Optimal Donor Selection, Pancreas Procurement, and Preservation for Successful Clinical Islet Isolation and Transplantation.

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Introduction

Over the last five years, outcomes of pancreatic islet transplantation have improved substantially. Islet transplantation has become an effective alternative to the whole pancreas transplant in type 1 diabetic patients. At experienced centers, 40-50% of patients remain insulin free 5 years after the transplant [1]. In Europe, Australia, and Canada, islet transplantation is an approved and reimbursed procedure for type 1 diabetics suffering from hypoglycemia unawareness. In England, donor pancreas is even distributed equally for pancreas and islet transplantation – there is only one common waiting list for islet and pancreas transplant candidates [2].

Despite the progress in clinical results, islet cell processing remains a very challenging procedure. Islet isolation is an expensive and time-consuming procedure. It must be carried out in a cGMP facility environment. In the USA, it costs more than $20,000 USD and takes 8-10 hours of at least 4-5 staff members' of diligent work for islet isolation from one pancreas [3, 4]. Despite that, even at the most experienced centers, only 40-50% of all islet isolations result in clinical transplant [5, 6]. At best, we can obtain 300-500 thousand islet equivalents (IEQ) from the 1million IEQ present in the pancreas.

Therefore, in order to maximize islet yield and chance for successful isolation with transplantation, it is critical to optimize each step of the pancreas and islet cell processing procedure. Those steps include donor selection, pancreas procurement, pancreas preservation and transportation, islet isolation and culture, patient selection, and treatment. Each of them is crucial, since a mistake at any of step leads to failure of the entire procedure.

Pancreas donor selection

First, in order to prevent transmission of infection or disease (e.g. cancer) to the recipient, each potential pancreas donor needs to meet the same criteria as for any other organ for clinical transplantation. In addition, due to islet cell pathology, we exclude donors with diabetes or pre-diabetes, those with HbAc1>6. Since islet transplantation is not a life saving procedure, CDC (Centers for Disease Control and

Prevention, www.cdc.gov) high-risk donors are excluded, too. They are described in table 1.

The optimal pancreas donor for islet transplantation has enough islet mass and quality, allowing for islet proper preservation. Based on the experience of the most active centers, several strong predicting factors for successful islet isolation have been identified. Higher islet yield is obtained from deceased brain dead donors comparing to DCD (Donation after Cardiac Death) donors and living donors. Preferred donor age ranges between 20 and 50 years old, however, the acceptable age range is wider (18-70). Fatty pancreas texture is preferred over fibrotic. Cold ischemia time should be as short as possible, preferably under 8 hours, and should definitely not exceed 12 hours. The chance for success is few times higher when the surgical team from the islet center procures the pancreas rather than a distant team.

In contrast, we should avoid: donors with prolonged hypotension and/or downtime over 10 minutes; donors on many or/and high dose of pressors; donors with evidence of acute pancreas or multiorgan damage; donors in metabolic distress- high serum lipase, amylase, creatinine, liver enzymes; donors with acute hyperglycemia resistant to treatment with insulin; and donors with prolonged hospital stay and CIT>12 hours. Pancreas with tumor, extensive pancreas hematoma, damage or capsular tear should be excluded as well. Based on analysis of the outcomes of over 300 isolations, the Edmonton group has established that it is not a single factor, but the constellation of all different factors, which better predicts the outcome of islet isolation [7]. Therefore, a donor scoring system was developed taking into consideration all above described above positive and negative donor factors. The sum of the points assigned for each donor factor correlated vey well with outcomes. When donor score was over 80 points, there was more than a 50% chance for successful islet isolation; a score below 50 practically leads to failure. The scoring system is a very helpful tool to tailor donor selection strategy depending on center budget and goals. It is also important that we validated this system also for a new, less experience islet isolation center [8].

It is important to remember that donor/pancreas criteria for islet transplantation are much wider then for whole pancreas transplantation. Therefore, any potential pancreas/donor, who was disqualified for whole organ transplant, can be considered for islet isolation.

Pancreas procurement

The goal of optimal pancreas procurement is to preserve islet function and organ integrity. The surgical technique of the pancreas procurement for islet isolation is essentially the same as for whole pancreas transplantation. Nevertheless, there are a few things that need to be remembered. First, pancreas is a fragile organ, and should be handled with special care to avoid a capsular tear. Second, it is sensitive to warm ischemia; therefore, it must be kept in cold ice slush during the entire procedure after the cross-clamp. If it is not cooled properly, islet yield decreased substantially [9].

Prior to incision, surgeons should always check donor documents including consent for donation, blood type, and brain death note. Midline incision from suprasternal

notch to pubis opening chest and abdomen allows for wide access to the organs. The liver team usually starts dissecting the liver, common bile duct, abdominal aorta and inferior mesentery vein (IMV). Next, standard pancreas dissection like for whole pancreas transplantation follows. The duodenum is washed with 100ml of Betadine infused through nasal-gastric tube. Next, the lesser sac is opened dividing the gastrocolic ligament; the spleen is freed by ligating short gastric vessels, dividing lienocolic and splenophrenic ligaments so it can be easily lifted and retracted further towards the midline. Next, we assess the pancreas: it should be well perfused, without any evidence of venous congestion, trauma or pathology. The presence of peripancreatic fat is acceptable. After the Kocher maneuver, we divide middle colic vessels, the first and fourth portions of the duodenum with a GIA linear cutting stapler (EndoGIA, U.S. Surgical, Norwalk, CN) preserving gastroduodenal artery (GDA). After dissection of the posterior pancreas from the retroperitoneum, we bring spleen and pancreas tail to midline. Next, 500u/kg heparin is injected intraveniously followed by aorta and IMV canulation.

During the entire surgical procedure, surgeons must carry out very careful dissection in order to minimize arterial vasospasm and vascular injury, which may compromise pancreas perfusion and the results of the isolation. Superior mesenteric artery (SMA), GDA and splenic artery should be kept intact, avoiding even placing vessel loops around these vessels. The venous cannula should be inserted into the IMV carefully to prevent the tip of the cannula from advancing proximally into the splenic or portal veins; this assures that the venous drainage of the pancreas is not impaired.

In the next step, we cross-clamp the supraceliac aorta in the abdomen or in the chest, transect the cavo-atrial junction exsanguinating donor into the chest cavity. We start simultaneously in situ a 3-5 liter aortic flush and 1 liter portal flush with chilled preservation solution- UW (Viaspan®, Barr Laboratories, Pomona, NY) or HTK (Chemie-GmbH, Alsbach-Hähnlein, Germany). The aortic flush bag should be 40 cm higher than the portal one to avoid venous congestion of the pancreas. Immediately, the spleen is brought to midline and copious iced-saline slush is packed behind and in front of the pancreas as well as over other organs to keep them cold.

After thorough perfusion, the pancreas in excised en bloc with the duodenum and spleen, before, with, or after the liver. The order is not crucial as long as the pancreas remains cool and embedded in iced saline. Liver with pancreas can removed en bloc and separated on the back table. As described before, it is crucial to avoid capsular damage to the pancreas during dissection (later in the lab, enzyme infused into the duct will leak and digestion of the pancreas and islet yield will be compromised). However, since there is no need for blood vessels during the islet processing, SMA, splenic artery and portal vein can remain with the liver, if necessary. In case aberrant right hepatic artery (RHA) exists, SMA can be easily reached distally, below to RHA take off without need for pancreas transection and organ waste. Presence of the aberrant RHA is not a contraindication and should not preclude pancreas retrieval for islet isolation.

Packing and transportation:

After procurement, pancreas is immediately transferred into 500 mL of chilled preservation solution (without ice) in a 1L Nalgen Jar. UW, HTK and Celsior (Genzyme, Cambridge, MA, USA) solutions are most commonly used for cold storage pancreas preservation and there is no proven benefit of one fluid over another. After packing in triple bags, the pancreas is placed in a styrofoam box including blood samples and donor chart and shipped as soon as possible to the islet isolation facility.

In case the procurement surgeon comes from the islet team, additional pancreas trimming is recommended on the back table prior to the packing. Excessive fat, duodenum, and spleen are removed for better penetration of the preservation solution during the transportation. In Dallas, Texas, and Japan, additionally intra-ductal injection of ET-Kyoto solution is performed for improved preservation [10,11].

Two layer method versus cold storage

In 1988, Kuroda *et al*. developed PFC (perfluorocarbon)/UW two layers method (TLM) for packing and transportation [12]. PFC is a hyper oxygen carrier and allows for extra oxygen delivery to the pancreas during the preservation, improving cell energy (tissue ATP concentration) and cell membrane integrity during ischemia [13]. In this method, the organ is immersed at the interphase of the PFC and UW solutions. In dynamic (continuous) TLM, oxygen is continuously provided to the system from an oxygen tank designed for organ salvage in the lab after prolonged transportation. In static TLM, PFC is pre-oxygenated (30 min) what allows pancreas shipping without need for oxygen tank.

TLM was shown to be superior to cold storage in animal models and in several initial clinical studies (low case number, TLM was usually applied by an islet team specialist)[14-17]. However, the advantage of this method was not confirmed in two large clinical studies (N>200) where TLM was applied routinely by the organ preservation team [18, 19]. Additionally, it was verified that oxygen could penetrate only through 1 mm of tissue, influencing only a small portion of the organ [20]. Therefore, it is possible that it was not TLM, but proper pancreas procurement and trimming by an islet center specialist in the initial TLM studies that allowed for better penetration of the preservation solution into the organ, improving outcomes. A prospective, randomized clinical trial is still necessary to assess the real advantage of TLM over simple cold storage.

Islet autotransplantation procurement

Some patients with chronic pancreatitis require total pancreatectomy due to intractable pain with subsequent islet autotransplantation to prevent diabetes. In order to improve pancreas preservation during pancreatectomy, blood flow in the splenic artery and vein should be maintained during the dissection until final ligation and pancreas removal. Ischemic injury to the islets should be limited. Next, at the back table, the pancreas should be perfused with preservation solution through the splenic

artery if feasible, and the angiocath should be placed in the pancreatic duct, if possible, for enzyme infusion during processing in the lab. After cleaning from adherent tissue, pancreas is sent to the lab in cold preservation solution.

Logistic consideration

Prior to starting an islet transplantation program, an islet center should develop a good relationship and communication with the local Organ Procurement Organization (OPO).

All logistics involving donor treatment and selection, pancreas procurement, and shipping should be planned ahead of time. The islet center should give OPO simple donor screening parameters, which should prompt calling with the organ offer (age range, HbA1c<6, no diabetes, no CDC high risk). At the same time, a surgeon or islet isolation team member should be available 24/7 for further over-the-phone donor evaluation. Once a donor is accepted, a surgeon, preferably from the islet center, should be sent for procurement, and bring the pancreas back as soon as possible and islet isolation should start promptly.

Conclusion

Proper donor selection and optimized pancreas procurement and preservation are essential for successful islet isolation.

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Table 1. Pancreas donor exclusion criteria

General exclusion criteria

- Clinical or active viral hepatitis A, B, C
- AIDS, HIV seropositivity (HIV-I/II), HTLV-I/II
- Syphilis
- Active viral encephalitis or encephalitis of unknown origin
- Creutzfeldt-Jacob disease
- Rabies, Tuberculosis, West Nile virus, Septicemia
- Malignancies, except primary brain tumor
- Serious illness of unknown etiology

CDC high risk- behavior/history exclusion criteria:

- Men who have had sex with another man in the preceding 5 years
- Persons who report non-medical intravenous, intramuscular or subcutaneous injection of drugs in preceding 5 years
- Persons with hemophilia or related clotting disorders who have received human-derived clotting factor concentrates
- Men and women who have engaged in sex in exchange for money or drugs in the preceding 5 years
- Persons who have had sex in the preceding 12 months with any person described in items 1-4 or with a person known or suspected to have HIV infection
- Persons who have been exposed in the preceding 12 months to known or suspected HIV-infected blood through percutaneous inoculation or through contact with an open wound, non-intact skin or mucous membrane
- Incarcerated for more than 72 hours (3 days) within the past 12 months
- Medical/Social history unobtainable

Pancreas specific exclusion criteria

- Diabetes mellitus
- Pre-diabetes, HbA1c>6mg%