

Technical Aspects Involved in the Harvesting and Preservation of the Pancreas Used for Pancreatic Islet Allograft Transplantation

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Rezumat

Aspecte tehnice cu implicații în prelevarea și prezervarea pancreasului în vederea allotransplantului de insule pancreatice

În studiul prezent au fost analizate recoltările de pancreas efectuate în perioada 2007-2012, în vederea transplantului de pancreas integral sau de insule pancreatice. Pancreasul a fost preluat de Institutul Clinic Fundeni, iar procesul de izolare a insulelor pancreatice a fost efectuat în Laboratorul de Terapii Celulare din cadrul acestuia. În cadrul recoltărilor multiorgan, s-au efectuat 20 de prelevări în bloc duodeno-pancreatico-splenic. În 18 din cele 20 de cazuri, pancreasul recoltat a fost folosit pentru izolare de insule pancreatice, iar în celelalte 2 cazuri a fost transplantat integral ca transplant solitar de pancreas și respectiv transplant simultan de rinichi și pancreas. Vârsta donatorilor a fost între 12 și 61 ani, cu o valoare mediană de 35 ani. Donatorii au fost 9 femei și 11 bărbați. Cauzele de deces au fost trauma cerebrală (8), ruptura de anevrism (7) și accidentul vascular cerebral (5). Distribuția lotului donatorilor în funcție de grupul sanguin a fost următoarea: 11 donatori de grup A(II), 7 donatori de grup O(I) și 2 donatori de grup B(III). Indicele de masă corporală calculat pentru lotul de donatori a avut valori

între 15,6 și 27,8, cu o valoare mediană de 24,1. Valoarea mediană calculată a scorului de predicție a acceptabilității pancreasului donat (scorul Vinkers) a fost de 11. Timpul de ischemie rece a avut valori între 1,5 și 8 ore, cu o durată mediană de 5 ore.

Cuvinte cheie: prelevarea de pancreas, prezervarea pancreasului, transplantul de insule pancreatice, donator de pancreas, diabetul zaharat

Abstract

The pancreas procurement from brain-dead donors used for pancreatic islet isolation and transplantation, was analyzed between 2007-2012. The pancreas was transported to the Fundeni Clinical Institute and the islet isolation process was performed in the Cell Therapy Laboratory. There were 20 en bloc pancreas-duodenum-spleen procurement during multi-organ harvesting. Eighteen pancreata were used for islet isolation and two were used for whole pancreas transplantation. One pancreas was used for whole pancreas transplant alone and the other one was used for simultaneous pancreas and kidney transplantation. Donor age ranged between 12 and 61 years, with a median age of 35 years old. The donors were 9 females and 11 males. The causes of death were in 8 cases – brain injury, in 7 cases – aneurism rupture, and in 5 cases – stroke. The donors' blood group was A(II) in 11 cases, O(I) in 7 cases, and B(III) in 2 cases. The calculated BMI of the donors ranged between 15.6 and 27.8, with a median value of 24.1. The median calculated Vinkers score for our study group was 11. Cold ischemia time ranged between 1.5 and 8 hours, with a median value of 5 hours.

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Key words: pancreas procurement, pancreas preservation, pancreatic islet transplantation, pancreatic donor, diabetes mellitus

Introduction

Diabetes mellitus type 1 and 2 is a condition characterized by relative or absolute deficiency of beta cells insulin production related to body insulin needs. There is no formulation of exogenous insulin available to date, able to mimic the physiological nictemeral rhythms of this hormone. Correction of this deficiency can be optimally achieved through beta cell replacement by total pancreas transplantation or pancreatic islet transplantation (1).

Pancreatic islet transplantation is a safe and effective method, involving a simplified surgery intervention, with lower morbidity and quasi-null mortality comparative with whole organ transplantation. However the method is highly dependent on the quality of pancreatic islet isolation, which is a complex and costly process (2).

The main objective of islet transplantation is to obtain a better control of glycaemia and consecutively to prevent the complications of diabetes and increase the patients' quality of life (3). Although five years post transplantation, only 15% of transplanted patients remain insulin-independent, this method provides a good glucose homeostasis (4).

The main disadvantage of islet transplantation is the fact that, during the isolation process and after transplantation, an important number of the islets is lost, which determines the use of multiple donors in order to achieve an insulin-free status (5). The standard donor-to-recipient ratio for islet transplantation is presently 2–4 : 1, in contrast to a ratio of 1 : 1 for whole-pancreas transplantation. A human pancreas contains $0.3\text{--}1.5 \times 10^6$ islets per pancreas of which only 30–50% can be isolated using current islet isolation protocols with a viability of 65% following isolation (6).

Given the shortage of pancreatic donors, there is a limited number of candidates who could benefit from pancreatic islet transplantation. These candidates for islet transplantation still remain at risks of immunosuppressant regimens (i.e. increased susceptibility to infection or neoplasia, deterioration of renal function), which are considered unacceptable by some authors (7, 8). Moreover, there is no available marker of islet rejection for proper monitoring of the graft function and immunosuppressive response (9).

Nevertheless, a better selection and matching of both donor and recipient, and an improved technique of isolation, transplantation, and immunosuppression have led to encouraging results of islet transplantation harvested from only one donor, which give hope for substitution of the whole pancreas transplantation with islet transplantation (9). A recent study from the group of Minnesota has showed high rates of islet engraftment, with 50% insulin independence at 5 years (19), similar to vascularized pancreas transplantation

(20). Pancreatic islet allotransplantation can actually reverse the type 1 diabetes and has been used even with no need of immunosuppression, making it an elective treatment option for diabetic children (11).

Regarding the selection of the pancreatic donor, the organ allocation from brain-dead donor must be designed to meet two main objectives: to minimize morbidity and to serve a larger number of recipients (10). The two methods (vascularized pancreas and islet transplantation) are complementary rather than in competition, each of them having specific indications for certain patients (11).

The success of islet transplantation depends on both careful selection of the recipient, and high quality isolation procedure of the harvested pancreas.

Exogenous insulin requirements for the patient is the first important guide for the estimation of beta cell number required to obtain the posttransplant insulin-free status. The lower the daily insulin requirement is, the smaller the number of transplanted beta cells needed to achieve insulin-free status and the higher the chance of using only one single donor pancreas. A high daily insulin requirement means that a large number of beta cells is needed for transplantation, and thus more donors must be used. These last patients are better candidates for a whole pancreas transplantation (5). If the surgical risk is great, due to comorbidities, in high dose insulin-requiring patients, then the islet transplantation become the best option and it can be repeated as necessary in order to achieve an insulin-independent status.

The cornerstone of successful islet transplantation remains the quality of the harvested pancreas, which depends on the donor, but also the procurement, preservation and transport process.

The quality of harvested tissues depends on some factors related to the donor: donor's age, body mass index (BMI), cause of death, the use of vasopressors, hemodynamic stability, length of hospitalization, blood levels of glucose, transaminases, and creatinine.

The quality of pancreatic islet isolation is determined not only by the quality of harvested tissue but also by the experience of the team and protocol used for organ procurement, preservation, and transport and islet isolation.

The experience of the procurement team is based mainly on solid knowledge of anatomical variants of intraabdominal vascularization, with great importance in case of multiorgan procurement, and of harvesting technique. Advances in organ harvesting and preservation techniques have played an important role in improving the results of pancreas transplantation. Combined liver, kidney, and whole-organ pancreaticoduodenal retrieval can be safely performed in virtually all donors irrespective of vascular anomalies. The harvesting techniques used are adjusted to the anatomical vascular variants and hemodynamic status of the donor and require proper identification, dissection, and cannulation of the vascular pedicles. All these techniques mandate the usage of no touch dissection of the pancreas (12). No touch procurement of the pancreas is meant to avoid the capsule damage of the organ, which otherwise could result in fluid leakage during pancreatic perfusion with

enzymatic solutions. There are three surgical techniques which can be used for pancreas retrieval: the classic, intermediate, and fast technique. The fast technique is preferred in a hemodynamic unstable or non-heart-beating donor and also in liver-pancreas en bloc harvesting. The fast technique begins with infrarenal abdominal aorta cannulation. The classic technique requires the initial dissection of all the vascular pedicles. The intermediate technique was adopted in our clinic and implies the identification, without complete dissection, of vascular pedicles before infrarenal aorta cannulation. The choice of any of these techniques is meant to shorten the warm and cold ischemia time.

Materials and Methods

The pancreas procurement from brain-dead donors used for islet isolation and transplantation was analysed between 2007-2012. The isolation process was done in the Cell Therapy Laboratory, at the Fundeni Clinical Institute.

Our exclusion criteria of the potential cadaveric pancreatic donor are in accordance with the International Pancreas Transplant Registry (Table 1).

There were 20 en bloc pancreas-duodenal and spleen procurements during multiorgan harvesting. In two cases, a combined liver-pancreas harvesting was performed (Fig. 1). The indication for en bloc liver-pancreas explantation was the existence of an accessory right hepatic artery, originating from the superior mesenteric artery. An accurate organ separation was performed in these cases on back table.

Table 1. Exclusion criteria for a potential cadaveric pancreatic donor

- Age > 65 or < 5 years old
- Preexisting conditions, such as: positive hepatitis serology A,B or C; AIDS; positive HIV-I or HIV-II; HTLV-III; viral encephalitis; syphilis; Creutzfeldt-Jacob disease; rabies; active tuberculosis; diabetes mellitus type 1 and 2; neoplasia (except skin or primary brain tumors); acute systemic infection; dementia; pituitary growth hormone treatment
- Chronic alcohol abuse
- Recent intravenous drug abuse
- Significant history with increased risk of infectious or transmissible diseases
- Pancreatic trauma
- History of diabetes mellitus, acute necrotizing pancreatitis, chronic pancreatitis, or previous pancreatic surgery
- Significant intra-abdominal contamination
- Prolonged episode of hypotension which determines significant biochemical changes (blood levels of creatinine > 1.5 x N or blood levels of transaminase > 2 x N)
- Cardiac arrest > 5 min, with hemodynamic instability, uncorrected during the first 48 h, or with significant biochemical changes
- ICU stay > 7 days
- Sodium > 160 mmol/l
- Amylase > 380 U/l or Lipase > 480 U/l
- Noradrenaline > 0.05 microg/kgBW/min or Dobuta-/dopamine > 10 microg/kgBW/min

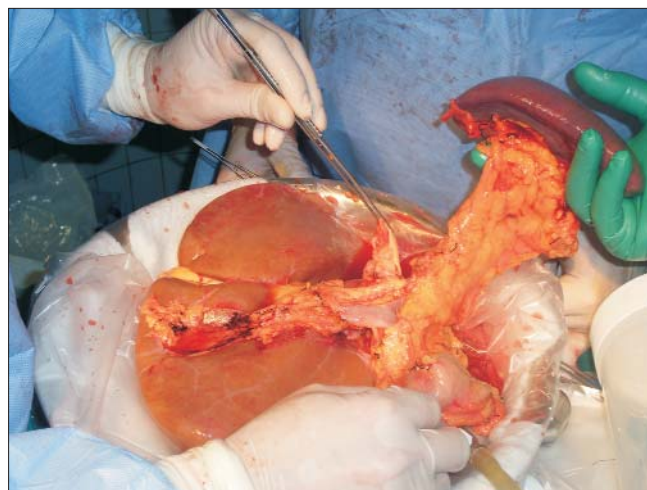


Figure 1. Combined liver-pancreas harvesting – back table dissection

In the other 18 cases the pancreas was explanted after liver and before kidney retrieval.

Donor age ranged between 12 and 61 years, with a median age of 35 years old, 3 donors younger than 20 years old and one older than 50 years old (61 years old). There were 9 females and 11 males. The donors' blood group was A(II) in 11 cases, O(I) in 7 cases and B(III) in 2 cases. The calculated BMI ranged between 15.6 and 27.8, with a median value of 24.1. The main causes of death were in 8 cases - brain injury, in 7 cases – aneurism rupture, and in 5 cases stroke.

Sternotomy and median xifo-pubian incision were used. Sternotomy was performed not only for heart removal, but also for an easier access and a better control of the intrathoracic aorta.

The pancreatic harvesting using a “no touch” technique was performed in all cases, with the spleen serving as a mechanical support for pancreas mobilization (Fig. 2).



Figure 2. No touch technique in which the spleen is hold without touching the pancreas

The first important step in organ harvesting is the exploration of the abdominal cavity and the check of the upper abdomen vascularization variants (accessory hepatic branches from the left gastric artery or superior mesenteric artery). The variants of upper abdominal vascularization are of less importance in pancreatic islet transplantation than in whole pancreas transplantation. The next step consists in the mobilization of the right colon, by retroperitoneum exposure and Kocher maneuver, with subsequent mobilization of the pancreatic head. Incision of the gastro-hepatic ligament is further performed by sparing the possible accessory hepatic arterial branch from left gastric artery. The operation is continued with the dissection of the common hepatic artery, gastroduodenal artery, splenic artery, left gastric artery, and portal vein. Caution should be exerted in clarifying the existence of a possible accessory hepatic branch from the superior mesenteric artery, which runs along the right side of the portal vein. The stomach is mobilized by incising the gastro-colic ligament. The maneuver allows the exploration of the omental bursa. The short gastric vessels, the right gastro-epiploic artery, and right gastric artery are ligated and sectioned. The mobilization of the pancreas begins with spleen mobilization, by incising the posterior and lateral peritoneal spleen reflections and spleno-colic ligament. The pancreas is medially retracted by sharp dissection, detaching it from left kidney and adrenal gland, and abdominal aorta. The superior mesenteric artery, the splenic and gastroduodenal arteries are spared for adequate perfusion of the pancreas with preservation solution. The left gastric artery is ligated but avoiding the ligation of the accessory arterial branch to the liver. The infrarenal aorta and inferior vena cava are dissected up to their bifurcations. The infrarenal aorta is taped near its bifurcation, after preliminary ligation of the inferior mesenteric artery and possible lumbar arteries, and prepared for cannulation. For cross clamping control, the aorta is taped subdiaphragmatically, after sharp dissection and incision of diaphragmatic pillars and periaortic serosa, and retroaortic blunt dissection. In unstable donors clamping of the aorta is done subdiaphragmatically, through an incision of the diaphragm and pericardium. The next step is transection with linear stapler of the first and the fourth segment of the duodenum after its previous lavage with Betadine solution through the nasogastric tube. The infrarenal aorta and portal vein - via the inferior mesenteric vein - are cannulated. After cross clamping (infrarenal aortic ligation, ligation of the subdiaphragmatic aorta, infrarenal inferior vena cava cutting) and systemic heparinization with 25,000 units or 300 units heparin/kg of bodyweight, the infusion of 40 Celsius Custodiol solution (histidine-tryptophan-ketoglutarate, HTK) is started (5,000 ml per aorta and 3,000 ml per portal vein). Starting with the warm ischemia time, the pancreas is packed in sterile ice for its quick cooling. Immediately after initiation of the infusion, the mesentery root together with the superior mesenteric vessels is stapled. At the end of perfusion, the gastroduodenal and splenic arteries are ligated (the splenic artery - close to its origin from the celiac trunk). The portal

vein is sectioned just above the pancreas, different to the whole pancreas transplantation, where the section is made at half the distance between pancreas and liver hilum. After its retrieval, the pancreas is transported in cold Custodiol preservation solution, in a container permanently cooled with ice.

Back table pancreas preparation and islets isolation are usually performed in the Cell Therapy Laboratory, under GMP conditions. Back table pancreatic preparation consists in removal of the fat tissue surrounding the pancreas, the vascular tissue, duodenectomy, and splenectomy, by gentle dissection and avoiding the damage of the pancreatic capsule. In our study there were 2 cases in which the pancreas retrieval was done in "en block liver and pancreas" manner, and separation on the back table was done in the operating room.

Cold ischemia time ranged between 1.5 and 8 hours, with a median value of 5 hours.

Eighteen pancreata were used for islet isolation, using different protocols, and two were used for whole pancreas transplantation. One pancreas procured from a 38 years old donor, with BMI of 27.8 and Vinkers score of 13 was used for pancreas transplant alone - PTA, and the other one procured from a 22 years-old patient, with BMI of 25.7 and Vinkers score of 9 was used for simultaneous pancreas and kidney transplantation - SPK.

Harvested pancreata showed no visible macroscopic lesions, even when increased blood levels of amylase and lipase were recorded. The pancreatic islets isolation was performed using different protocols, aiming to obtain a high yield of viable pancreatic islets. The isolated pancreatic islets were used in research purposes in animal and in vitro studies.

The main result of the present study was the formation of national qualified teams for pancreas harvesting and pancreatic islet isolation. The experience of the procurement team for pancreas transplantation is doubled by its previous experience in liver harvesting. Having the same team for liver and pancreas procurement, any difficulty raised by anatomical variants is better surmounted in this way. Moreover a combined liver-pancreas procurement does not represent a limitation in achieving a successful liver and pancreatic transplantation, and can be followed by the back table dissection in the operating room. Our important advantage consists in the possibility to perform pancreatic islet isolation and transplantation, whole pancreas, liver, and kidney transplantation in the same institute. The team involved in pancreatic procurement consists in three surgeons and one assistant. The team involved in pancreatic islet isolation has three surgeons, two biochemists, and two laboratory technicians.

Discussions

The quality of the harvested tissue depends on the harvesting technique and factors related to the donor such as cause of death, age, BMI, hemodynamic stability, vasopressor doses

used to maintain hemodynamic stability, period of stay in the Intensive Care Unit (ICU), and other blood parameters.

Due to the vicinity of the endocrine tissue to digestive enzymes in the pancreas, a fast organ procurement from cadaveric donors is necessary.

There are two different techniques of abdominal organs harvesting: the warm dissection and the cold dissection. In the warm dissection technique, dissection is done prior to preservation solution perfusion. It has the disadvantage of higher parenchymal and vascular injuries of the harvested organs, knowing the fact that dissection before perfusion causes vasospasm and increased oxygen consumption by the organs. There is also the risk of pedicles' torsion. These facts may lead to a poor quality of the harvested pancreas. The cold dissection is used in case of unstable donors. In this technique, the first step is cannulation, which decreases the operating time.

In our department an intermediate technique was used. It consists in incomplete dissection of the hepatic pedicle (dissection of only the right side of portal vein for identification of a possible accessory right hepatic artery with its origin in the superior mesenteric artery) and incomplete dissection of the celiac trunk origin from infra-diaphragmatic aorta, before cannulation, followed by a thorough back table dissection.

Multiple studies have shown the impact of donor's age on deterioration of beta cell function, leading to decreased glucose tolerance and type 2 diabetes. Some authors have shown on large series of pancreas transplantation that decreasing insulin production with age is a process independent of BMI, sex, cause of death, or methods of organ retrieval and isolation. Therefore, to exclude an undiagnosed type 2 diabetes, especially in donors aged over 40 years, glycosylated hemoglobin determination is required prior to the pancreas procurement (13). Potential cadaveric donors with history of diabetes type 1 and 2 are excluded. Also excluded are the cadaveric donors with a history of acute or chronic pancreatitis. It should be remembered that after brain death the serum amylase levels are often high in the absence of pancreatitis and hyperglycemia may occur in the absence of diabetes, due to severe insulin resistance in the donor, a problem that does not occur in the recipient. Therefore the presence of hyperglycemia or hyperamylasemia are not absolute contraindications to pancreas donation and the direct inspection of the pancreas is justified before deciding whether or not to use it.

In clinical islet transplantation, a large number of islets is required to achieve normoglycemia. The number of the islets needed for transplantation depends on the quantity and quality of islet preparation. The quantity of pancreatic islets obtained is expressed as the number of islet equivalents (IEQ), which is calculated based on the number and diameter of the islets present in the preparation, mathematically corrected for islet volume. IEQ is considered an islet with diameter of 150 μm . Generally more than 900,000 IEQ are needed for islet transplantation (6). The quality of the preparations is expressed as per cent islet purity and per cent

trapped islets. The per cent purity is the percentage of islets compared to all issue present in the islet preparation (islets, acinar, and ductal cells), determined by visual inspection of a representative sample of the islet preparation. The per cent trapped is the percentage of islets that are embedded or trapped in acinar tissue compared to all islets (free and trapped), determined as visual inspection of a representative sample of islet preparation. Free islets are considered those that have less than 25% of their borders attached to acinar tissue. Trapped islets have 25% or more of their borders attached to acinar tissue.

Donors younger than 50 years are optimal for pancreas harvesting and obtaining free insulin status by transplantation of islets using a single donor. Proportional with donor's age a greater mass of islets are required to be isolated and transplanted. Donors over 50 years are considered suboptimal for whole pancreas transplantation because graft survival at 5 years is lower than the survival of the graft obtained from donors between 18 and 34 years old (60% versus 73%). However donors over 50 years should be considered for islet transplantation. Due to the declining pool of donors, even donors 65-70 years old are accepted for islet transplantation, as marginal donors, under the condition of obtaining a greater number of IEQ or harvesting the pancreas from multiple donors.

Pediatric donors and young donors less than 18-20 years old are considered optimal candidates for whole pancreas transplantation but not for islet transplantation due to the existence of a denser extracellular matrix structure which elicits a cumbersome enzymatic digestion, the first stage of the isolation procedure. However there are highly experienced centers which use these young donors for islet isolation, with encouraging results.

In term of BMI, it is known that the obese patients (BMI over 30), with no history of diabetes, represent a better group of islet donors than lean patients, because beta cell mass is increased in accordance with increased need of obesity-associated insulin. Thus isolated beta cell mass is proportional to the donor weight. In many islet transplant centers, BMI of the donor must be over 22. With the advance in islet isolation technology, an increment of the number of IEQ that remain viable for transplant has been observed, if apoptosis inhibitors are used after isolation (34). These encouraging results give hope of a larger number of IEQ isolated from donors with BMI increasingly smaller. It is stated that it is easier to isolate a large number of islets from an older organ donor with a high BMI, due to the loss of extracellular matrix structure, but this does not mean that these islets have an optimal function. Islet function decreases with increasing age, fact that was proved by measuring insulin secretion and expression of the pancreatic gene PDX-1. Therefore, in most of the islet transplantation programs, the cut-off age is 60 years. However, the initial intraoperative inspection of the organ is justified for deciding the pancreas allocation, knowing that several human conditions, like obesity, aging, obstruction of the pancreatic duct, and presence of congenital disorders are associated with pancreatic lipomatosis (21-24). A fat infiltrated pancreas is considered suboptimal for whole organ

transplantation, due to the high risk of early graft loss. In contrast, it is preferred for islet transplantation, in accordance with some authors who reported a positive correlation between the high fat content of the pancreas and high islet yield (25, 26). The explanation of a more successful islet isolation from a pancreas with fat infiltration has not been clearly determined yet. It seems that there is a less amount of acinar tissue in a fat infiltrated pancreas, with consecutively less endogenous enzymes released from acinar tissue. That is a favorable condition for pancreas dissociation during islet isolation (27, 28). Some authors has even showed that digestion of pancreas with heavy fat infiltration resulted in remarkably less volume of digested tissue with preserved islet mass, rendering subsequent purification procedure unnecessary (2).

In addition to improvement of the efficiency of isolation technique, there are other strategies that lead to the establishment of an insulin-free status after transplantation of a small number of islets (17). Among these is the usage of a nondiabetogenic regimen of immunosuppression, with no steroids, nor calcineurin inhibitors (16).

It is documented that brain-dead donors generally have a long hospitalization in the ICU, frequently presenting episodes of hypotension which need vasopressors administration, and usually develop increased levels of serum creatinine, glucose, and/or transaminases. All these factors affect the quality of the collected pancreatic tissue. However, due to a decreased donor pool, there are accepted donors with less than 7 days in ICU, small doses of administered vasopressors, blood glucose < 16.7 mmol/l (3 g/l), serum amylase, lipase, transaminases, and creatinine < 2x normal range.

Factors that correlate with a high percentage of low quality harvested pancreas are hospitalization in ICU > 7 days, cardiac arrest > 5 minutes, serum Na > 160 mmol/l, amylase > 390 U/L, lipase > 480 U/L, administration of norepinephrine > 0.05 µg/kg/min or Dobutamine/Dopamine > 10 µg/kg/min (9, 12, 45-47). Based on these adverse factors, Vinkers settled a score of accepted pancreas donors of less than 17 (18). (Table 2)

The median calculated Vinkers score for our study group was 11. The highest Vinkers score was 16 points calculated in only one case.

A significant factor for obtaining a high quality isolation is the length of warm and cold ischemia time. Warm ischemia damages pancreatic tissue more than cold ischemia, with

worse repercussion in the isolation process. Therefore, the existence of some preservation solutions which facilitate rapid cooling of the pancreas and thus minimize warm ischemia time concomitant with sterile ice packaging must be reminded (48). Lakey et al. showed that it was very important for the pancreas to have its temperature kept below 10° Celsius during harvesting, transportation, and preservation (25). Immediately following cross clamping, before cardiac arrest, the pancreas is perfused with cold preservation solution (e.g. University of Wisconsin (UW), Celsior, Custodiol).

Japanese authors have developed their own system of "in situ" regional cooling, used for NHB donors. The system was adapted from the continuous infusion of the kidneys, which is done by inserting two catheters. The first catheter with two balloons is inserted via the femoral artery, before cardiac arrest, with one balloon being placed above the celiac trunk and the other just below the renal artery. The second catheter is inserted into the inferior vena cava via the femoral vein. The infusion is made with cold Ringer's solution immediately after cardiac arrest, during 3 minutes, thus avoiding the vasospasm induced by high concentration of potassium, that may occur when UW solution is used (14).

The accepted cold ischemia time for the whole pancreas transplantation is maximum 18-24 hours, and for islet transplantation maximum 8-12 hours, with an optimum time less than 8 hours.

During harvesting and transportation several types of preservation solutions can be used (e.g. UW, Celsior, Custodiol, and M-Kyoto solution). UW solution still remains the gold standard for pancreas procurement.

A recent study done by the Japanese authors proved that UW inhibits collagenase activity during enzymatic digestion, resulting in a smaller number of isolated IEQ with low viability (35,36). Therefore they have considered the use of other solutions. They studied M-Kyoto (MK) solution which contains ulinastatin, which exerts an inhibitory effect on trypsin activity during preservation and modest inhibitory effect on collagenase activity (37,38). As an advantage, due to the chemical stability of the effective components and other ingredients, MK solution can be stored at room temperature for a long period of time. The high potassium concentration in UW solution causes vasospasms and insulin release from pancreatic β cells (39) and its high viscosity may impair the

Table 2. Vinkers score for selection criteria of the pancreatic donor

Parameter	1 point	2 points	3 points
Age (years)	< 30	30-40	> 40
BMI (kg/m ²)	< 20	20-25	> 25
Serum sodium (mmol/l)	< 155	155-160	> 160
ICU stay (days)	< 3	3-7	> 7
Cardiac arrest (min)	No	< 5	> 5
Amylase (U/L) or	< 130	130-390	> 390
Lipase (U/L)	< 160	160-480	> 480
Noradrenaline (µg/KgBW/min)	No	< 0.05	> 0.05
Dobutamine/dopamine (µg/KgBW/min)	No	< 10	> 10

proper flushing and pancreatic ductal injection. In both porcine and human islet isolation, the islet yield was significantly higher in the MK group compared with the UW group (40). These findings showed that MK solution could be a more effective cold-storage solution for pancreas preservation in islet isolation than UW solution.

Other studies showed similar efficiency using UW or Custodiol preservation solution (HTK), when cold ischemia time was less than 10 hours. Instead, when cold ischemia time was over 10 hours, Custodiol solution had worse results comparing with UW solution.

Some authors compared MK solution with modified Custodiol solution, enhanced with ulinastatin (M-HTK). MK was showed to be superior to M-HTK due to the higher levels of ATP in the islets and similar anti-collagenase activity.

MK solution was also superior to modified Celsior solution containing hydroxyethyl starch (HES) -HNC (14,41).

Some studies have shown increased number of isolated islets by intraductal infusion with MK preservation solution (1 ml/g pancreas), immediately after explantation. The result is justified by the trypsin inhibitor activity of MK solution and its lower inhibition of collagenase activity. Otherwise, trypsin activity could result in damage of the ductal system and consecutively reduction of the efficiency of collagenase activity during enzymatic digestion stage (15).

For better preservation of the pancreas in case of prolonged cold ischemia time, the "two layer" method (TLM) is recommended. The method consists in pancreas preservation with UW solution concomitant with normobaric oxygenation and perfluorocarbon (PFC) solution as carrier of oxygen. The pancreas is placed between two immiscible media, with 2/3 of the pancreas covered by PFC solution. However, there is a controversy regarding its effectiveness (19, 29-32). Papas et al. showed that oxygen penetration into pancreatic tissue is up to 1 mm depth. The percentage of oxygen seems to be dependent on the thickness of the tissue. In this regard the pancreas fat should be removed before TLM preservation (33).

Besides mechanical damage during the islet isolation procedure, islets are separated from their nourishing microenvironment and subjected to devascularization, denervation, and hypoxia. The brief culture period after isolation may provide the islet with a much-needed recovery period prior to transplantation and may also allow for depletion of passenger leukocytes and deactivation of intracellular stress signalling pathways with consecutively diminished allo rejection (6).

Given the strict criteria for pancreatic allocation for islet transplantation, the number of donors is insufficient for the candidates on the waiting list. The donor pool can be increased by including also the marginal, NHB, and living donors. Due to the sensitivity of pancreatic islets to ischemia, it is difficult to harvest pancreas from NHB donors. Japanese authors have improved "in situ" cooling technique with a system adapted from kidney procurement, which decreases the warm ischemia time (42,43,44). In marginal donors, a recent meta-analysis showed an increased number of isolated islets,

using the TLM method, provided by highly specialized pancreas harvesting teams. The reported unsuccessful experiences regarding TLM method could be explained by its inappropriate use, with less caution to details (31). The first pancreatic islet transplantation from a living donor was performed by Japanese authors after distal pancreatectomy (49). The transplant was indicated in an unstable diabetic patient. A sufficient number of IEQ were isolated from the pancreas of the living donor and transplanted to the recipient who consecutively obtained an insulin-free status. The donor maintained normal levels of HbA1c and peptide C. The metabolic effect of the living islet transplant derived from just a half of a living pancreas seems similar to that achieved by using the islet isolation from two or more cadaveric pancreas. The difference in the required IEQ may indicate the greater potency of islets prepared from living than from cadaveric donors (14).

Conclusions

The organ allocation for vascularized pancreas versus islet transplantation should be made by matching both the donor and recipient criteria. The cornerstone of successful pancreatic islet transplantation remains the quality of the harvested organ. Recent advances in pancreatic islet transplantation such as the utilization of NHB, single, or living-donor were based on the improvement of pancreas procurement, preservation, transportation, and islet isolation methods. Several critical donor factors should be looked for, including donor's age, BMI, cause of death, vasopressor use, hypotensive episodes, duration of hospitalization, blood glucose, transaminases and creatinine levels, and cold preservation time. The experience of the procurement team regarding knowledge of the pancreas anatomy and surgical techniques is important in obtaining optimal warm and cold ischemia time. Pancreatic islet isolation was performed using different protocols, aiming to obtain a high yield of viable pancreatic islets. The isolated pancreatic islets were used on research purposes in animal and in vitro studies. The main result of the present study was the formation of national qualified teams for pancreas harvesting and pancreatic islet isolation, which represents an essential first step for clinical implementation of the Romanian pancreatic islet transplantation program.

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