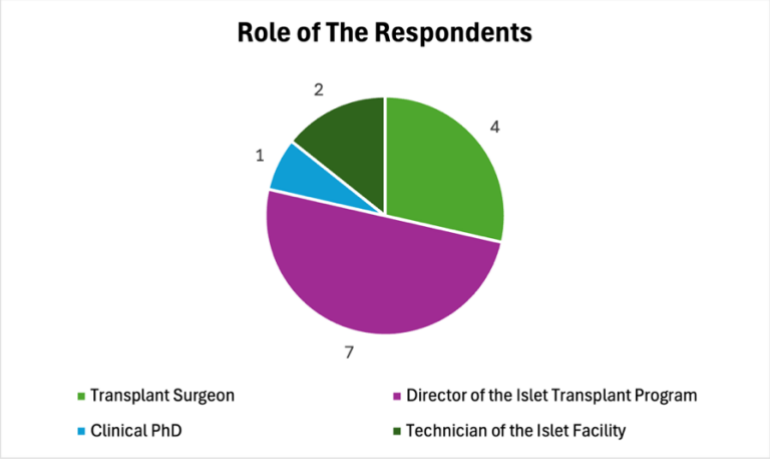

Figure 5 - Graph representing the roles of the respondents

There is notable variability in the reimbursement models for the transplant procedure. In 3 out of 14 cases, the program is completely funded by the national healthcare systems (Sweden, Italy, and France). In one case (Norway), only partial reimbursement is provided by the national health insurance. In contrast, in 8 instances, the entire cost is covered privately, often by the institution itself; this applies to LUMC and Dresden University Hospital.

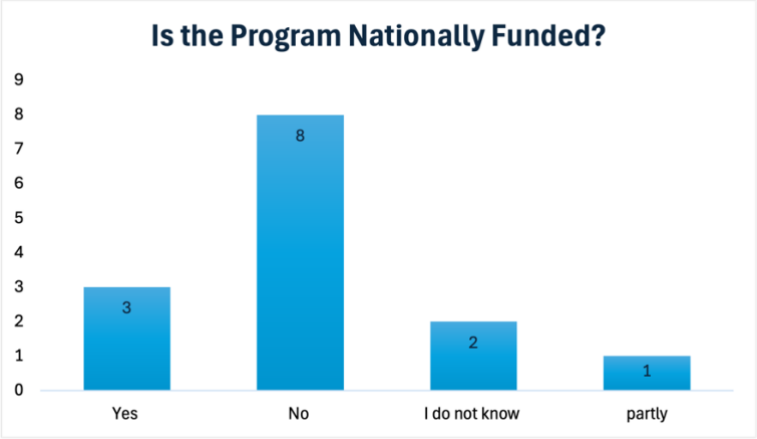

Figure 6 - National funding status of transplant program

Among the institutions from which responses were received, the timeframe during which the IAT procedure was conducted for the first time varies widely, ranging from 1977 to 2023. The leading institute is the University of Minnesota (USA), the earliest implementation was reported here in 1977, followed by the UPenn (USA) approximately a decade later. The most recent adherent is the University of Padua (Italy), which has initiated its IAT activity in 2023.

Complete list of universities and the relative starting year:

- 1977: 1 (University of Minnesota, USA)
- 1990: 1 (UPenn, USA)
- 2009: 1 (University of Chicago, USA)
- 2010: 1 (University of Lille, France)
- 2012: 2 (University Hospital “Carl Gustav Carus” Dresden, Germany and NNCIT, Sweden)

- 2014: 1 (LUMC, Netherlands)
- 2015: 2 (University of Louisville and Hospital of the University of Pennsylvania, USA)
- 2017: 1 (University of Oslo, Norway)
- 2018: 3 (UZ Brussel, LBCB Brussel, Vrije Universiteit Brussel, Belgium)
- 2023: 1 (University of Padua, Italy)

Overall, the participating IAT centers can be classified into three groups based on the number of IAT procedures performed in 2024:

a. < 5: 8 institutes
b. 5 – 10: 3 institutes
c. ≥ 10: 2 institutes

The *Table II* elucidates the average number of IAT procedures performed in the last year (2024) by each center, specifying the adult and pediatric cases. One center does not provide the average number of IAT procedures. The head of the line is captained by the University of Minnesota that completed 22 interventions. Furthermore, as it is possible to extrapolate from the results, the majority of facilities (n = 9) perform IAT exclusively in adult patients (≥18 years). The remaining 5 centers offer the procedure to both pediatric and adult population, regardless of the patient's age.

Table II - Table of average number of IAT performed in 2024 by each institute, specifically defining adult and pediatric cases

IAT Centers	*Number of IAT performed in 2024*	*Adult Cases*	*Pediatric Cases*
University of Minnesota	22	17	5

Dresden University Hospital	10	9	1
LUMC	7	7	0
University of Chicago	5	4	1
University of Lille	5	5	0
NNCIT	4	4	0
Oslo University Hospital	2-4	2-4	0
Hospital of the University of Pennsylvania	3	3	0
UPenn	3	3	0
University of Padua	3	3	0
Vrije Universiteit Brussel	1-5	1-5	0
UZ Brussel	1	1	0
LBCB – Brussel	1	1	0

The responses we gathered regarding the timing of the islet infusion from different centers highlight a predominance of postoperative transplantation (n = 7), most commonly performed on the day following surgery via a percutaneous approach, or on postoperative day 2 through a catheter placed intraportally from the supra-mesocolic area during the pancreatectomy. This latter approach is singularly reported in this study by one center only. Conversely, few centers (n = 5) infuse the

islet during the primary surgery in the OR, while the remaining institutions (n = 2) employ both techniques.

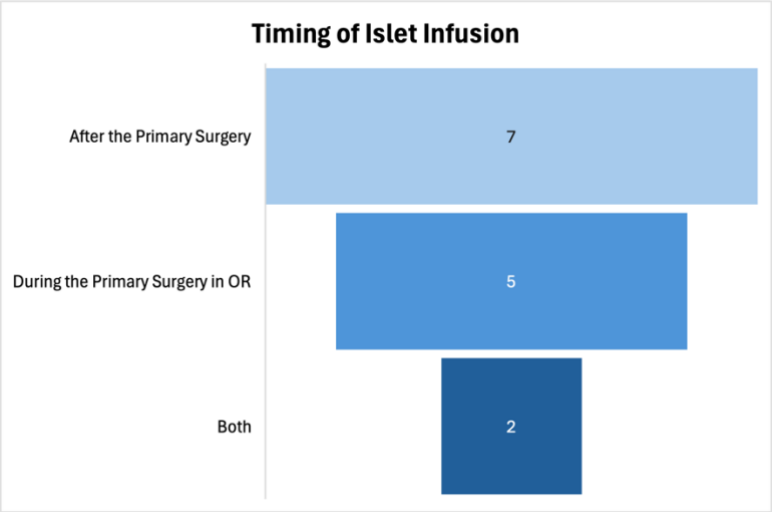

Figure 7 - Islet infusion timing

The responses regarding indications for IAT are revealed by the histogram shown in *Figure 8*. CP represents the principal indication, with unanimous agreement among all surveyed facilities (n = 14), similarly, hereditary pancreatitis with gene mutations is commonly treated with IAT in many of the interviewed institutions (n = 11). On the other hand, upon closer analysis, the category labeled "other" identifies a distinct indication, adenoma of the head of the pancreas, which is taken into consideration exclusively by one center.

Below the list of the possible indications, along with the number of facilities performing IAT for each:

- Chronic pancreatitis: 14
- Hereditary pancreatitis with gene mutations: 11
- Benign/borderline pancreatic tumours localized in the pancreas head (e.g., intraductal papillary mucinous neoplasms, neuroendocrine neoplasms): 2
- High-risk pancreatic resections with a high risk of postoperative anastomotic or stump leak: 5

- Postoperative completion pancreatectomy: 7
- Pancreatic trauma: 7
- Other: Adenocarcinoma of the area of the head of the pancreas that necessities PD with high-risk of POPF: 1

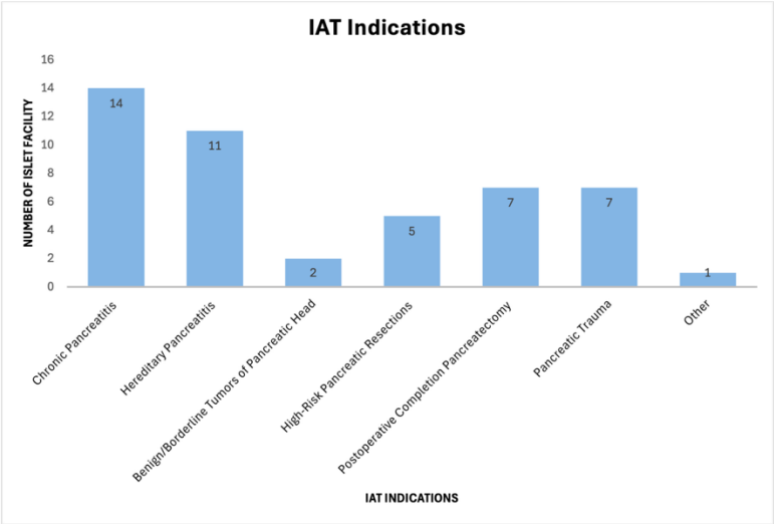

Figure 8 - Institutions performing IAT by indication

Remote islet processing is an area of great concern because it allows to increase the possibilities of performing IAT and the number of treatable patients. Out of the 14 facilities questioned, 9 possess an on-site isolation islet laboratory, while the remaining five rely on a specialized reference laboratory performing remote islet processing.

A relevant point of inhomogeneity concerns the type of vascular flush utilized prior to islet isolation. Although the majority of centers (n = 10) agree on the use of a vascular flush as preservation solution; a minority do not apply this technique (n = 2), while the remaining respondents are uncertain (n = 2). However, consensus on the specific type of flush solution remains unclear, as evidenced by the diversity of responses, the solutions the various institutions have reported to be using include: Institut Georges Lopez-1 (IGL-1) solution, Pulmolast, ringer-based solution (i.e., ringer acetate), Servator B, UW solution, and cold serum prior than IGL-1 flush. IGL-1 is part of the protocol of three institutes, ringer-based solution of two

institutes, as well as UW solution, while Pulmolast, Servator B, and cold serum are indicated only by one center.

Similarly, nearly the totality of expert centers (n = 13) performs pancreatic perfusion with collagenase in the reference specialized laboratory, while only one respondent was indeterminate regarding this procedure.

The *Table III* represents the mean ischemia time (in min) per IAT procedure. According to the data, the average mean ischemia time per IAT procedure is ≤ 60 min in three centers, between 60 and 120 min in three centers, between 120 and 240 min in three centers, and between 240 and 360 min in the remaining three centers. Among the reported values, the minimum mean ischemia time is 30 min and it has been indicated only by a single institution; on the other hand, the longest ischemia duration is 360 min, which has been reported by one institute as well. Two respondents do not know this value.

Table III - Mean ischemia time

Mean Ischemia Time	Number of Facility
≤ 60 min	3
60-120 min	3
120-240 min	3
240-360 min	3

There is variability regarding the presence of minimum and maximum volumes of islets infused reflected in the survey responses, in particular 6 out of 10 respondents identify the presence of thresholds, while 7 out of 10 declare no limit, and one respondent is uncertain. Among the centers that indicate a lower and an

upper limit of islets to be infused, different answers have been obtained, expressed as IEQ, mL, and mL/kg of recipient body weight. The minimum volume is specifically reported as 20,000 IEQ or 2-3 mL. On the other hand, the maximum volume of islets is cited as either 10-15 mL or 0.15 mL/kg of recipient weight.

The volume of islet infused is closely related to the possibility of performing purification of the digested tissue once the islet isolation step has occurred, in order to reduce the risk of complications secondary to infusion of a large volume of tissue into the portal vein. Islet purification is routinely performed by 64% (n = 9) of the respondents, while the remaining 36% (n = 5) do so only according to the volume of the digested tissue.

Concordance is observed on the use of heparin, with almost the totality of institutes (n = 13) employing it during the process of islet infusion. However, the results show that the dosage of heparin is not uniform, and it can be categorized into two classes:

a. Fixed dosage: variable answers are recorded, 2,000 Internation Unit (IU) (n = 1), 5,000 IU (n = 2), 2,500 IU as starting bolus before the infusion and 500 IU after every 50 mL of infused preparation (n = 1), and a fixed volume in the medium (n = 1).
b. Weight-based dosage: 70 IU/kg (n = 7). Additionally, another center employes the weight-based approach but it does not specify the amount of heparin per kg.

Furthermore, the method of heparin administration differs among centers. Approximately 43% (n = 6) of centers add heparin to the infusion bag, with half doing so at the time of transplantation preparation and the other half immediately before islet infusion. Among these centers, 4 out of 6 centers use a fixed dosage of heparin ranging from 2,000 IU to 5,000 IU. Only one center administer heparin intravenously directly inside the patient through a precise scheme: starting bolus of 2,500 IU and 500 IU every 50 mL of infusion. The last 43% (n = 6) employ both methods. Focusing on the latter modus, half of the total dose (35 IU/kg) is divided

among the infusion bags, and the rest (35 IU/kg) is delivered to the recipient intravenously; moreover, the dosage can be adjusted as clinically indicated.

The monitor of portal pressure is performed by 79% (n = 11) of the institutes, of the remaining 21%, 7% (n = 1) do not include this step in their protocol, and 14% (n = 2) of the respondents are unaware of whether this requirement is part of their institutional practice. Among the eleven centers that assess the pressure to determine when it is necessary to halt islet infusion in order to prevent IAT complications such as PVT; four centers define the threshold at 20 mmHg, and 3 centers at 25 mmHg. A single center discontinues the transplantation when the pressure increases by 5 mmHg from the baseline, while a different one when ΔPP increases by 50% relative to the initial value.

Anticoagulation therapy after the surgery is administered by 86% (n = 12) of the institutes, whereas the remaining 14% (n = 2) of the respondents report not knowing whether this is part of their practice.

Below the list of the anticoagulants included in the protocols and the number of centers reporting their use:

- LMWH (n = 5), specifically Fragmin, Fraxiparine, enoxaparin (Lovenox)
- Heparin (n = 6)
- UFH (n = 1)
- Low molecular weight dextran (LMWD) (n = 1)
- ASA (n = 2): one of these centers reports to administer ASA in combination with heparin

Spleen preservation remains a doubtful topic, as *Figure 9* visually unveils. The results of the survey reveal an approximately equal distribution of responses: 29% (n = 4) of centers perform Spleen-Preserving Total Pancreatectomy-Islet Autotransplantation (SPTP-IAT), 21% (n = 3) regularly execute TP-IAT with

splenectomy, 21% (n = 3) consider both approaches depending on the case, finally, the remaining 29% (n = 4) of the respondents are unsure.

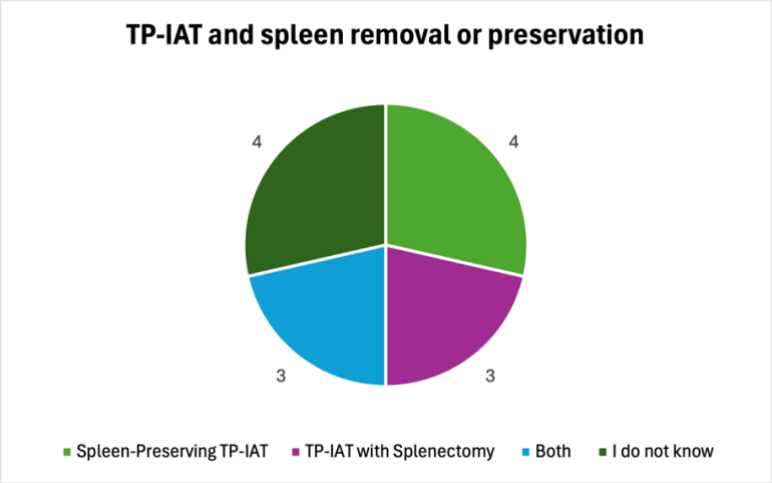

Figure 9 - Graph illustrating TP-IAT approaches: with or without splenectomy

Splenectomy may be complicated by postoperative thrombocytosis. Among the six institutes that routinely perform spleen removal, this condition is typically treated in the adult population with ASA, as more than half of the centers define (n = 4), principally when the platelet count exceeds $10^6/\mu L$. Additionally, one center specifies that pediatric patients are managed with a combination of ASA and hydroxyurea. On the other hand, two respondents are not certain about the presence of a therapy.

DISCUSSION

The variability of responses obtained from this international survey, despite its modest sample size, offers a clear insight into the absence of uniform and standardized guidelines for IAT procedure. This lack of consensus grants individual centers the autonomy to make decisions based on their own experience and expertise. Accordingly, the results can be categorized in two groups based on the degree of inter-institutional harmony on each specific topic: concordance and discordance. Positively, some topics of concordance are present mainly regarding the procedural intervention, but not only, among them, the data extracted from the present survey identify the presence and the use of on-site isolation islet laboratory, the average ischemia time for IAT procedure, the need of cold preservation solution for the storage and for the optimal maintenance of the organ, the pancreatic perfusion of collagenase in the reference specialized laboratory rather than in the OR, the intra-operative and post-operative anticoagulant administration, and finally the monitor of portal pressure. At the same time, the analysis of the responses reveled incongruence on main topics further highlighting the necessity of clearer IAT protocol. Disagreement ranges from the indications for IAT performance, with the only exception of CP, to the timing of islet infusion, the type of vascular flush utilized prior to islet processing, islet purification, minimum and maximum volume of islet infused in a single procedure, heparin dosage and techniques of administration, up to the decision to remove or preserve the spleen in combination with TP.

Existing literature is concordant in describing IAT procedure as a low-volume intervention,[3] performed in a limited number of specialized centers worldwide, mainly in the USA. The Collaborative Islet Transplant Registry (CITR) published its second report on autologous islet transplant (Auto-Itx) recipients in February 2022, covering procedures performed worldwide between the year 1999 and 2020. Data were submitted by 15 centers in North America, along with 5 European and Australian islet transplant facilities. In total, the registry documented 1,233 Auto-

Itx recipients, of these 1,123 recipients were in North America, 98 in Europe, and 12 in Australia. Additionally, 9 of the total recipients received a second auto-islet transplant. This information underlies the predominant role of North America centers in the field and the overall rarity of IAT practice.

The data collected in the present survey illustrate nicely its paucity of experience on a global scale. The promoting institutes of IAT procedure in the world panorama are the University of Minnesota (USA) and San Raffaele Institute in Milan (Italy) as evidenced by the volume of publications originating from these centers. At the University of Minnesota Medical Center, a total of 817 TP-IATs were performed between 1977 and 2021,[86] averaging approximately 19 procedures per year. In comparison, the Islet Process Facility at the San Raffaele Institute, serving as a central hub for collaborating centers in the country, collected 114 IAT procedures from 2008 to 2023, an average of approximately 23 procedures annually.[27] Eventually, based on these figures, a center performing between 10 and 20 procedures annually may reasonably be classified as a high-volume center. In this context, the data obtained through the present questionnaire suggest that, in addition to the University of Minnesota, the Dresden University can be considered a medium-to-high volume center. In the field of transplant surgery, IAT is still a niche intervention, it has been elucidated by the beforementioned paragraph which perfectly mirrors the paucity of responses registered through this survey. The scarcity of experience can explain the variability of protocols and their performance on a center-based according to the knowledge and expertise of the singular institution. Eventually, the data variability found in the literature is in line with the results of this international survey.

The indications for IAT remain a central topic of ongoing debate among IAT experts in the field, and contrast approaches are applied. Traditionally, IAT has been chiefly employed for the management of intractable pain due to CP, which historically represents the first reason for IAT performance and the most common indication for the procedure.[45] Accordingly, the totality of the respondents (n = 14) of the questionnaire report CP as an indication for IAT. However, increasing evidence supports the benefit of performing IAT in conjugation with TP or subtotal

pancreatic surgery for a broader number of indications beyond CP. Describing the indications in decrescent order according to their frequency of acceptance among centers, hereditary pancreatitis with gene mutations is cited by 11 centers, while pancreatic trauma is reported by 7 centers. These responses are congruent with the literature, since over the past decades there have been technical improvements and growing utilization of TP-IAT in patients though to be at risk of PDAC development, such in the case of hereditary pancreatitis.[52] The same tendency has been observed for pancreatic trauma. To the same extent, "postoperative completion pancreatectomy" is included in the protocol of 7 centers. Previous studies[52] have emphasized the importance of performing rescue pancreatectomy whenever feasible because of the marvelous metabolic benefit it provides. Moreover, 5 institutes report to perform IAT for high-risk pancreatic resections with a high risk of postoperative anastomotic or stump leak. Supporting literature demonstrates[54] that patients at high risk of POPF can be efficiently and safely treated via TP-IAT, and it may become a future standard of care. Contrarily, benign or borderline pancreatic tumors localized in the pancreas head are considered possible indications for undergoing IAT only by two centers. This limited use likely reflects concern over the possible infusion of occult malignant cells, reason why the adoption of this procedure has been kept limited in the oncologic setting.

The broadening of the indications for IAT procedure has been guided principally by the research group at San Raffaele Institute in Milan. In 2013 the research group held by Balzano medical doctor published a study on the largest cohort of patients who underwent TP or subtotal pancreatectomy with IAT for indications other than CP and RAP, precisely: completion pancreatectomy for severe complications after pancreatic surgery (i.e., grade C pancreatic fistula), TP for high-risk pancreatic stump, and extensive DP for benign/borderline neoplasm of the pancreatic body neck. On the other hand, multifocal pancreatic neoplasm diagnosed either on preoperative imaging or on intraoperative evaluation, multiple endocrine neoplasm, involvement of the pathologic pancreatic transection margin, and any pathological state that could interfere with safety IAT procedure, represent contraindications to TP-IAT. The study demonstrated the safety, feasibility and efficacy of IAT for

additional indications other than CP. Noteworthy, the decision to perform IAT necessities the evaluation of the risk-benefit ration in each individual case in the context of multidisciplinary discussion.[17] The Milan Protocol represents a notable advancement in the IAT field, aiming to extend its application to a wider patient population with both malignant and non-malignant pancreatic diseases.[26,27] The study published by Piemonti et al. in 2023 is the largest study for the evaluation of the extended IAT indications, however the heterogeneity of the patient population limits its applicability, further researches are necessary to optimize IAT indications panorama.[27] Nonetheless, outcomes remain divergent and inconclusive. The role of TP-IAT in patients with pancreatic and peripancreatic malignant neoplasms is still controversial, and IAT nowadays does not represent the standard of care in this setting.[1] This likely explains the limited number of institutions and islet isolation facilities offering IAT for these purposes.

Additionally, a single European center reports to perform IAT in case of adenocarcinoma of the head of the pancreas, including the duodenum and the ampullary region, that requires PD and with high-risk POPF. Balzano et al. confirmed the efficacy of preemptive TP-IAT as alternative to PD in selected patients at high risk for POPF. TP may avoid deterioration of QoL connected to POPF. This is significant for patients affected by adenocarcinoma, since a complex disease trajectory may preclude or defer the beginning of the oncological therapy, having an impact of the oncological history of the patient.[54]

IAT protocol lacks homogeneity in many of its phases. Clarification can be made in a stepwise fashion on the points of agreement and disagreement.

First of all, following the retrieval of the pancreas, nowadays the organ can be processed either in an on-site isolation islet laboratory or sent to a geographically remote specialized facility. Remote processing represents a great opportunity to widen IAT program, enabling hospitals without a dedicated isolation laboratory to offer IAT to the patients. This is particularly relevant considering that only approximately 20 specialized centers worldwide currently perform pancreatic resection and in-house islet isolation.[71] The results of the study indicate that the

majority of participating centers (n = 9) own their dedicated private isolation facilities. Upon closer analysis, it is noticeable that particularly medium-to-high volume centers rely on their own institutional facility. This element underlies the importance of a strict collaboration between the surgical team and laboratory technicians in maintaining high procedural standards, and it highlights the necessity of highly trained personnel. Furthermore, in many instances, the transplant surgeon is responsible for both the islet isolation and the subsequent islet infusion, emphasizing the multidisciplinary nature of the procedure.

Once pancreatectomy is completed, almost the totality of the respondents (n = 10) declares to perform in situ vascular perfusion of the organ with a vascular flush optimal for the conservation of the organ. However, a significant point of disagreement among centers concerns the type of vascular flush solution utilized prior to islet isolation. This discordance is accurately elucidated by the survey results, the responses collected are numerous including IGL-1 solution, Pulmolast, ringer-based solution (i.e., ringer acetate), Servator B, cold serum, and UW solution. UW solution is a colloid solution made of hydroxyethyl starch with a high potassium/sodium ratio. Historically, since the late 1980s, UW solution has been considered the gold standard mainly for abdominal organ conservation; for example, the pancreas. IGL-1 preservation solution contains polyethylene glycol, and it has an inversed potassium/sodium contents in respect to UW.[87] Ringer-based preservation solutions are usually adopted for organ storages. Ringer's solution is balanced, non-alkalizing, and isotonic. It is a crystalloid solution that contains physiologic concentrations of sodium, potassium, calcium, and chlorine. Its characterizing features, isotonicity and balance, minimize osmotic stress on tissues and cells allowing an optimal tissues and cells preservation. Servator B is a sterile solution having the same formula of UW solution, which is predominantly adopted during the process of pancreas, kidney, and liver retrieval, storage, and transport. Finally, Pulmolast is commonly used for lung preservation, however, a single center declares to adopt this flush for the pancreas.

The selection of an appropriate preservation solution is critical, and it affects transplant success, because of the high pancreatic vulnerability to ischemia-

reperfusion injury. Therefore, reaching a consensus on the most effective preservation solution is a priority. Literature on the preferred vascular flush adopted for IAT is scarce, while the information regarding pancreas transplant preservation solution is more easily available. A study[88] investigates and compares the effects of the four most commonly used preservation solutions in pancreas transplantation i.e., UW, IGL-1, Celsior (CS), and Histidine-Tryptophan-Ketoglutarate (HTK). While these solutions are not all cited in our survey responses, they are widely utilized in clinical practice. CS solution is a low viscosity crystalloid solution with low potassium/sodium ration, primarily used for the storage and maintenance of thoracic organs, since it was developed for heart preservation, and abdominal organs. HTK solution is characterized by a low viscosity and intense buffering of the extracellular space through histidine/histidine hydrochloride; its composition resembles that one of the intracellular fluid. Originally, HTK solution was intended for cardiac graft protection as well as CS solution. The outcome of the research discovered a trend towards better graft and patient survival in IGL-1 pancreas recipients mainly in the long-term; while in the first year similar graft survival rates were observed among the four solutions. Additionally, IGL-1 provides up to 17h of cold ischemia time.[88] A further study evaluated the impact of CS, IGL-1, and UW solutions on islet isolation outcome and early graft function. Eventually, it demonstrated that the results of isolation and transplantation do not depend on the preservation solution; IGL-1 is equivalent to UW and CS solutions for pancreas perfusion and storage before islet transplantation. No differences in total islet number, IEQ number, nor IEQ number per gram of pancreas among the three solutions have been identified. Equally, early graft function is not affected by the type of preservation solution.[87] Differently, the study conducted by Hubert et al. found that the composition of the preservation solution impacts islet recovery, and in particular the colloid-free CS solution may cause pancreatic edema which alters islet recovery by improper collagenase diffusion.[89] Although the beforementioned information, UW solution presents some disadvantages, firstly it must be kept at cold temperature until its use and it is expensive. Its high viscosity complicates the initial organ flush, moreover it has been observed that it inhibits collagenase digestion phase resulting in poor islet yields, in particular the main inhibitory

element is the low sodium and high potassium concentration. On the other hand, CS solution induces cell swelling and pancreas edema after 4 hours of cold storage.[90] Emerging clinical case series further support the safety and efficacy of IGL-1 in pancreas transplantation. However, as IGL-1 is a relatively recent addition to the preservation solution landscape and limited data are available, further large-scale multicenter investigations are warranted to declare the "holy grail" of the preservation solution.[91] Currently, available data indicate that the selection of vascular perfusion solution primarily relies on established practices and habits of individual centers.

Proceeding through the steps of IAT protocol, almost the totality of the surveyed institutes agrees on the place where the pancreas must be trimmed off from the excess of fat, and from the potential duodenum and spleen, decontaminated through antiseptic and lavage solutions, and eventually perfused with collagenase. Collagenase perfusion is a critical moment in the enzymatic digestion of the organ, which in combination with the gentle agitation of the Ricordi chamber ensures the optimal separation of islets from acinar tissue. The reference specialized laboratory is concordantly identified as the location where these phases take place, as previously mentioned the majority of centers own their in-house specialized islet laboratory. Since the islet product is vulnerable to contamination, the handling of it, it is safer in specialized structures called Facilities. These facilities must respect specific rules on *Good Manufacturing Practice* and on *Good Tissue Practice*, which guarantee high standards of quality, purity, potency, and safety through the processes of cellular product manufacture. The facility must respect some requisites: positive differential pressure in respect to the adjacent rooms, biological safety cabinets in which the level of air cleaning must respect the standard for particles of Class 100 [*International Standards Organization* (ISO) 5], HEPA-filter for air furniture, controlled temperature and air quality system, furthermore, the floor, the sealing and the walls must be non-porous and smooth to ensure sterility.

Cold ischemia time is defined as the interval between cross-clamping during pancreas retrieval and the placement of the cut organ into the Ricordi chamber for islet mechanical digestion process. A study conducted by the Diabetes Institute for

Immunology and Transplantation in the University of Minnesota[92] illustrates the disparity in cold ischemia duration between islet allotransplantation and autotransplantation. The former is characterized by a significantly longer mean cold ischemia time (5.8 ± 3.4 hrs) compared to islet autotransplantation (58 ± 24 min). Similarly, the report published by CITR identified a mean duration of cold ischemia time for IAT of 1.6 hrs based on the data recorded between 2019 and 2022. Data from the present survey indicate that the shortest mean cold ischemia time per IAT procedure is 30 min while the longest is 360 min, both numbers have been indicated by two institutions, while homogenous values are identified between these two extremes by the other centers. A more detailed analysis reveals that medium-to-high volume centers tend to report shorter cold ischemia durations. Notably, the University of Minnesota case with 22 IAT procedures and average cold ischemia time of 45 min, and the Dresden example with 10 IATs reports 60 min of cold ischemia duration. Furthermore, centers equipped with their on-site islet isolation facility describe shorter cold ischemia times, reflecting the logistical advantage of in-house laboratory.

Eventually, islet digestion can be followed by the purification of the post-digested tissue, however, as the results of the present survey declare, discordance is found on the regulation of this process, precisely on the threshold of post-digested volume that designates purification as compulsory. Islet purification is a step that according to the results of the present survey is performed routinely by 64% of the institutes (n = 9), while 36% of the centers (n = 5) undertake purification selectively, depending on the volume of the digested tissue. Notably, two respondents specified thresholds for initiating purification: one center recommends purification when the digested tissue volume exceeds 0.25 cc/kg (mL/kg), and another when the volume is above >10 mL. The results of the questionnaire indicate the absence of consensus regarding the optimal threshold of volume for purification. The decision to undergo purification or not is individualized and dependent on the post-digest volume, and it should be considered case-by-case rather than predetermined. This variability is a perfect representation of the current literature, which reports differing threshold values. For example, Sutherland and co-workers recommended to perform

purification when digest tissue exceeds 20 cm^3,[45] while Shinichi Matsumoto et al. when tissue volume is above 15-20 mL.[28] In light of these discrepancies, there is a clear need to establish a universally accepted threshold above which islet purification should be considered mandatory. The verdict would help to minimize the risk of portal pressure increase and the subsequent complications (i.e., PVT and bleeding) associated with it, when large tissue volume is infused in the portal system.

The ending phase of islet transplant consists in the infusion of the final product inside the patient. The timing of islet infusion differs among centers accordingly to clinical and logistic indications. The results of this international study highlights discordance on this issue. In particular, the infusion can be pursed intraoperatively, during the same anesthesia session of the primary surgery, while the patient is still in the OR, or postoperatively within 48 hours. Timing of islet infusion cannot be decided a priori, rather it should be evaluated on a case-by-case basis. The decision depends on several factors, including the patient's hemodynamic stability and overall conditions, the characteristics of the pancreas itself, principally quality and consistency, and the potential necessity of tissue purification and islet culture.

Islet culture is important for the appropriate assessment of islet viability, sterility, and QC. When the indication for pancreatic resection is a malignancy clear resection margin must be left. According to a San Raffaele Scientific Institute study, 1 cm of pancreatic remnant proximal to the pancreatic margin is resected and sent for frozen section examination to confirm negativity and to take decision on the appropriate candidacy for IAT; thus this step extends the pre-infusion time.[17] Moreover, purified islets can be incubated for 24-48 hours at 37°C in culture medium according to the pathology the patient is affected by. A study[51] found that culture time ranging from 1 hour to 14 hours has a favorable effect on endocrine outcome, as the higher prevalence of insulin independence among recipients of islet product that has been cultured for a longer period of time demonstrates.[51] Overall, as reported by the research group at San Raffaele Institute, the average islet culture time ranges from 14 to 16 hours.[27] However, due to limited data and lack of general consensus on the most appropriate culture duration, further studies are

warranted. Additionally, given the current concern regarding the potential infusion of malignant cells, a future frontier involves the analysis of islet preparation aliquots during culture phase, specifically for the detection of oncogenic mutations such as K-ras.[17]

The results of this international study reveals the complete absence of consensus on the volume (mL, IEQ, or mL/kg) of islet infused in a single procedure. Interestingly, half of the respondent facility (n = 7) denies the presence of reference thresholds below or above which transplantation is excluded. Only three centers define a lower limit below which IAT is not completed, they are: 2 mL, 3 mL, and 20,000 IEQ. The maximum value, which represents the opposite end of the spectrum, is identified as 10 mL, 15 mL, or 0.15 mL/kg by 5 institutes. Criteria determining the required volume of islet for infusion vary across centers. Examples from the literature can be consulted, in the study conducted by Piemonti et al., at San Raffaele Institute in Milan[27] the average volume of infused islet tissue intraportally was 1.5 mL (range 1-2.5 mL); differently, another research conducted by B. Jablonska and S. Mrowiec[1] identified as a threshold for safe intraportal IAT a islet tissue volume less than 0.25 cm^3/kg. Additionally, Balzano et al.,[17] in 2013 recommended to conclude islet infusion exclusively when more than 1,800 IEQ/kg are available. Emerging evidence suggests that the mass of islets transplanted, expressed as IEQ or IEQ/kg, is the predominant factor minimizing the effect of iatrogenic diabetes following pancreatectomies.[19] Rates of insulin independence range from 12% in patients receiving <2,500 IEQ/kg to 72% in subjects transfused >5,000 IEQ/kg. These scenarios underscore the absence of strict rules in the contest of islet volume infusion, and the necessity of additional multi-center studies to better correlate the results of IAT procedure to the quantity of islet volume infused.

The process of islet infusion is accompanied by heparin administration to prevent islet aggregation, thrombotic complications, and to enhance islet engraftment. This approach is supported by near-unanimous agreement among the survey respondents, with only one center that avoids its use. However, both the dosage and the mode of administration vary across institutions, reflecting different clinical experience and expertise. The addition of heparin to the islet preparation has been

shown to reduce the risk of tissue aggregation,[19] and its avoidance may increase the likelihood of complications such as PVT and DIC. Specifically, infusion of significant tissue volume into the portal vein causes portal pressure rise and blood flow reduction and the consequent PVT.[39]

Heparin administration is of paramount importance; however, despite its fundamental role, agreement on its optimal dosage is scarce. Survey responses are categorized into two classes based on heparin dosing strategies: (A) fixed dosage, (B) weight-balance dosage. Among centers employing a fixed heparin dose, the amount administered ranged from 2,000 IU to 5,000 IU. Instead, centers using weight-based dosing showed remarkable concordance, with nearly all reporting a dose of 70 IU/kg. Notably, protocols with fixed dosing typically administer heparin exclusively within the infusion bag, either immediately prior to transplantation or during the preparation of the transplant product. An exception to this approach is observed in the protocol of one hospital, which consists in a starting intravenous bolus of 2,500 IU administered before the first islet infusion, followed by an additional 500 IU bolus after every 500 mL of infused tissue. In contrast, centers utilizing weight-based dosing most commonly combine two methods: heparin administration within the transplantation bag and systemic intravenous infusion. Specifically, half of the total dose (35 IU/kg) is incorporated into the infusion bag, divided among multiple bags if necessary, and the remaining half is given as intravenous bolus to the recipient. Among these centers, two report administering heparin solely within the islet infusion bag, deviating from the combined approach. It is difficult to find uniform guide in the literature which defines the optimal dosage of heparin. Different examples exist which are extrapolated from several studies and they are in line with the classification mentioned in our study; (A) fixed dosage, (B) weight-balance dosage: 5,000 IU administered intravenously immediately before infusion,[53] 2,000 IU added to the islet preparation,[51] 5,000 IU added to the islet preparation,[93] 35 IU/kg up to 70 IU/kg if the infused tissue volume exceeds 5 mL added to the islet suspension prior to infusion.[94] A study[95] analyzed the intraoperative heparin dosing to optimize post-operative surgical outcomes since a dilemma challenges the medical staff; while PVT is the major complication

associated with islet infusion, postoperative bleeding can influence negatively the convalescence, and a balance between these extremes must be identified. The findings provided evidence that the administration of <60 IU/kg is optimal because it does not increase neither the risk of PVT nor the risk of postoperative hemorrhage. Upon heparin administration, it is appropriate to take into consideration hypercoagulability disorders that could affect the patient and adjust the therapy in a personalized manner.[96] It is essential for the medical personnel to tailor heparin dosage based on individual patient factors and institutional protocols to optimize IAT outcomes.

The additional requirement during islet infusion is the measurement of portal pressure, whose monitoring is of paramount importance. An increase in portal pressure, ΔPP, is directly associated with a higher risk of complications rates, in particular of PVT. Because of this reason, 11 out of 14 centers report to regularly monitor the pressure during the islet infusion. Literature reports a variety of portal pressure thresholds, ranging from 20 to 31 cmH_2O, as cut-off values for halting islet infusion,[19] specifically in the study[1] intraportal infusion is temporarily stopped when portal pressure exceeds 25 cm H_2O; or when portal hypertension (> 25-28 mmHg) is observed. If intraportal infusion cannot proceed, the remaining islet volume is dispersed into other sites.[97] These values are in line with the ones collected from the responses of the present survey.

The indication for anticoagulation extends beyond the pre-infusion phase to include the postoperative period to further reduce the risk of PVT and DVT, as confirmed by 86% (n = 12) of the institutes replying to the survey. Regarding the type of anticoagulant medication, heparin and its derivates play a central role. Specifically, standard heparin is reported to be used by 6 centers, LMWH by 5 centers (e.g., Fragmin, Fraxiparin, enoxaparin), UFH by one center, and LMWD by one center as well. Additionally, ASA is used by 2 centers. The findings collected through the survey underscore the variability in anticoagulation strategies employed during IAT. To underly the absence of uniform guidelines, surgeon preferences and postoperative bleeding risk determine the way of anticoagulant therapy

administration and different studies show alterative preferences in its management. Balzano study group,[51] defined that the patient must receive 40 mg of enoxaparin 6 hours after the end of the elective surgery and since then once daily for at least 30 days. Alternative but equivalent anticoagulation options are heparin drip prior to the end of surgery adjusted based on the portal pressure (5-10 U/kg/hr) and transition to enoxaparin at 0.5 mg/kg approximately one week after the transplant when duplex US shows absence of thrombosis.[19] Additionally, either the continuous infusion of UFH or the intermittent subcutaneous enoxaparin (LMWH) has been shown to reduce PVT risk equally. Specifically, LMWH is preferred for oncology patients, while UFH is more effective in patients affected by renal insufficiency, since enoxaparin relies on kidneys for its elimination.[46] In the Milan Procol study[17] DVT prophylaxis consists in LMWH according to risk stratification of the patients, normally 4,000 IU/day from day 0 to 28.

The last aspect we should mention is the possibility to remove the spleen simultaneously to pancreatic resection. Pancreatic resection and IAT with splenectomy or spleen-preservation should be considered case-by-case since the process is affected by multiple biases which can occur during the surgical procedure that can conditionate the removal or the maintenance of the spleen. The data collected from the survey show discordance among the respondents, 4 centers preserve the spleen when TP is conducted, 3 institutes perform splenectomy, 3 institutes consider both approaches, while 4 respondents are unsure about this step in their protocol.

First of all, it is necessary to elucidate that different surgical equipes may collaborate for the cure of same patient-case, thus the respondents may be unaware about the reasons that lead to the removal or the preservation of the spleen, because the surgeon who removed the pancreas can differ from the surgeon and technician who isolate the islets or the surgeon who infuse the final product. Furthermore, the different occupational role of the figures who completed the survey may have influenced the variety of responses. Moreover, the decision to retain or remove the spleen depends on different factors, extending from the individual anatomy of the

patient to the pathology the patient is affected to, to the quality of the splenic tissue, and to the surgical indications.

In some circumstances, the anatomic characteristics of the person are favorable to preserve the spleen on the short gastric vessels performing the Warshaw technique, vice-versa in other instances, for example when the splenic vessels are lesioned, splenectomy is mandatory.[39] The decision to attempt spleen preservation depends on the inflammatory milieu, adhesions, and calcifications surrounding the splenic vessels, with the intent to spare the spleen whenever technically feasible. The benefits achieved through the preservation of the splenic vessels have been analyzed by a research study.[58] They are the reduction or absence of gastric varices consequence of left-sided portal hypertension, and the minority of splenic infarction. At the same time, spleen-preserving procedure increases the surgical time because the preservation of splenic artery and vein necessities the meticulous and individual ligation of all the tributary vessels of the beforementioned vessels. It results in warm ischemia of the tail of the pancreas, where most islets reside, reason why ligation of the vascular branches near the pancreas tail is done lastly. The longer warm ischemia time to the islet tissue is in contrast with the fundamental theory according to which the pancreatic blood supply should be clamped at the end of the pancreatic resection just before the retrieval of the organ, in particular the gastroduodenal artery, together with the origin of the splenic artery and the termination of the splenic vein[6]. However, despite the prolongation of the warm ischemia time, TP-IAT with arterial and venous preservation of the spleen has not controversy on islet yield and glycemic control. Additionally, the same study compares the group of patients undergoing TP-IAT with splenectomy in respect to the patients in which the spleen has been preserved. The results reveal the absence of alarming differences in the hospital length of stay, median blood transfusion units, 30-days morbidity, and endocrine metabolic function between patients performing splenectomy and patients who don't. However, insulin requirement is better in SPTP patients. In conclusion, TP-IAT is technically feasible and it can be safely performed with spleen conservation;[58] however, it is necessary to notice that cases of splenic vein thrombosis have been reported in SPTP, which in turn

increases the risk of left-side portal hypertension, resulting in short gastric varices and late gastrointestinal tract bleeding or painful splenomegaly.[45]

In addition, there are oncologic indications that require splenectomy concurrently to TP to reduce the risk of local recurrence and to ensure clear oncologic margins; these include PDAC, PNET, and metastasis that invade the splenic hilum, or that involve adjacent lymph nodes. However, since pancreatic cancer is a rare indication for the execution of TP-IAT, few studies have investigated the feasibility and the outcomes of SPTP compared to TP with splenectomy. Conversely, spleen preserving DP can be pursued when benign of low-grade malignant tumors reside in pancreas body and tail.[98,99]

Moreover, when the splenic vessels are injured secondary to a trauma or a vascular compromise, splenectomy is mandatory.

Additionally, when CP presents with inflammation involving the splenic hilum or with other complications (i.e., pseudocysts or vascular thrombosis near the splenic hilum), the removal of the spleen is necessary.

Finally, DP is commonly associated with splenectomy since pancreas resection often disrupts splenic blood flow, however, recently different studies reveal the possibility to perform spleen-preserving DP safely, specifically spleen-preserving DP is achieved by either splenic vessel resection or preservation.[100]

In conclusion there are no real consensus on the need to perform spleen preservation. It is necessary to consider case-by-case, the primary intent should be spleen preservation to eliminate the fatal, although rare, complications of infections from encapsulated bacteria.[101]

Postoperative RT may follow splenectomy, in this scenario, among the six centers that possibly remove the spleen contemporarily to pancreatic resection, four centers administer ASA when platelets count is above $10^6/\mu L$. Additionally, one respondent specifies the childhood therapeutic procedure: combination of ASA and

hydroxyurea. RT may cause PVT in the postoperative outcome. Differently from the answers of this survey, the study[1] recommends heparin infusion aimed at reducing thrombotic complications.

CONCLUSIONS

This international survey clearly represents the significant paucity of information currently available in the literature regarding IAT procedure, and its heterogeneity. Moreover, it underscores the lack of unified, evidence-based guidelines on numerous technical aspects of the procedure, including elements of critical importance for the clinical outcomes. Nevertheless, areas of agreement have been identified, particularly regarding the need of vascular flushing, intra- and postoperative heparin administration, and portal pressure monitoring during islet infusion, offering hope that a comprehensive standardized protocol may be developed in the future. On the other side, considering the areas where less heterogeneity is identified, the urgent need for large-scale multicenter clinical studies aimed at analyzing the correlation between individual protocols and patient outcomes, rises. Such research is essential to determinate the most effective techniques, which could then serve as standardized models for broader implementation of the IAT practice.

Furthermore, CP still represents the primary indication for IAT procedure, and although the Milan Protocol has laid the groundwork for expanding its use to other pathological conditions, general consensus on these additional indications is still lacking. In particular, skepticism is present on the feasibility of IAT in oncological settings. Consequently, additional research is required to validate these expanded indications and to address concerns surrounding its application in cancer patients.

Lastly, the development of international guidelines is critical to standardize the entire IAT process, streamline its implementation, and facilitate broader adoption. The survey underscores the need for harmonization of procedural steps, especially concerning islet purification, islet infusion volume, and heparin dosage. As demonstrated in the literature and in the survey results, centralized islet processing in reference laboratories is a valuable, although not the preferable, alternative to on-site isolation islet facility, potentially enabling more institutions to offer IAT. The

ultimate aim should be to increase the global volume of IAT procedures worldwide, thereby increasing collective knowledge and improving clinical outcomes. Additionally, standardization would enable more robust comparison of results across centers, leading to stronger validation of best practices and achievement of higher-quality outcomes.

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