Functional islets and where to find them

Doppenberg, J.B.

CHAPTER 3. PANCREATIC ISLET ISOLATION FROM DONATION AFTER CIRCULATORY DEATH PANCREAS

Jason B. Doppenberg[1,2], Marten A. Engelse[1,2], Eelco J.P. de Koning[1,2]

Leiden University Medical Center, departments of [1]Transplantation, [2]Internal Medicine

Chapter 40 - Pancreatic islet isolation from donation after circulatory death pancreas, Editor(s): Giuseppe Orlando, Lorenzo Piemonti, Camillo Ricordi, Robert J. Stratta, Rainer W.G. Gruessner. Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas Academic Press. 2020. Pages 471-482. ISBN 9780128148334. doi.org/10.1016/B978-0-12-814833-4.00040-X.

Abstract

In many countries there is an insufficient number of organs retrieved from donation after brain death (DBD) procedures in order to keep time on deceased donor organ waiting lists acceptable. Organs from donation after circulatory death (DCD) procedures could help to relieve this pressure. In some countries DCD pancreas are increasingly used for both vascularized pancreas and islet transplantation. With more stringent donor pancreas acceptance criteria, preliminary results indicate that islet isolation from DCD pancreas results in a somewhat reduced number of isolated islets, but no consistent difference in functionality *in vitro* or clinical outcome in islet transplant recipients. Although more data on clinical outcome are clearly needed, islets from DCD donors can be considered for clinical islet transplantation.

Introduction

In order to keep up with the demand for donor organs in an era of changing donor demographics, there is an increasing focus on the use of organs from donors with "marginal" or "expanded criteria" donor (ECD) characteristics[1]. These donors are generally defined by a higher age, higher BMI, hemodynamic instability, and other factors[2]. The precise criteria can differ between countries, institutions, and organ type[3–5]. As acceptance criteria in the context of vascularized pancreas transplantation broaden, the availability and quality of donor pancreas for islet isolation and transplantation also decreases in most countries[3]. A potential strategy to ensure a sufficient supply of islets for transplantation is the use of pancreas from donation after circulatory death (DCD). But what are the consequences for both islet isolation and islet transplant graft outcome using DCD pancreas? This chapter will focus on the history, the technique of procurement, and finally the utilization of DCD pancreas for islet isolation and transplantation.

History

In 1954 the first successful kidney transplantation between two identical twins took place[6]. This living donor kidney transplantation procedure occurred at a time before immunosuppression was available. It was not until 1959 that Schwartz and Dameshek published on the immunosuppressive drug azathioprine that paved the way for non-related kidney donation and transplantation[7]. In 1960, the first living donor kidney transplantation in non-identical twins was performed followed by the first successful cadaveric kidney transplantation in 1962[8,9]. Although there are few details on donor characteristics, prevailing disease and cause of death, procurement of the kidney occurred after cardiocirculatory arrest[8]. During these early days, the dead donor rule was strictly adhered to. One was deceased when "cold, blue, and stiff", i.e. without circulation or ventilation[10]. It was clear that such cadaveric organs, with extended periods of ischemia before retrieval, were less than ideal for transplantation. A possible alternative source of cadaveric organs from deceased donors with intact circulation was proposed.

Donation after Brain Death

In 1963 the first donation after brain death (DBD) procedure was performed in Brussels, Belgium[11]. In the United States, as new criteria for death were discussed in the late 1960s, a proposal for a definition of brain death was documented in 1968[12]. Almost all U.S. organ

procurement agencies switched to DBD procedures and abandoned all other donation procedures promptly after the DBD guidelines were published[13]. This quickly became the standard worldwide as acceptance of the criteria for brain death grew[14].

Nowadays, neurological assessment of brain death is conducted via several tests determining irreversible loss of consciousness in the absence of brainstem reflexes. In brain death there is no longer the capacity for autonomous respiration, but cardiac arrest and/or circulatory standstill have not yet occurred[15]. The donor, who is at that point on mechanical ventilation, can be taken to the operating theater. Pharmaceutical agents are given in order to combat hypovolemia, hemodynamic instability, pulmonary edema, hyperglycemia, hypernatremia, coagulopathy, and musculoskeletal reflexes. Exact protocols differ per institutions[16]. During procurement itself, there is sufficient preparation time for a rapid retrieval and cooling of the organs[17]. After aortic perfusion with a preservation solution, procurement can commence almost immediately. Thus, DBD procedures offer the potential to maintain advantageous hemodynamic conditions in the organs prior to retrieval[18]. However, brain death has been shown to negatively affect the circulatory and hormonal state of organs[19,20]. At the onset of brain death vasoconstrictive hormones are released, endothelial adhesion molecules are upregulated, heat shock proteins are formed, and a marked influx of leukocytes in the donor graft is observed, suggesting a state of stress in the donor[20–23]. These factors can alter the engraftment and/or primary function of an organ.[22]

Donation after Circulatory Death

In the United States waiting lists for solid organ transplantations increased yearly by 7.9% from 1990 until 2015[24]. It is estimated that transplantation of cadaveric organs only meets 10% of the global need[25]. The number of pancreas transplantations in the Eurotransplant region and the United States have declined by 2.5% and 3.3% per year since 2004, respectively, and by 1.0% in the UK since 2009[26]. To maintain or increase the number of suitable organs for transplantation organs from expanded criteria donors are increasingly utilized[27]. In case of pancreas from organ donors in the Eurotransplant region, age >50 years and/or BMI ≥30 are considered expanded criteria donor characteristics[28]. As the average age of the population in most societies increases, and consequently the age of potential donors, many countries have broadened the criteria for expanded criteria donors to include donors from donation after circulatory death (DCD)[29]. Four categories of DCD were proposed during the *First International Workshop on DCD* in Maastricht, the Netherlands, in 1995.[30]

Category 1 DCD was defined as “Dead on arrival”, category 2 DCD as “Unsuccessful resuscitation”, category 3 DCD as “Awaiting cardiac arrest”, and category 4 DCD as “Cardiac arrest while brain dead”.[30] These categories could be further divided in uncontrolled DCD (category 1 and category 2) or controlled DCD (category 3 or category 4). In 2012 subcategories for category 2 DCD based on location (cardiac arrest occurring out of hospital or in hospital) were proposed[31]. Recently a fifth category, euthanasia, (medically assisted cardiocirculatory death) was added to indicate organ procurement after controlled termination of life upon request in countries such as Belgium and the Netherlands. Further revisions of the Maastricht classification are presented in table 1.

(Modified) Maastricht Classification Of Donation After Circulatory Death

CATEGORY	General Definition	Control	Further Subdivisions
1	Dead on arrival [1]	Uncontrolled [30]	
A		Uncontrolled [11]	Non-witnessed death outside hospital [11]
B		Uncontrolled [11]	Witnessed death outside hospital and rapid resuscitation attempt [11]
2	Unsuccessful resuscitation [30]	Uncontrolled [30]	
A		Uncontrolled [11]	Unexpected cardiac death in ICU [11]
B		Uncontrolled [11]	Unexpected cardiac death in hospital (ER or ward), with witnesses and rapid resuscitation attempt [11]
3	Awaiting cardiac arrest [30]	Controlled [30]	
A		Controlled [11]	Expected cardiac death in ICU [11]
B		Controlled [11]	Expected cardiac death in OR (agonal phase >30 min) [11]
C		Controlled [11]	Expected cardiac death in OR (agonal phase ≤ 30 min) [11]
4	Cardiac arrest while brain dead [30]	Controlled [30]	
A		Uncontrolled [11]	Unexpected cardiac arrest in a brain-dead donor (in ICU) [11]
B		Controlled [11]	Expected cardiac arrest in a brain-dead donor (in OR or ICU) [11]
5	Euthanasia [11]	Controlled [11]	
A		Controlled [11]	Medically assisted cardiac death in ICU or ward [11]
B		Controlled [11]	Medically assisted cardiac death in OR [11]

Table 1. In the original 1995 classification, four categories of DCD were proposed (General Definition,[30]). Later modifications included a fifth category and subdivisions[11]. Abbreviations: ICU (intensive care unit), ER (emergency room), OR (operating theater). Table adapted from references 30 and 11.

Ischemic time intervals

The key difference between DBD and DCD is a period of a warm ischemia during DCD procedures. In controlled DCD procedures, this warm ischemia time consists of several time intervals (figure 1). The cardiopulmonary system gradually deteriorates when life support is switched off. This is the start of the total warm ischemia time (tWIT) and agonal phase. At a certain moment, there is a critical reduction in hemodynamics and oxygen delivery to tissues. The functional warm ischemia time (fWIT) starts at this point. When cardiac arrest occurs the agonal phase ends. Then a "no-touch" period is applied followed by a transfer to an operating theater. Cannulation of the aorta is performed as quickly as possible and the donor is perfused with cold preservation solution, ending tWIT and fWIT.

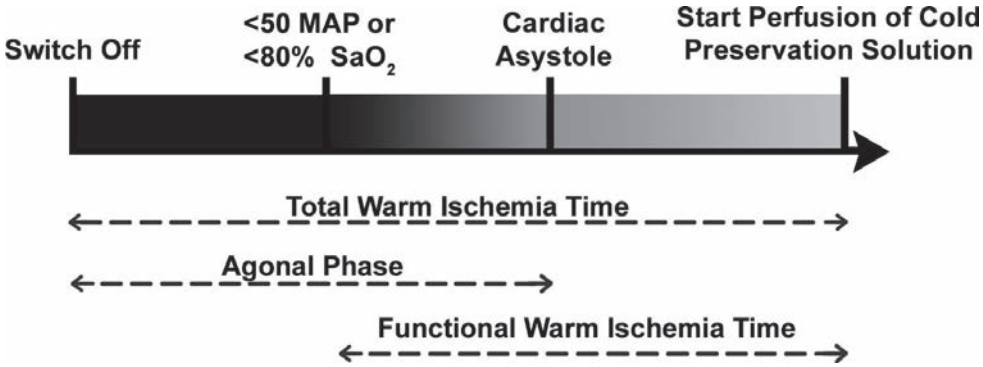

Figure 1. Ischemic time intervals of controlled DCD procurement. The greyscale gradient becomes increasingly lighter, representing cooler temperatures. Critical moments in the agonal phase (from switch to the start of perfusion of cold preservation solution) are indicated with vertical lines. Below the bar definitions of the different relevant time periods are provided.

The time between withdrawal of life support and cardiac arrest is known as the agonal phase which can differ widely per donor[32]. A long agonal phase is not uncommon, and a procurement team will stand down when a maximum time interval is reached which can differ between organ and country. For pancreas procurement, the maximum length of the agonal phase can be 30 minutes (Australia), 60 or 120 minutes (in the Netherlands for vascularized pancreas transplantation or islet transplantation, respectively), or 180 minutes (UK)[33–36]. In the UK approximately 40% of intended DCD procedures are abandoned due to the agonal phase exceeding the maximum time limit[36]. There is evidence that this period can even be longer than 4 hours for kidney procurement and transplantation[37]. Concerns have been raised that waiting more than 2 hours until cardiac arrest may also pose an additional burden on the next of kin[38].

The first time interval which can be distinguished in the agonal phase is from the moment of withdrawal of life support until the moment at which the organs become ischemic. There is no consensus on the definition of this point[39]. Either the mean arterial pressure or systolic pressure must drop below 35 to 70 mm Hg[16,32,40] and/or the oxygen saturation must drop below 25% to 80%[36,41]. Before this time point it is believed that there is still sufficient blood flow and oxygen delivery to prevent warm ischemic damage to the organs. Careful documentation is imperative as values of oxygen saturation and blood pressure can fluctuate rapidly[42].

Previous research has shown that a prolonged agonal phase with adequate hemodynamics until cardiac arrest, still associates with good organ quality[43]. In a clinical setting, the variability in factors such as age, cold ischemia time, BMI and others that affect organ quality have a greater effect on graft outcome than functional warm ischemia time with varying definitions, which has made it very difficult to statistically define precise values for inadequate hemodynamics[42]. Despite the lack of definitive criteria, an increasing number of centers use some definition of compromised hemodynamics to indicate the start of the period of functional warm ischemia instead of total warm ischemia time[44]. In our center, either the MAP must drop below 50 mmHg or the oxygen saturation must drop below 80%.

After cardiac arrest there is a strict adherence to a time period known as the “no-touch period”, after which death is pronounced. This policy, although resulting in longer warm ischemia times, is based on ethics, respect, and legal necessity. The length of this period differs from 2 to 20 minutes dependent on the country and/or center[29].

The period of warm ischemia continues during the transportation of the donor to the operating theater. It is possible in some areas to observe the no-touch period during this transfer time[13]. It is of vital importance to commence with the perfusion of cooled preservation solution as soon as possible to cease the period of warm ischemia[36].

In some countries, besides a limit on the time of the agonal phase, organs are no longer offered for allocation if the fWIT exceeds a specific time limit. UK guidelines mandate an organ specific maximum fWIT of 30 minutes for pancreas retrieval [37]. Therefore, theoretically, a UK team can wait up to 210 minutes for the procurement of a pancreas before standing down (180 minutes until the onset of fWIT, another 30 minutes until cardiac arrest)[33]. For uncontrolled DCD the WIT starts essentially at the moment of cardiac arrest, which is often uncertain. In the case of known cardiac downtime, reports have documented up to 120 minutes WIT before perfusion with cold preservation solution[45].

Controlled DCD Perfusion Technique

Rapid laparotomy and direct cannulation of the aorta is the preferred technique for controlled DCD donors[46]. This technique allows for a high flow rate of cooled perfusion solution with topical cooling of the organs[47]. Most Japanese DCD donors are brain dead, and before the heart stops beating, a system for *in situ* regional organ cooling is inserted: a double-balloon catheter is placed above the celiac axis in the aorta via the femoral artery and a venous catheter is placed in the inferior vena cava. Cooled perfusion solution is administered immediately after cardiac arrest, allowing for a much shorter period of warm ischemia during DCD procedures than in other countries where such interventions prior to cardiac arrest are not allowed[48,49]. One series reported a WIT of 4.2±0.7 mins[50], much shorter than data reported from other controlled DCD series[51,52].

Prevalence of DCD

DCD is predominantly performed in Spain, Belgium, UK, the Netherlands, Australia, and the USA[53] and to a lesser extent in a number of other countries[25,54–56]. Table 2 shows an overview of countries where at least one DCD procedure was reported in the Global Observatory on Donation and Transplantation registry in 2016. In Western Europe, the recent rise in DCD has largely been due to a dwindling number of suitable DBD donors[57]. In fact during the last 5 years DCD increased to 51.6% of all organ retrieval procedures in the Netherlands, and to 26.1% of retrieval procedures in Belgium. Most of these procedures are category 3 DCD[58].

On the other hand in Spain most DCD procedures are uncontrolled category 1 or 2[29]. In the United Kingdom, approximately 40% of organ retrievals are category 3 DCD[59]. In Japan the majority of procedures most closely resemble category 4 DCD[60].

Countries With High Number Of Dcd Per Million Population	Countries with lower number of DCD per million population
Spain (10.7)	Canada (4.8)
Belgium (9.5)	China (2.4)
United Kingdom (9.3)	Switzerland (1.8)
Netherlands (7.7)	France (1.4)
Australia (5.3)	New Zealand (1.3)
United States of America (5.0)	Portugal (1.0)
	Austria (0.7)
	Lithuania (0.7)
	Ireland (0.6)
	Singapore (0.5)
	Russian Federation (0.4)
	Czech Republic (0.4)
	Norway (0.4)
	Italy (0.3)
	Japan (0.3)
	Israel (0.2)
	Poland (0.2)
	Saudi Arabia (<0.1)
	South Africa (<0.1)

Table 2. Countries performing DCD. Countries in which there are where DCD donations represent more than 5.0 or more DCD donations per million population (pmp) are listed in the first column (rounded to the nearest 0.1 million. Countries that allow in which DCD but have is allowed but fewer than 5.0 DCD donations pmp rare are listed in the second column[53].

Category 5 DCD procedures are heavily regulated and protocolized. In the Netherlands, euthanasia is performed by a physician only upon a patient's request with adherence to strict procedures and in light of unbearable suffering and the absence of treatment alternatives[61]. Consultations with a second physician are necessary in order to confirm that conditions are met[62]. Firstly, a coma is induced with a sedative (most often thiopental or propofol), followed by a high dose muscle relaxant (rocuronium or atrcurium), causing the complete paralysis of all striated muscles[63]. Cardiac arrest soon follows and the 5 minute "no-touch" period is honored. Thereafter, organ retrieval is performed in the same manner as category 3 DCD.

Graft outcomes in DCD kidney and liver transplantation

Using more stringent donor selection criteria for DCD compared to DBD, graft outcome is in general quite adequate for different vascularized organs[64]. Results of DCD kidney transplantations show that primary non-function, graft survival and patient survival at 1, 5

and 10 years differ only slightly compared to DBD kidney transplantations. However, there is an almost two fold increased rate of delayed graft function in DCD kidneys. Also, DCD kidneys are more susceptible to cold ischemia damage than DBD kidneys. It has been recommended that the CIT for DCD organs should therefore not exceed 12 hours[65].

Results of DCD liver transplantations have been less promising than DCD kidney transplantations. Several centers have pursued DCD liver transplantation and have observed higher rates of biliary complications and ischemic cholangiopathy resulting in retransplantation[66]. Therefore, there has been hesitation to accept DCD liver grafts with otherwise favorable donor criteria, due to the dire consequences of organ failure[67]. However, leaving patients without a transplant on the waiting list may pose even greater risks for the patient[66].

DCD vascularized pancreas transplantation

Slow acceptance of DCD pancreas has been attributed to concerns of post-operative dysfunction and pancreatitis, as it is believed that pancreas are especially vulnerable to ischemic damage and damage related to factors such as older age, obesity, alcohol and cardiovascular disease[41]. In a 2000-2008 European wide survey on DCD pancreas only 3% of pancreas offered for procurement were transplanted[29]. Since then rates have increased. Over the past 5 years in the United Kingdom this conversion rate for DCD pancreas reached 56%[68]. Vascularized pancreas transplantations with pancreas from DCD donors have been performed in only few centers since 1993[64].

Donor selection for pancreas retrieval in DCD donors

Generally speaking, the selection of DCD donors for pancreas procurement is more stringent than DBD donors. Extended warm ischemia times are avoided and combinations with other potentially disadvantageous factors are kept at a minimum[36]. Although no clear consensus exists on the nature of these other disadvantageous factors, most centers take into account: age, predicted CIT, BMI (or body surface area), cause of death (anoxia, trauma, cerebral vascular), hospital stay, vasopressor use, blood glucose, and blood chemical analysis (Na^+, ALAT, AST, lipase, amylase, HbA1c)[69–71].

In 2009, the P-PASS (pre-procurement pancreas suitability score) was introduced in the Eurotransplant region in order to facilitate recognition of a suitable pancreas donor. A combination of nine clinical parameters available at time of donor reporting provide a P-

PASS between nine and 27 for each donor (table 3)[72]. It was recommended that a pancreas with a P-PASS of less than 17 should be considered for donation and transplantation. However, it has been shown that the P-PASS is a poor predictor of long term graft survival or ischemia-reperfusion injury[73,74]. DCD procurement is not one of the components of PASS. Therefore, the pancreas donor risk index (pDRI), a continuous score, was developed as a tool to predict graft survival[75]. DCD procurement is one of its eight variables, and this score has recently been shown to predict pancreas graft survival[76].

Item	1 Point	2 Points	3 Points	Weight Of Score
Age (Years)	<30	30-40	≥ 40	2
Bmi (Kg/M2)	<20	20-25	≥ 25	2
Icu-Stay (Days)	<3	3-7	≥ 7	1
Cardiac Arrest	No	Yes, <5 min	Yes, ≥ 5 min	1
Sodium (Mmol/L)	<155	155-160	≥ 160	1
Amylase (U/l) Or Lipase (U/l)	<130	130-390	≥ 390	1
	<160	160-480	≥ 480	1
(Nor)Adrenaline (Γ) Or Dobuta-/Dopamine (Γ)	No	<0.05	≥ 0.05	1
	No	<10	≥ 10	1

Table 3. Calculation of P-PASS. The P-PASS was used to introduced in order to better facilitate recognition of a suitable pancreas donor. A score of <17 is predictive of favorable pancreas graft outcome after transplantation. Adapted from[72].

Clinical outcome of vascularized DCD pancreas transplantation

A recent review compared DCD and DBD pancreas transplantations from five different studies[14]. No difference was found in patient survival rates (96.5% for the DCD group and 95.3% for the DBD group, p=0.502), the 1-year graft survival rate (86.5% in the DCD group and 87.2% in the DBD group, p=0.741), nor HbA1c after 1-year(5.6% for DCD and 5.4% for DBD, no meta-analysis performed). However, there was a higher incidence of graft thrombosis in DCD transplantations (9.3% in the DCD group and 5.3% in the DBD group, p=0.030). In our center there was no difference in pancreas graft survival between DCD and DBD pancreas transplantation after 7 years[77].

Donor Selection for DCD pancreatic islet isolation

It is still very difficult to judge whether a pancreas is suitable for islet isolation and transplantation. Donor risk scores have been developed to predict islet isolation success (with varying definition of “success” from >100,000, >250,000, ≥315,000 to >400,000 IEQ post-

purification[69,70,78,79]) or the total IEQ[80]. DCD is not included in these scores due to insufficient data.

A more direct approach would be to judge the quality of the organ itself, such as the "Remuzzi" score, that is based on analysis of pre-transplant kidney biopsies[81]. However, taking pancreas biopsies prior to isolation damages the capsule making distention with an enzyme solution more difficult due to enzyme solution leakage.

A cut-off point for functional warm ischemia time that indicates too much damage for the pancreatic islets must still be determined. Recommendations for a maximal total warm ischemia time before pancreas procurement for islet processing vary between 30 and 60 minutes. This was based on either data from kidney transplantations or arbitrary choice[41,44,51,82–84]. Evidence in a porcine research model has shown that blood flow varies greatly in different vessels during the agonal phase and that it is difficult to predict where this flow is insufficient, and leads to hypoxia[39].

Pancreatic islet isolation from DCD pancreas

The first report of human pancreatic islet isolation from a DCD pancreas was in 1976[85]. In an era prior to the semi-automated method, the results state merely "the isolation procedure yielded satisfactory islets." In 1994, a case report showed the feasibility of DCD islet isolation from a pancreas of a donor with diabetes yielding 132,000 IEQ[86].

Since then, there have been several reports on islet isolations of controlled DCD procedures [51,52,82,87–90].

Three studies with relatively small numbers of DCD pancreas (≤ 15 per study) reported similar islet yields obtained from DCD and DBD donors[51,52,82]. In one of these studies islet number instead of IEQ was used as outcome parameter for yield, making comparisons to other studies difficult, regardless, a similar yield was found between DCD and DBD pancreas[52] and in another study approximately 100,000 IEQ more was isolated from DBD pancreas compared to DCD pancreas, but this was not statistically significant[51]. On the other hand, in one small study there was approximately 100,000 IEQ higher islet yield from DCD pancreas compared to DBD pancreas that was attributed by the authors to a shorter hospital stay of the DCD donors[87].

Two larger studies have been presented results using controlled, category 3 DCD pancreas islet isolations. We compared 93 category 3 DCD and 193 DBD isolations and found a reduction of about 100,000 IEQ resulting in an overall 25% lower islet yield [88]. These data are in line with a large study from Australia comparing 27 DCD and 73 DBD isolations where also 100,000 IEQ less was isolated from DCD donors, resulting in a 33% lower islet yield.[89].

Several studies have emerged from controlled DCD procedures from Japan, which probably involved category 4 DCD, using the rapid *in situ* regional organ cooling technique with excellent results[50,91–93]. With these very short warm ischemia times, initial results from 10 pancreas yielded a mean IEQ > 400,000[92]. In 2010 the Japanese Islet Transplant Registry presented their cumulative results from 64 DCD isolations[94]. This study reported an average 427,119 IEQ for the 35 transplanted pancreas and 270,278 IEQ for the 30 pancreas that were not transplanted.

There has been one report on the use of uncontrolled (closest resembling category 1) DCD donors for islet isolation[95]. In that study potential donors were out-of-hospital deaths, less than 50 years old, with less than 15 min of cardiac arrest without cardiac massage, with a known etiology of death, and without general contraindications for donation. After arrival in the hospital, a cardiopulmonary bypass was performed and maintained for approximately 4 hours before organ retrieval. There is mention of 31 pancreas used for islet isolation, although no further details are given.

Islet in vitro function after islet isolation from DCD pancreas

Although the acceptable long-term endocrine function after pancreas transplantation is reassuring, there are concerns related to the hypoxic damage to the islets during the warm ischemia time. Pancreatic islets are highly vascularized and receive approximately 10% of blood flow while constituting only 1-2% of pancreas mass[96]. Among the most metabolically active cells in the human body, insulin-producing islet cells are very sensitive to dysfunction following hypoxic conditions[97]. Also, the isolation process itself elicits stress to islets[98]. Proinflammatory cytokines are released and apoptosis occurs during the isolation[99]. The additional stress from hypoxia during procurement could add to functional impairment after isolation [100,101]. Indeed, in a porcine model 30 minutes of warm ischemia impaired islet function after isolation[102].

Few data exist on the functionality of isolated human DCD islets *in vitro*. We showed no difference in insulin response to a dynamic glucose stimulated insulin secretion (GSIS) test in isolated islets from 93 controlled DCD pancreas[88]. Zhao et al[87] compared islet characteristics after 10 DCD and 12 DBD islet isolations and found that islets in both groups were similar *in vitro* with regard to morphology, viability, insulin release and β-cell ultrastructure. Importantly, ATP and GTP content were lower in the DCD islets which correlated inversely to longer warm ischemia times. This has led to concerns that DCD islets possibly engraft more poorly in portal vein transplantation[103].

The proportion of category 5 DCD versus category 3 DCD has been steadily rising in the Netherlands: from 0.6% of all DCD procedures in 2014 to 4% in 2018. Out of all allocated DCD pancreas for islet isolation to our center the proportion of category 5 DCD pancreas has increased from 2.5% in 2014 to almost 18% in 2018[104]. There has been concern that the use of rocuronium as a muscle relaxant in the euthanasia procedure may interact with paracrine signaling in islets cells for maintaining glucose homeostasis[105,106]. We presented that islet yield in terms of IEQ and IEQ/gram pancreas was similar between category 5 DCD (n=16) and category 3 DCD pancreas (n=48). However, preliminary data indicate that insulin release from category 5 DCD islets after a glucose challenge is impaired *in vitro*[104].

Clinical outcome of islet transplantation using DCD pancreas

Controlled DCD islet transplantation was first reported in 2003[51]. Immediate graft function was observed with a reduction in daily insulin requirement, improved glycemic control, absence of hypoglycemic events, and a normal C-peptide level. Insulin independence followed at day 52.

Since then the scarce reports on clinical outcome of DCD islet transplantations showed no obvious difference. One study examined insulin independence and the decrease in insulin requirement at 1-month post-transplantation. No difference was found for the DCD group (n=9) compared to the DBD group (n=196)[82]. In the Netherlands, three months post-transplantation mixed meal tests were given to recipients receiving a single or combined (single DBD, single DCD, double DBD, double DCD, or combined DBD and DCD, n=29). No differences in C-peptide production or blood glucose level during the mixed meal test were found among the groups[88].

With only controlled category 4 DCD pancreas used in the Japanese Islet Transplantation Registry, 18 recipients received DCD islet infusions[94]. Ten of these recipients received more than one islet infusions. After three years, 33.6% maintained a C-peptide level more than or equal to 0.3 ng/mL. All recipients remained free of severe hypoglycemic events and three achieved insulin independence for 14, 79, and 215 days. There is no data on clinical outcome of uncontrolled DCD islet transplantation.

Future Perspectives

With the rekindled interest in DCD transplantation, more attention is being paid to improve the quality of the procured organs. Because of the period of warm ischemia and hypoxia during DCD procurement, methods to improve preservation and combat hypoxic damage are being investigated.

Before the start of islet isolation, the pancreas is subject to multiple warm and cold ischemic insults (figure 2). After perfusion of cold preservation solution there is a period of so-called lukewarm ischemia time until pancreatectomy. Despite using copious amounts of ice for packing the abdominal cavity and omenta, we measured pancreas temperatures of approximately 18°C (unpublished results). We found no correlation between graft dysfunction and extended lukewarm ischemia time (unpublished results). After pancreatectomy and putting the pancreas on ice which is the start of the cold ischemia time, the pancreas is transported to the human islet isolation unit until perfusion with enzymes commences and the cold ischemia time ends.

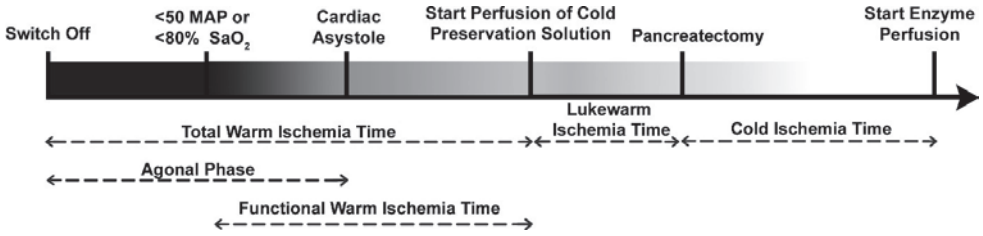

Figure 2. Warm and cold ischemic time intervals during a DCD procedure until the start of enzyme infusion during the islet isolation procedure. The greyscale gradient becomes increasingly lighter, representing cooler temperatures. Critical moments in both warm ischemia and cold ischemia periods are indicated with vertical lines. Below the bar definitions of the different relevant time periods are provided.

The static cold storage of pancreas in UW solution before islet isolation has been the gold standard for the past 25 years[107]. Other solutions have been used but comparisons of preservation solutions have mainly focused on DBD pancreas[108–113]. Retrospective analysis

has shown no difference in islet yield, *in vitro* function (GSIS), viability, and *in vivo* function (insulin need, C-peptide:glucose ratio, β-score) when the pancreas was preserved in either Institut Georges Lopez-1 (IGL-1, n=95), UW (n=204), or Celsior (n=77). The most optimal solution for static cold storage of DCD pancreas for islet isolation has yet to be established. Due to the increased ischemic damage, the optimal preservation solution for DCD pancreas may turn out to be different from DBD pancreas.[114]. A new solution, SCOT 15, contains a polyethylene glycol colloid and a low K^+ concentration (to avoid cell membrane depolarization) and ATP depletion[115]. It is hypothesized that this solution would be more favorable for DCD pancreas as it is thought to be more immunoprotective to pancreas subjected to warm ischemia[116].

In an effort to deliver oxygen to the pancreas tissue during preservation to combat hypoxia, a two-layer method was developed that involves the suspension of the pancreas at the interface between a layer of oxygenated perfluorcarbon (PFC) solution of and a layer of UW solution[117]. This interface is achieved by a specific gravity difference between the two solutions and a grid is used to keep the pancreas, which is devoid of excess fat, duodenum and spleen, in place[117]. It was shown that by increasing oxygen tension in the PFC layer, tissue ATP content was elevated after 3-5 hours of preservation (at 20°C)[118]. Initially some groups reported favorable results on islet yield using the two-layer method[119–121]. But detailed analyses have shed doubt on whether these improvements result from the oxygenated PFC layer[122–124]. A randomized study in 200 pancreas comparing the two-layer method and only UW storage showed no difference in islet yield, isolation purity, GSIS, or *in vivo* function in the recipient[124]. In agreement with these observations a mathematical model showed that oxygen penetration into pancreatic tissue is only 1 mm using the two-layer method at 8°C[123]. It has been speculated that early promising results are due to additional attention during packing and handling[125]. An alternative, more simple technique is the use of perfluorohexyloctane emulsified in polydimethylsiloxane in UW solution[126]. More oxygen can be stored in the solution and the lipophilicity is believed to improve penetration of oxygen into the tissue. The lower density of the solution, which is similar to that of the pancreas, avoids the use of custom-made transport vessels, as required for the two-layer method.

Another method to optimize pancreas quality after pancreas procurement from expanded criteria donors including DCD donors could be machine perfusion. Hypothermic machine perfusion (HMP) of the pancreas was first attempted in the 1970's and soon afterwards the

first pancreatic islet isolations were performed after hypothermic pancreas perfusion[127,128]. Formation of edema due to inaccurate pressure maintenance was linked to poorer isolation results[129,130]. Interest faded during the following decades until the appearance of next generation machinery. Using very low flow rates (20-32 mL/min) and pressure (2-30 mmHg) it was shown that a reasonable islet yield could be achieved[131,132]. Also edema in the peri-insular area surrounding the islets may actually be beneficial in the process of islet isolation by reducing the amount of trapped islets by providing a disrupted extracellular space for digestive enzymes[133]. HMP has been shown to raise ATP content of DCD pancreas to the level of DBD pancreas[134]. Recently, the first case of human islet transplantation after hypothermic machine perfusion was presented[135]. In that report sixteen pancreas were perfused with HTK. Half of the pancreas were oxygenated during HMP via the splenic and superior mesenteric arteries at a pressure of 25 mmHg using a custom made peristaltic pump. One pancreas (DBD) yielded 703,746 IEQ and was used was for allogeneic transplantation. The recipient did not become insulin independent.

Normothermic machine perfusion (NMP) is a technique using a pancreas preservation solution at 34-37°C. As the pancreas becomes metabolically active, oxygenation and nutrient administration become essential to increase energy reserves and prevent ischemia. Recent studies have shown that normothermic machine perfusion (NMP) is a promising technique for the preservation of DCD livers[136], kidneys[137], and hearts[138]. Additionally, NMP could be used to recondition the pancreas using mesenchymal stromal cells[139] or gene editing[140] prior to islet isolation. Barlow et al.[141] performed NMP on 4 human pancreas (3 DBD and 1 DCD) with cold ischemic times longer than would be acceptable for clinical transplantation.. In all pancreas blood flow and pH was maintained throughout perfusion and insulin release was shown. However, all pancreas showed signs of edema and necrosis after NMP. No pancreas were transplanted and islets were not isolated in this study. It has been proposed that the sensitivity of the pancreas to cold ischemia may have played a role in the observed edema and necrosis in this study and a transportable NMP system may be key in achieving success[142].

Especially interesting to marginal DCD organs is the emerging technique of normothermic regional perfusion (NRP). First described in uncontrolled DCD donors[143], this technique has also been used in controlled DCD donors[144]. After the no-touch period, when normally the perfusion of cold preservation solution would be administered (see figure 2), the aorta (or common iliac artery) and inferior vena cava are cannulated. An ECMO (Extra Corporeal

Membrane Oxygenation)-like perfusion system is then started using the donor's own blood[145]. Successful pancreas transplantations[144,146–148] and islet isolations[144] have been documented. Strong evidence of the beneficial effects of NRP in kidney and liver transplantation will likely spur more research into the use of NRP pancreas for islet isolation and transplantation[41].

Alternatively, persufflation, may be a simpler method of pancreatic tissue preservation. In this method, after perfusion with preservation solution, the native pancreas vasculature is cannulated and perfused with a gaseous oxygen mixture. Theoretically, more oxygen per gram can be delivered than through HMP[149]. Using a DCD rat venous oxygen persufflation model, a higher islet yield, higher islet viability and an improved islet morphology (larger, more well-rounded islets) were achieved compared to static cold storage or hypothermic machine perfusion[150]. It has been shown using magnetic resonance spectroscopy, that the bioenergetic status was higher in persufflation storage than in static cold storage or in two-layer method stored human pancreas[151].

Conclusion

As the number of high quality organs dwindles there is an increasing pressure to use organs from expanded criteria donors including DCD donors. There may be a somewhat lower islet yield from DCD pancreas, but viability and functionality *in vitro* and *in vivo* appear to be largely maintained. The increasing use of organs from expanded criteria donors also spurs interest in the field of organ preservation techniques and replenishment of energy reserves. Advances in these fields can stimulate the use of DCD pancreas for islet isolation and increase the much needed donor pool.

References

1. Salvalaggio PR, Davies DB, Fernandez LA, Kaufman DB. Outcomes of pancreas transplantation in the United States using cardiac-death donors. *Am J Transpl*. 2006;6(5 Pt 1):1059-1065. doi:10.1111/j.1600-6143.2006.01310.x
2. Proneth A, Schnitzbauer AA, Zeman F, et al. Extended pancreas donor program – the EXPAND study rationale and study protocol. *Transplant Res*. 2013;2(1):12. doi:10.1186/2047-1440-2-12
3. Ris F, Toso C, Veith FU, Majno P, Morel P, Oberholzer J. Are criteria for islet and pancreas donors sufficiently different to minimize competition? *Am J Transplant*. 2004;4(5):763-766. doi:10.1111/j.1600-6143.2004.00409.x
4. Transplantation from donors after circulatory death: British Transplantation Society Guidelines. http://bts.org.uk/wp-content/uploads/2016/09/15_BTS_Donors_DCD.pdf. Published 2015. Accessed July 16, 2019.
5. EuroTransplant Pancreas Allocation System (EPAS). http://www.eurotransplant.org/cms/mediaobject.php?file=chapter7_epas1.pdf. Published 2012. Accessed July 16, 2019.
6. Reese PP, Boudville N, Garg AX. Living kidney donation: Outcomes, ethics, and uncertainty. *Lancet*. 2015;385(9981):2003-2013. doi:10.1016/S0140-6736(14)62484-3
7. Schwartz R, Dameshek W. Drug-induced Immunological Tolerance. *Nature*. 1959;183(4676):1682-1683.
8. Murray JE, Merrill JP, Harrison JH, Wilson RE, Dammin GJ, Waldemar G. Johanson, M.D., Alan K. Pierce, M.D. PSMD. Prolonged survival of human-kidney homografts by immunosuppressive drug therapy. *N Engl J Med*. 1963;281(24):1315-1323. doi:10.1056/NEJM196306132682401
9. Merrill JP, Murray JE, Harrison JH, Friedman EA, Dealy JB, Dammin GJ. Successful Homotransplantation of the Kidney between Nonidentical Twins. *N Engl J Med*. 1960;281(25):1251-1260. doi:10.1056/NEJM196006232622501
10. Truog RD, Miller FG. The Dead Donor Rule and Organ Transplantation. *N Engl J Med*. 2008;359(7):674-675. doi:10.1056/NEJMp0804474
11. Detry O, Le Dinh H, Noterdaeme T, et al. Categories of donation after cardiocirculatory death. *Transplant Proc*. 2012;44(5):1189-1195. doi:10.1016/j.transproceed.2012.05.001
12. Ad Hoc Committee of the Harvard Medical School. A Definition of Irreversible Coma. *JAMA*. 1968;205(6):85-88.
13. Morrissey PE, Monaco AP. Donation After Circulatory Death: Current Practices, Ongoing Challenges, and Potential Improvements. *Transplantation*. 2014;97(3):258-264. doi:10.1097/01.TP
14. van Loo ES, Krikke C, Hofker HS, Berger SP, Leuvenink HGD, Pol RA. Outcome of pancreas transplantation from donation after circulatory death compared to donation after brain death. *Pancreatology*. 2017;17(1):13-18. doi:10.1016/j.pan.2016.11.002
15. Wijdicks EFM, Varelas PN, Gronseth GS, Greer DM. Evidence-based guideline update: Determining brain death in adults: Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*. 2010;74(23):1911-1918. doi:10.1212/WNL.0b013e3181e242a8
16. Anderson TA, Bekker P, Vagefi PA. Anesthetic considerations in organ procurement surgery: a narrative review. *Can J Anesth*. 2015;62(5):529-539. doi:10.1007/s12630-015-0345-8
17. Starzl TE, Hakala TR, Shaw BW, et al. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet*. 1984;158(3):223-230. doi:10.1016/j.bbi.2008.05.010
18. McKeown DW, Bonser RS, Kellum JA. Management of the heartbeating brain-dead organ donor. *Br J Anaesth*. 2012;108(SUPPL. 1):96-107. doi:10.1093/bja/aer351
19. Lopau K, Mark J, Schramm L, Heidbreder E, Wanner C. Hormonal changes in brain death and immune activation in the donor. *Transpl Int*. 2000;13 [Suppl:S282-5. doi:10.1007/s001470050342
20. Nijboer WN, Schuurs TA, van der Hoeven JAB, et al. Effect of Brain Death on Gene Expression and Tissue Activation in Human Donor Kidneys. *Transplantation*. 2004;78(7):978-986.

doi:10.1097/01.TP.0000135565.49535.60
21. Amado JA, López-Espadas F, Vázquez-Barquero A, et al. Blood levels of cytokines in brain-dead patients: Relationship with circulating hormones and acute-phase reactants. *Metabolism*. 1995;44(6):812-816. doi:10.1016/0026-0495(95)90198-1
22. Koo DDH, Welsh KI, McLaren AJ, Roake JA, Morris PJ, Fuggle S V. Cadaver versus living donor kidneys: Impact of donor factors on antigen induction before transplantation. *Kidney Int*. 1999;56(4):1551-1559. doi:10.1046/j.1523-1755.1999.00657.x
23. Nijboer WN, Moers C, Leuvenink HGD, Ploeg RJ. How important is the duration of the brain death period for the outcome in kidney transplantation? *Transpl Int*. 2011;24(1):14-20. doi:10.1111/j.1432-2277.2010.01150.x
24. Organ Donation and Transplantation Statistics. https://www.organdonor.gov/statistics-stories/statistics/data.html. Accessed November 25, 2018.
25. Miñambres E, Rubio JJ, Coll E, Domínguez-Gil B. Donation after circulatory death and its expansion in Spain. *Curr Opin Organ Transplant*. 2018;23(1):120-129. doi:10.1097/MOT.0000000000000480
26. Benjamins S, Margreiter C, de Koning EJ, Leuvenink HGD, Pol RA. Decline in Pancreas TransplantationNumbers is Accompanied with Lower Publication Rates. *Transplantation*. 2018;102(7S):S78-S79.
27. Krieger NR, Odorico JS, Heisey DM, et al. Underutilization of pancreas donors. *Transplantation*. 2003;75(8):1271-1276. doi:10.1097/01.TP.0000061603.95572.BF
28. Stegall MD, Dean PG, Sung R, et al. The rationale for the new deceased donor pancreas allocation schema. *Transplantation*. 2007;83(9):1156-1161. doi:10.1016/j.amc.2010.05.089
29. Domínguez-Gil B, Haase-Kromwijk B, Leiden H Van, et al. Current situation of donation after circulatory death in European countries. *Transpl Int*. 2011;24:676-686. doi:10.1111/j.1432-2277.2011.01257.x
30. Kootstra G, Daemen JH, Oomen AP. Categories of non-heart-beating donors. *Transplant Proc*. 1995;27(5):2893-2894. http://www.ncbi.nlm.nih.gov/pubmed/7482956. Accessed November 16, 2015.
31. Donation after circulatory death in Spain: Current situation and recommendations. National Consensus Document. [In Spanish]. Available at: http://www.ont.es/infesp/DocumentosDeConsenso/donación en asistolia en españa. Situación actual y recomendaciones.pdf. Published 2012.
32. Allen MB, Billig E, Reese PP, et al. Donor Hemodynamics as a Predictor of Outcomes After Kidney Transplantation From Donors After Cardiac Death. *Am J Transplant*. September 2015:n/a-n/a. doi:10.1111/ajt.13432
33. Peters-Sengers H, Houtzager JHE, Heemskerk MBA, et al. DCD donor hemodynamics as predictor of outcome after kidney transplantation. *Am J Transplant*. 2018;18(8):1966-1976. doi:10.1111/ajt.14676
34. Nederlandse Transplantatie Stichting. Protocol for deceased organ and tissue donation [In Dutch]. https://www.transplantatiestichting.nl/sites/default/files/modelprotocol_post-mortale_orgaan-_en_weefseldonatie.pdf.
35. National Protocol for Donation after Cardiac Death. Australian Government: National Health and Medical Research Council. http://www.donatelife.gov.au/sites/default/files/files/DCD protocol 020311-0e4e2c3d-2ef5-4dff-b7ef-af63d0bf6a8a-1.PDF. Published 2010.
36. Bradley JA, Pettigrew GJ, Watson CJ. Time to death after withdrawal of treatment in donation after circulatory death (DCD) donors. *Curr Opin Organ Transplant*. 2013;18(2):133-139. doi:10.1097/MOT.0b013e32835ed81b
37. Manara AR, Murphy PG, Ocallaghan G. Donation after circulatory death. *Br J Anaesth*. 2012;108(SUPPL. 1):i108-i121. doi:10.1093/bja/aer357
38. Sohrabi S, Navarro A, Wilson C, et al. Renal Graft Function After Prolonged Agonal Time in Non-Heart-Beating Donors. *Transplant Proc*. 2006;38(10):3400-3401.

doi:10.1016/j.transproceed.2006.10.080
39. Rhee JY, Alroy J, Freeman RB. Characterization of the withdrawal phase in a porcine donation after the cardiac death model. *Am J Transplant*. 2011;11(6):1169-1175. doi:10.1111/j.1600-6143.2011.03567.x
40. le Dinh H, de Roover A, Kaba A, et al. Donation after cardio-circulatory death liver transplantation. *World J Gastroenterol*. 2012;18(33):4491-4506. doi:10.3748/wjg.v18.i33.4491
41. Berney T, Boffa C, Augustine T, et al. Utilization of organs from donors after circulatory death for vascularized pancreas and islet of Langerhans transplantation: Recommendations from an expert group. *Transpl Int*. 2015:7-9. doi:10.1111/tri.12681
42. Coffey JC, Wanis KN, Monbaliu D, et al. The influence of functional warm ischemia time on DCD liver transplant recipients' outcomes. *Clin Transplant*. 2017;31(10):1-6. doi:10.1111/ctr.13068
43. Abt PL, Praestgaard J, West S, Hasz R. Donor hemodynamic profile presages graft survival in donation after cardiac death liver transplantation. *Liver Transplant*. 2014;20(2):165-172. doi:10.1002/lt.23777
44. Muthusamy ASR, Mumford L, Hudson A, Fuggle S V., Friend PJ. Pancreas transplantation from donors after circulatory death from the United Kingdom. *Am J Transplant*. 2012;12(8):2150-2156. doi:10.1111/j.1600-6143.2012.04075.x
45. Sánchez-Fructuoso AI, Prats D, Torrente J, et al. Renal transplantation from non-heart beating donors: a promising alternative to enlarge the donor pool. *J Am Soc Nephrol*. 2000;11(2):350-358. http://www.ncbi.nlm.nih.gov/pubmed/10665943. Accessed August 7, 2018.
46. van Heurn LWE, Talbot D, Nicholson ML, et al. Recommendations for donation after circulatory death kidney transplantation in Europe. *Transpl Int*. 2016;29(7):780-789. doi:10.1111/tri.12682
47. Snoeijs MGJ, Dekkers AJE, Buurman WA, et al. In situ preservation of kidneys from donors after cardiac death: Results and complications. *Ann Surg*. 2007;246(5):844-852. doi:10.1097/SLA.0b013e318142cb1b
48. Noguchi H. Pancreas procurement and preservation for islet transplantation: personal considerations. *J Transplant*. 2011;2011:783168. doi:10.1155/2011/783168
49. Nagata H, Matsumoto S, Okitsu T, et al. Procurement of the human pancreas for pancreatic islet transplantation from marginal cadaver donors. *Transplantation*. 2006;82(3):327-331. doi:10.1097/01.tp.0000228886.15985.62
50. Matsumoto S, Okitsu T, Iwanaga Y, et al. Successful islet transplantation from nonheartbeating donor pancreata using modified Ricordi islet isolation method. *Transplantation*. 2006;82(4):460-465. doi:10.1097/01.tp.0000231710.37981.64
51. Markmann JF, Deng S, Desai NM, et al. The use of non-heart-beating donors for isolated pancreatic islet transplantation. *Transplantation*. 2003;75(9):1423-1429. doi:10.1097/01.TP.0000061119.32575.F4
52. Clayton H, Swift S, Turner J, James R, Bell PRF. Non-heart-beating Organ Donors: A Potential Source of Islets for Transplantation? *Transplantation*. 2000;69(May):2094-2098.
53. Global Observatory on Donation and Transplantation. http://www.transplant-observatory.org/countdcd/. Published 2017.
54. Gómez MP, Pérez B, Manyalich M. International Registry in Organ Donation and Transplantation—2013. *Transplant Proc*. 2014;46(4):1044-1048. doi:10.1016/j.transproceed.2013.11.138
55. Bendorf A, Kelly PJ, Kerridge IH, et al. An international comparison of the effect of policy shifts to organ donation following cardiocirculatory death (DCD) on donation rates after brain death (DBD) and transplantation rates. *PLoS One*. 2013;8(5):e62010. doi:10.1371/journal.pone.0062010
56. Chen GD, Shiu-Chung Ko D, Wang CX, et al. Kidney transplantation from donors after cardiac death: An initial report of 71 cases from China. *Am J Transplant*. 2013;13(5):1323-1326. doi:10.1111/ajt.12190
57. Rudge C, Matesanz R, Delmonico FL, Chapman J. International practices of organ donation.

Br J Anaesth. 2012;108(SUPPL. 1):i48-i55. doi:10.1093/bja/aer399
58. Eurotransplant. Donors used in Netherlands, by year, by donor type. statistics.eurotransplant.org: 1092P_Netherlands: 10.04.2015.
59. Johnson S, Forsythe J, Gardiner D. *Organ Donation and Transplantation Activity Report 2017/18*.; 2018. http://www.odt.nhs.uk. Accessed August 8, 2018.
60. Talbot D, D'Alessandro AM, Muiesan P. *Organ Donation and Transplantation after Cardiac Death*. Oxford University Press; 2013. doi:10.1093/med/9780199217335.001.0001
61. Ruijs CDM, Van Der Wal G, Kerkhof AJFM, Onwuteaka-Philipsen BD. Unbearable suffering and requests for euthanasia prospectively studied in end-of-life cancer patients in primary care. *BMC Palliat Care*. 2014;13(1):1-11. doi:10.1186/1472-684X-13-62
62. Verkerk M, van Wijlick E, Legemaate J, de Graeff A. A National Guideline for Palliative Sedation in The Netherlands. *J Pain Symptom Manage*. 2007;34(6):666-670. doi:10.1016/j.jpainsymman.2007.01.005
63. KNMG/ KNMP. 2012 Guidelines for the Practice of Euthanasia and Physician-Assisted Suicide. https://www.knmg.nl/web/file?uuid=c56c038c-ffcd-486e-a774-07f7de104f94&owner=5c945405-d6ca-4deb-aa16-7af2088aa173&contentid=223&elementid=152959. Published 2012.
64. Wai P, Kim J, Niederhaus S, et al. Donation after cardiac death: A 29-year experience. *Surgery*. 2011;150(4):692-702. doi:10.1016/j.surg.2011.07.057
65. Summers DM, Watson CJE, Pettigrew GJ, et al. Kidney donation after circulatory death (DCD): state of the art. *Kidney Int*. 2015;88(2):241-249. doi:10.1038/ki.2015.88
66. Mateo R, Cho Y, Singh G, et al. Risk Factors for Graft Survival After Liver Transplantation from Donation After Cardiac Death Donors: An Analysis of OPTN/UNOS Data. *Am J Transplant*. 2006;6(4):791-796. doi:10.1111/j.1600-6143.2006.01243.x
67. Monbaliu D, Pirenne J, Talbot D. Liver transplantation using Donation after Cardiac Death donors. *J Hepatol*. 2012;56(2):474-485. doi:10.1016/j.jhep.2011.07.004
68. *NHS Interim Report On Pancreas And Islet Transplantation 5 Year Report (1 October 2012 – 30 September 2017)*.; 2018.
69. O'Gorman D, Kin T, Murdoch T, et al. The Standardization of Pancreatic Donors for Islet Isolations. *Transplantation*. 2005;80(6):801-806. doi:10.1097/01.tp.0000172216.47547.d5
70. Wang LJ, Kin T, Gorman DO, et al. A Multicenter Study: North American Islet Donor Score in Donor Pancreas Selection for Human Islet Isolation for Transplantation. *Cell Transplant*. 2016;25(8):1-30. doi:10.3727/096368916X691141
71. El-Shahawy M, Luis V, Dafoe D, et al. Sodium levels of human pancreatic donors are a critical factor for determination of islet efficacy and survival. *Am J Physiol Metab*. 2014;308(5):E362-E369. doi:10.1152/ajpendo.00443.2014
72. Vinkers MT, Rahmel AO, Slot MC, Smits JM, Schareck WD. How to Recognize a Suitable Pancreas Donor: A Eurotransplant Study of Preprocurement Factors. *Transplant Proc*. 2008;40(5):1275-1278. doi:10.1016/j.transproceed.2008.03.142
73. Woeste G, Moench C, Hauser IA, Geiger H, Scheuermann E, Bechstein WO. Can the preprocurement pancreas suitability score predict ischemia-reperfusion injury and graft survival after pancreas transplantation? *Transplant Proc*. 2010;42(10):4202-4205. doi:10.1016/j.transproceed.2010.09.021
74. Schenker P, Vonend O, Ertas N, Wunsch A, Viebahn R. Preprocurement Pancreas Allocation Suitability Score Does Not Correlate With Long-Term Pancreas Graft Survival. *Transplant Proc*. 2010;42(1):178-180. doi:10.1016/j.transproceed.2009.12.036
75. Axelrod DA, Sung RS, Meyer KH, Wolfe RA, Kaufman DB. Systematic evaluation of pancreas allograft quality, outcomes and geographic variation in utilization. *Am J Transplant*. 2010;10(4):837-845. doi:10.1111/j.1600-6143.2009.02996.x
76. Blok JJ, Kopp WH, Verhagen MJ, et al. The Value of PDRI and P-PASS as Predictors of Outcome After Pancreas Transplantation in a Large European Pancreas Transplantation Center.

Pancreas. 2016;45(3):331-336. doi:10.1097/MPA.0000000000000485
77. Kopp WH, Verhagen MJJ, Blok JJ, et al. Thirty Years of Pancreas Transplantation at Leiden University Medical Center: Long-term Follow-up in a Large Eurotransplant Center. *Transplantation*. 2015;99(9):e145-e151. doi:10.1097/TP.0000000000000604
78. Lakey JR, Warnock GL, Rajotte R V, et al. Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation*. 1996;61(7):1047-1053. doi:10.1097/00007890-199604150-00010
79. Nano R, Clissi B, Melzi R, et al. Islet isolation for allotransplantation: Variables associated with successful islet yield and graft function. *Diabetologia*. 2005;48:906-912. doi:10.1007/s00125-005-1725-3
80. Doppenberg J, Kopp W, Putter H, Braat A, Engelse M, de Koning E. Islet Donor Risk Score: an evidence-based IEQ prediction model. In: *6th EPITA Symposium & 35th AIDPIT Workshop*. Innsbruck; 2016. https://link.springer.com/content/pdf/10.1007%2Fs00592-015-0831-z.pdf.
81. Remuzzi G, Grinyò J, Ruggenenti P, et al. Early experience with dual kidney transplantation in adults using expanded donor criteria. Double Kidney Transplant Group (DKG). *J Am Soc Nephrol*. 1999;10(12):2591-2598. http://www.ncbi.nlm.nih.gov/pubmed/10589699. Accessed August 8, 2018.
82. Andres A, Kin T, O'Gorman D, et al. Clinical islet isolation and transplantation outcomes with deceased cardiac death donors are similar to neurological determination of death donors. *Transpl Int*. 2015;29(1):34-40. doi:10.1111/tri.12650
83. Munksgaard B, Bernat JL, D'Alessandro AM, et al. Report of a National Conference on Donation after Cardiac Death. *Am J Transplant*. 2006;6:281-291. doi:10.1111/j.1600-6143.2005.01194.x
84. Tanioka Y, Hering BJ, Sutherland DE, et al. Effect of pancreatic warm ischemia on islet yield and viability in dogs. *Transplantation*. 1997;64(12):1637-1641. http://www.ncbi.nlm.nih.gov/pubmed/9422394. Accessed July 30, 2015.
85. Andersson A, Borg H, Groth CG, et al. Survival of isolated human islets of Langerhans maintained in tissue culture. *J Clin Invest*. 1976;57(5):1295-1301. doi:10.1172/JCI108397
86. Rilo H, Carroll P, Trucco M, et al. Human pancreatic islet isolation from a diabetic non-heart-beating donor. *Transpl Proc*. 1994;26:598.
87. Zhao M, Muiesan P, Amiel SA, et al. Human islets derived from donors after cardiac death are fully biofunctional. *Am J Transplant*. 2007;7(10):2318-2325. doi:10.1111/j.1600-6143.2007.01937.x
88. Doppenberg J, Putter H, Nijhoff M, Engelse M, de Koning E. Good Functionality But Lower Yield After Islet Isolation From Donation After Circulatory Death Pancreata. In: *2015 IPITA-IXA-CTS Joint Congress*. 2016.
89. Hawthorne WJ, Chew YV, Haron C, et al. Outcomes for Islet Transplantation in Donation After Circulatory Death compared with Donation after Brain Death in Australia. In: *27th International Meeting of the Transplantation Society*. ; 2018.
90. London NJ, Swift SM, Clayton HA. Isolation, culture and functional evaluation of islets of Langerhans. *Diabetes Metab*. 1998;24(3):200-207. http://www.ncbi.nlm.nih.gov/pubmed/9690051.
91. Matsumoto S, Yamada Y, Okitsu T, et al. Simple evaluation of engraftment by secretory unit of islet transplant objects for living donor and cadaveric donor fresh or cultured islet transplantation. *Transplant Proc*. 2005;37(8):3435-3437. doi:10.1016/j.transproceed.2005.09.045
92. Matsumoto S, Tanaka K. Pancreatic islet cell transplantation using non-heart-beating donors (NHBDs). *J Hepatobiliary Pancreat Surg*. 2005;12(3):227-230. doi:10.1007/s00534-005-0978-z
93. Liu X, Matsumoto S, Okitsu T, et al. Analysis of donor- and isolation-related variables from non-heart-beating donors (NHBDs) using the Kyoto islet isolation method. *Cell Transplant*. 2008;17:649-656. doi:10.3727/096368908786092711
94. Saito T, Gotoh M, Satomi S, et al. Islet transplantation using donors after cardiac death: report of the Japan Islet Transplantation Registry. *Transplantation*. 2010;90(7):740-747. doi:10.1097/TP.0b013e3181ecb044

95. Alvarez J, del Barrio R, Arias J, et al. Non-Heart-Beating Donors From The Streets: An Increasing Donor Pool Source. *Transplantation*. 2000;70(July):314-317.
96. Bonner-Weir S. The microvasculature of the pancreas, with emphasis on that of the islets of Langerhans: anatomy and functional considerations. In: Go VLW, Dimagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA, eds. *The Pancreas: Biology, Pathobiology and Disease*. Vol 2. New York: Raven Press; 1993:759-768.
97. Gerber PA, Rutter GA. The Role of Oxidative Stress and Hypoxia in Pancreatic Beta-Cell Dysfunction in Diabetes Mellitus. *Antioxid Redox Signal*. 2017;26(10):501-518. doi:10.1089/ars.2016.6755
98. Bottino R, Balamurugan AN, Tse H, et al. Response of human islets to isolation stress and the effect of antioxidant treatment. *Diabetes*. 2004;53(10):2559-2568. doi:10.2337/diabetes.53.10.2559
99. Paraskevas S, Maysinger D, Wang R, Duguid WP, Rosenberg L. Cell loss in isolated human islets occurs by apoptosis. *Pancreas*. 2000;20(3):270-276. doi:10.1097/00006676-200004000-00008
100. Lakey JRT, Burridge PW, Shapiro AMJ. Technical aspects of islet preparation and transplantation. *Transpl Int*. 2003;16(9):613-632. doi:10.1007/s00147-003-0651-x
101. Pileggi A, Damaris Molano R, Berney T, et al. Heme Oxygenase-1 Induction in Islet Cells Results in Protection from Apoptosis and Improved in Vivo Function after Transplantation. *Diabetes*. 2001;50(9):1983-1991. doi:10.2337/diabetes.50.9.1983
102. Brandhorst D, Iken M, Bretzel RG, Brandhorst H. Pancreas storage in oxygenated perfluorodecalin does not restore post-transplant function of isolated pig islets pre-damaged by warm ischemia. *Xenotransplantation*. 2006;13(5):465-470. doi:10.1111/j.1399-3089.2006.00340.x
103. Matsumoto S, Noguchi H, Yonekawa Y, et al. Pancreatic islet transplantation for treating diabetes. *Expert Opin Biol Ther*. 2006;6(1):23-37. doi:10.1517/14712598.6.1.23
104. Steffen A, Engelse MA, de Koning EJ. Organ donation after Termination of Life on Request and Assisted Suicide: A good source for islet transplantation? In: *8th EPITA Symposium & 37th AIDPIT Workshop*. 2018.
105. Amisten S, Salehi A, Rorsman P, Jones PM, Persaud SJ. An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans. *Pharmacol Ther*. 2013;139(3):359-391. doi:10.1016/j.pharmthera.2013.05.004
106. Somm E. Nicotinic cholinergic signaling in adipose tissue and pancreatic islets biology: Revisited function and therapeutic perspectives. *Arch Immunol Ther Exp (Warsz)*. 2014;62(2):87-101. doi:10.1007/s00005-013-0266-6
107. Squifflet JP, Ledinh H, De Roover A, Meurisse M. Pancreas preservation for pancreas and islet transplantation: A minireview. *Transplant Proc*. 2011;43(9):3398-3401. doi:10.1016/j.transproceed.2011.09.052
108. Potdar S, Malek S, Eghtesad B, et al. Initial experience using histidine-tryptophan-ketoglutarate solution in clinical pancreas transplantation. *Clin Transplant*. 2004;18(6):661-665. doi:10.1111/j.1399-0012.2004.00262.x
109. Englesbe MJ, Moyer A, Kim DY, et al. Early Pancreas Transplant Outcomes with Histidine-Tryptophan-Ketoglutarate Preservation: A Multicenter Study. *Transplantation*. 2006;82(1):136-139. doi:10.1097/01.tp.0000225764.21343.e3
110. Fridell JA, Mangus RS, Powelson JA, Fridell JA. Histidine-Tryptophan-Ketoglutarate for Pancreas Allograft Preservation: The Indiana University Experience. *Am J Transplant*. 2010;10:1284-1289. doi:10.1111/j.1600-6143.2010.03095.x
111. Manrique A, Jiménez C, Herrero ML, et al. Pancreas Preservation With the University of Wisconsin Versus Celsior Solutions. *Transplant Proc*. 2006;38(8):2582-2584. doi:10.1016/j.transproceed.2006.08.058
112. Nicoluzzi J, Macri M, Fukushima J, Pereira A. Celsior Versus Wisconsin Solution in Pancreas Transplantation. *Transplant Proc*. 2008;40(10):3305-3307. doi:10.1016/j.transproceed.2008.05.080
113. Boggi U, Vistoli F, Del Chiaro M, et al. Pancreas preservation with University of Wisconsin and Celsior solutions: a single-center, prospective, randomized pilot study. *Transplantation*.

2004;77(8):1186-1190. http://www.ncbi.nlm.nih.gov/pubmed/15114082. Accessed August 5, 2018.
114. Barlow AD, Hosgood SA, Nicholson ML. Current state of pancreas preservation and implications for DCD pancreas transplantation. *Transplantation*. 2013;95(12):1419-1424. doi:10.1097/TP.0b013e318285558f
115. Hubert T, Gmyr V, Arnalsteen L, et al. Influence of preservation solution on human islet isolation outcome. *Transplantation*. 2007;83(3):270-276. doi:10.1097/01.tp.0000251723.97483.16
116. Giraud S, Hauet T, Eugene M, Mauco G, Barrou B. A New Preservation Solution (SCOT 15) Improves the Islet Isolation Process From Pancreata of Non-Heart-Beating Donors: A Murine Model. *Transplant Proc*. 2009;41(8):3293-3295. doi:10.1016/j.transproceed.2009.08.042
117. Fujino Y, Kuroda Y, Suzuki Y, et al. Preservation of canine pancreas for 96 hours by a modified two-layer (UW solution/perfluorochemical) cold storage method. *Transplantation*. 1991;51(5):1133-1135.
118. Matsumoto S, Kuroda Y, Hamano M, et al. Direct evidence of pancreatic tissue oxygenation during preservation by the two-layer method. *Transplantation*. 1996;62(11):1667-1670. http://www.ncbi.nlm.nih.gov/pubmed/8970625. Accessed August 5, 2018.
119. Noguchi H, Levy MF, Kobayashi N, Matsumoto S. Pancreas preservation by the two-layer method: Does it have a beneficial effect compared with simple preservation in University of Wisconsin solution? *Cell Transplant*. 2009;18(5-6):497-503.
120. Matsumoto S, Rigley TH, Qualley SA, Kuroda Y, Reems JA, Stevens RB. Efficacy of the oxygen-charged static two-layer method for short-term pancreas preservation and islet isolation from nonhuman primate and human pancreata. *Cell Transplant*. 2002;11(8):769-777. http://www.ncbi.nlm.nih.gov/pubmed/12588109.
121. Qin H, Matsumoto S, Klintmalm GB, De Vol EB. A meta-analysis for comparison of the two-layer and university of Wisconsin pancreas preservation methods in islet transplantation. *Cell Transplant*. 2011;20(7):1127-1137. doi:10.3727/096368910X544942
122. Agrawal A, Gurusamy K, Powis S, Gray DW, Fuller B, Davidson BR. A meta-analysis of the impact of the two-layer method of preservation on human pancreatic islet transplantation. *Cell Transplant*. 2008;17(12):1315-1322. doi:10.3727/096368908787648065
123. Papas KK, Hering BJ, Gunther L, Rappel MJ, Colton CK, Avgoustiniatos ES. Pancreas oxygenation is limited during preservation with the two-layer method. *Transplant Proc*. 2005;37(8):3501-3504. doi:10.1016/j.transproceed.2005.09.085
124. Caballero-Corbalán J, Eich T, Lundgren T, et al. No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation. *Transplantation*. 2007;84(7):864-869. doi:10.1097/01.tp.0000284584.60600.ab
125. Kin T, Mirbolooki M, Salehi P, et al. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. *Transplantation*. 2006;82(10):1286-1290. doi:10.1097/01.tp.0000244347.61060.af
126. Brandhorst H, Asif S, Andersson K, et al. A new oxygen carrier for improved long-term storage of human pancreata before islet isolation. *Transplantation*. 2010;89(2):155-160. doi:10.1097/TP.0b013e3181c9266c
127. Toledo Pereyra LH, Valgee KD, Castellanos J, Chee M. Hypothermic Pulsatile Perfusion: Its Use in the Preservation of Pancreases for 24 to 48 Hours Before Islet Cell Transplantation. *Arch Surg*. 1980;115(1):95-98. doi:10.1001/archsurg.1980.01380010081022
128. Tersigni R, Toledo Pereyra LH, Pinkham J, Najarian JS. Pancreaticoduodenal preservation by hypothermic pulsatile perfusion for twenty four hours. *Ann Surg*. 1975;182(6):743-748. doi:10.1097/00000658-197512000-00016
129. Mahler R, Franke FE, Hering BJ, et al. Evidence for a significant correlation of donor pancreas morphology and the yield of isolated purified human islets. *J Mol Med*. 1999;77(1):87-89. doi:10.1007/s001090050308
130. Kaddis JS, Danobeitia JS, Niland JC, Stiller T, Fernandez L a. Multicenter analysis of novel and established variables associated with successful human islet isolation outcomes. *Am J Transplant*.

2010;10(3):646-656. doi:10.1111/j.1600-6143.2009.02962.x
131. Leeser DB, Bingaman AW, Poliakova L, et al. Pulsatile pump perfusion of pancreata before human islet cell isolation. *Transplant Proc*. 2004;36(4):1050-1051. doi:10.1016/j.transproceed.2004.04.041
132. Karcz M, Cook HT, Sibbons P, Gray C, Dorling A, Papalois V. An ex-vivo model for hypothermic pulsatile perfusion of porcine pancreata: Hemodynamic and morphologic characteristics. *Exp Clin Transplant*. 2010;8(1):55-60.
133. Taylor MJ, Baicu S, Leman B, Greene E, Vazquez A, Brassil J. Twenty-Four Hour Hypothermic Machine Perfusion Preservation of Porcine Pancreas Facilitates Processing for Islet Isolation. *Transplant Proc*. 2008;40(2):480-482. doi:10.1016/j.transproceed.2008.01.004
134. Leemkuil M, Engelse M, Ploeg R, de Koning E, Krikke C, Leuvenink H. Hypothermic Machine Perfusion Improves the Quality of Marginal Donor Pancreata. *Am J Transplant*. 2015;15 (suppl(https://atcmeetingabstracts.com/abstract/hypothermic-machine-perfusion-improves-the-quality-of-marginal-donor-pancreata/). https://atcmeetingabstracts.com/abstract/hypothermic-machine-perfusion-improves-the-quality-of-marginal-donor-pancreata/. Accessed August 6, 2018.
135. Yakovets N, Fedoruk A, O, Rummo, et al. The Methods Of Pancreas Preservation Before Isolation Of Islets For Transplantation. *Surg East Eur*. 2016;5(2):247-257.
136. Nasralla D, Coussios CC, Mergental H, et al. A randomized trial of normothermic preservation in liver transplantation. *Nature*. 2018;557(7703):50-56. doi:10.1038/s41586-018-0047-9
137. Hosgood SA, Thompson E, Moore T, Wilson CH, Nicholson ML. Normothermic machine perfusion for the assessment and transplantation of declined human kidneys from donation after circulatory death donors. *Br J Surg*. 2018;105(4):388-394. doi:10.1002/bjs.10733
138. Messer S, Page A, Colah S, et al. Human heart transplantation from donation after circulatory-determined death donors using normothermic regional perfusion and cold storage. *J Hear Lung Transplant*. 2018:1-5. doi:10.1016/J.HEALUN.2018.03.017
139. Orlando G, Murphy S, Bussolati B, et al. *Rethinking Regenerative Medicine From a Transplant Perspective (and Vice Versa)*.; 2018. doi:10.1097/TP.0000000000002370
140. Kuan KG, Wee MN, Chung WY, et al. Extracorporeal machine perfusion of the pancreas: technical aspects and its clinical implications – a systematic review of experimental models. *Transplant Rev*. 2016;30:31-47. doi:10.1016/j.trre.2015.06.002
141. Barlow AD, Hamed MO, Mallon DH, et al. Use of Ex Vivo Normothermic Perfusion for Quality Assessment of Discarded Human Donor Pancreases. *Am J Transplant*. 2015;15(9):2475-2482. doi:10.1111/ajt.13303
142. Dholakia S, Royston E, Sharples EJ, Sankaran V, Ploeg RJ, Friend PJ. Preserving and perfusing the allograft pancreas: Past, present, and future. *Transplant Rev*. 2018;(2017):1-5. doi:10.1016/j.trre.2018.02.001
143. Fondevila C, Hessheimer AJ, Ruiz A, et al. Liver Transplant Using Donors After Unexpected Cardiac Death: Novel Preservation Protocol and Acceptance Criteria. *Am J Transplant*. 2007;7(7):1849-1855. doi:10.1111/j.1600-6143.2007.01846.x
144. Oniscu GC, Randle L V., Muiesan P, et al. In situ normothermic regional perfusion for controlled donation after circulatory death - The United Kingdom experience. *Am J Transplant*. 2014;14(12):2846-2854. doi:10.1111/ajt.12927
145. Johnson LB, Plotkin JS, Howell CD, Njoku MJ, Kuo PC, Bartlett ST. Successful emergency transplantation of a liver allograft from a donor maintained on extracorporeal membrane oxygenation. *Transplantation*. 1997;63(6):910-911. http://www.ncbi.nlm.nih.gov/pubmed/9089236. Accessed August 6, 2018.
146. Miñambres E, Suberviola B, Dominguez-Gil B, et al. Improving the outcomes of organs obtained from controlled donation after circulatory death donors using abdominal normothermic regional perfusion. *Am J Transplant*. 2017;38(1):42-49. doi:10.1111/ajt.14214
147. Farney AC, Singh RP, Hines MH, et al. Experience in Renal and Extrarenal Transplantation with Donation after Cardiac Death Donors with Selective Use of Extracorporeal Support. *J Am Coll*

Surg. 2008;206(5):1028-1037. doi:10.1016/j.jamcollsurg.2007.12.029
148. Magliocca JF, Magee JC, Rowe SA, et al. Extracorporeal support for organ donation after cardiac death effectively expands the donor pool. *J Trauma*. 2005;58(6):1095-1101; discussion 1101-2. doi:10.1097/01.TA.0000169949.82778.DF
149. Suszynski TM, Rizzari MD, Scott WE, Tempelman LA, Taylor MJ, Papas KK. Persufflation (or gaseous oxygen perfusion) as a method of organ preservation. *Cryobiology*. 2012;64(3):125-143. doi:10.1016/j.cryobiol.2012.01.007
150. Reddy MS, Carter N, Cunningham A, Shaw J, Talbot D. Portal Venous Oxygen Persufflation of the Donation after Cardiac Death pancreas in a rat model is superior to static cold storage and hypothermic machine perfusion. *Transpl Int*. 2014;27(6):634-639. doi:10.1111/tri.12313
151. Scott WE, Weegman BP, Ferrer-Fabrega J, et al. Pancreas oxygen persufflation increases ATP levels as shown by nuclear magnetic resonance. *Transplant Proc*. 2010;42(6):2011-2015. doi:10.1016/j.transproceed.2010.05.091