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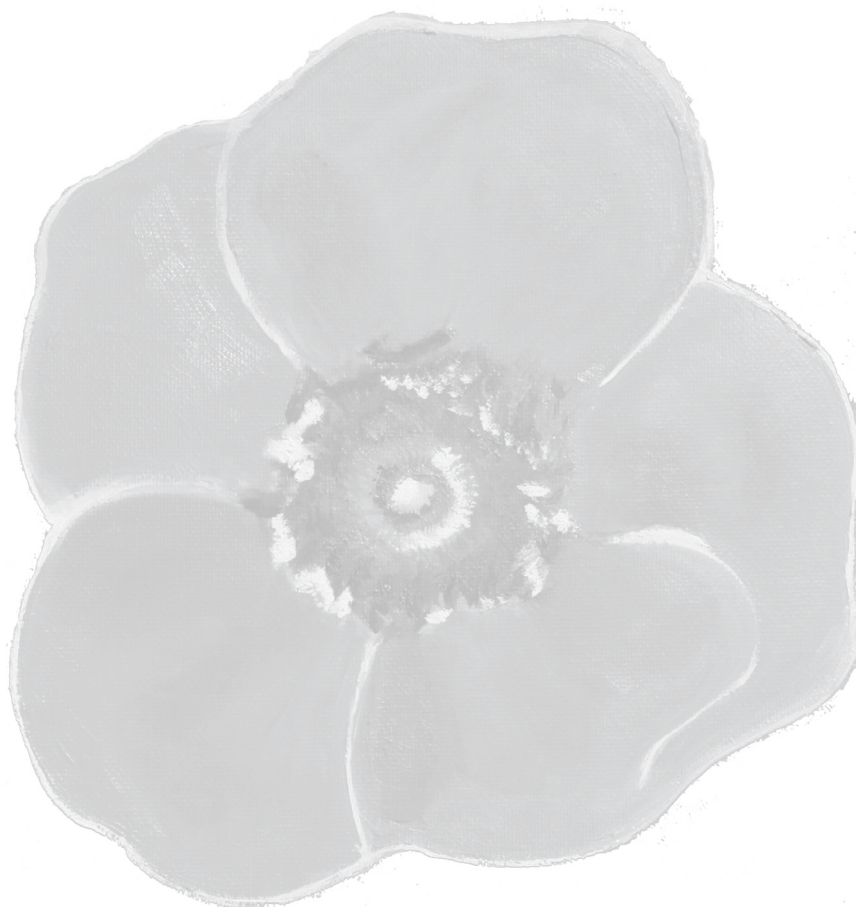
Author: Hilling, Denise Eline

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Chapter 9

Summary and discussion



SUMMARY AND DISCUSSION

Pancreas transplantation and islet of Langerhans transplantation are potential solutions to treat patients with type 1 diabetes. Both procedures have shown to abolish the need for exogenous insulin and to restore normoglycemia. However, pancreas grafts are scarce and there is a shortage of donor pancreata relative to the number of patients needing a transplant (1). Therefore, optimal use of the available donor organs is essential.

The aim of this thesis was to contribute to further optimization of pancreas graft survival in pancreas transplantation and optimization of islet isolation outcomes in islet of Langerhans transplantation, leading to better use of available organs. Over the last years, human islet transplantation has become routine clinical practice in approximately 30 centers worldwide. In the Leiden University Medical Center it is performed on a regular basis and currently making the transition to becoming routine clinical practice. Porcine islet transplantation is also, slowly but surely, making a transition from the laboratory to clinical practice worldwide. To achieve this aim, we first assessed the importance of several factors that may optimize the outcome of either pancreas or islet transplantation, using both registry-based comparative effectiveness research and systematic review techniques. This was combined with biomedical research to study the mechanisms through which some factors may influence the outcomes of transplantation or isolation outcome in more detail.

In the Netherlands, the Leiden University Medical Center is the largest center performing pancreas transplantations. In 2011, 86% of all pancreas transplantations in the Netherlands were performed in our center. In **chapter 2** we have shown that both donor and recipient characteristics as well as donor-recipient matching influence graft survival. Pancreas graft survival was reduced in female patients who received a graft from a donor with a similar BMI with enteric drainage of the graft. Recipient factors remain most important and explain the largest proportion of the variance in both 1-year and overall survival whereas donor factors were less important in both short-term and long-term pancreas graft survival.

Our method of quantifying the impact of donor versus recipient factors has not been shown before. The advantage of our method is that besides the assessment of which factors significantly influence pancreas graft survival, their importance in terms of their contribution to graft survival can also be established. Optimizing recipient factors thus seems more important for long-term survival than optimizing donor factors. This seems logical when considering that pancreas donors are highly selected prior to procurement and transplantation. Because of this selection, the variation in donor factors (e.g. variation in age) is much smaller than in recipient factors. This smaller variation in donor factors is likely to result in a smaller variation in survival,

thus explaining a smaller part of the differences in pancreas graft survival. Recipients on the other hand are selected to a smaller extent, in particular in more recent years in which pancreas transplantation is also offered to more high-risk patients (e.g. older patients with comorbidity) so that they differ far more in various characteristics that may influence survival. Further research may lead to an improvement of this model by including other factors, which may result in an even higher ability to explain differences in survival.

Apart from donor or recipient factors, the procurement technique of a pancreas graft has also been shown to influence pancreas graft and patient survival. Surgical injuries that occur during pancreas procurement may lead to complications after transplantation, impaired function of the allograft, graft loss or even death of the patient. In **chapter 3** we determined how often pancreata were refused for transplantation after procurement during back-table inspection, which type of problems were responsible for the decision not to transplant the pancreas and whether different problems were encountered in transplanted versus refused pancreata. Reasons to refuse pancreata for transplantation were for example: severe atherosclerosis, severe injuries of the pancreas parenchyma, superior mesenteric or splenic vein and of the splenic or dorsal pancreatic artery such that reconstruction and transplantation became impossible. We evaluated all procured pancreata transported to our center for transplantation in the period February 2002 until May 2008. Of these, 82.8% were transplanted while 17.2% were refused for transplantation during back-table inspection, regardless of procurement region. Thirteen percent of the pancreata were refused solely due to surgical injuries. As one would expect, in refused pancreata a higher number of critical and non-critical problems per pancreas were found than in transplanted pancreata. Chances of refusal increased in pancreata from older donors procured by centers not performing pancreas transplantations. When pancreata were procured by these centers, chances of refusal were eight times higher compared with centers that did perform pancreas transplantations. These results have important implications for current practice in pancreas procurement. More extensive and recurrent training of pancreas procurement surgeons might lead to a better quality of the organs and thus to a reduction in refusal rates. Surgeons with experience in pancreas transplantation may be excellent teachers in such a training program. Another possibility to reduce pancreas refusal may be to leave pancreas procurement to those centers also performing pancreas transplantations, but this seems difficult (if not impossible) to implement in practice. It seems better to complement training with annual feedback to each center on the proportion of procured organs that could be transplanted compared to other centers, which may lead to further improvement if rates are lower than expected.

In selected patients, the alternative to pancreas transplantation is transplantation of isolated islets as a free graft. Sufficient islet numbers can be obtained from a

single-donor, but even in the most successful studies multiple transplantations were necessary to obtain (temporary) normalization of hyperglycemia in the recipients (2-7). Furthermore, islet isolation outcome is highly variable. Given the relative shortage of donor pancreata, this emphasizes the need to optimize isolation yields so that sufficient islet numbers are routinely obtained from a single-donor.

In **chapter 4** we present a systematic review of donor, pancreas and isolation procedure related factors shown to influence islet isolation outcome. Higher pre- and post-purification islets yields and a higher proportion of successful islet isolations were obtained when pancreata were preserved with the “two-layer method”, rather than with the University of Wisconsin solution in donors with shorter cold ischemia times (one hour longer cold ischemia time resulted in an average decline of pre-purification post-purification yields, and proportion of successful isolations of 59 IEQ/g, 54 IEQ/g and 21%, respectively). Higher pre-purification yields and a higher percentage of successful islet isolations were found in younger donors with higher BMI. Lower yields were found in donation after brain death (DBD donors) compared to donation after cardiac death (DCD donors). Higher post-purification yields were found in isolations with Serva collagenase.

However, these results were obtained by including only a selection of studies, as not all studies reported the same factors. To obtain more reliable evidence, standardized reporting of these factors would be necessary. In univariate analysis 66.3-87.5% of the available pancreata were analyzed on the effect on pre- and post-purification yield or percentage of successful isolation. In multivariate analysis this percentage was only 10.3-20.6%, due to missing data on at least 1 of the variables included. This indicates that the studies differ to such a great extent in the variables that are reported, even when only those variables reported in most studies were selected. Standardized reporting of a minimal set of variables in all future studies would also lead to a better fit of the model used in any meta-analysis. In the current analyses on post-purification yield 19% of the variance in islet isolation outcome could be explained by the variables included. In pre-purification islet yield and proportion of successful isolations, this percentage was better, but still only 50% of the variance could be explained. This suggests that besides the variables included in our systematic review, other factors also influence isolation outcome.

Among these other factors influencing isolation outcome may be the occurrence of hyperemic islets (HIs), for which the mechanism describing its origin as well as their relevance for islet isolation outcome is unknown. In **chapter 5** we studied histological characteristics of the human pancreas in relation to islet isolation. HIs were found in approximately half of the assessed pancreata. It is most likely that the HIs arose shortly before or during pancreas procurement and that a rise in blood pressure in combination with hemodynamic instability (associated with prolonged ICU stay),

are responsible for the formation of HIs. In addition, besides the pre-procurement hemodynamic status, the handling of the pancreas during surgery could also be a contributing factor in the development of HIs.

With respect to the consequences of HI occurrence, we found substantially lower yields in pancreata with HI (HI+) than in pancreata without HI (HI-). It can be speculated that besides the reduced isolation outcome this phenomenon could also provide a possible explanation for the variable, unexplained loss of islets during culture and after transplantation. When islets are obtained from donor pancreata containing HIs, these islets would appear to be “normal” (since only a small proportion of the assessed islets was hyperemic) when in fact, the entire islet population of these pancreata may be affected to some extent. Therefore, these islets are more likely to fail in culture or have impaired function when transplanted. To establish the importance of HIs for islet transplantation, further research is needed focused on the impact on post-purification yield, purity and viability. Systematic reporting of the presence of hyperemic islets in future studies (by taking a biopsy prior to the isolation procedure for example) would make it possible to include this as a factor in a meta-analysis to determine their relevance on isolation outcome compared to other known factors.

A potential solution for the shortage of human donor pancreata is xenotransplantation of porcine islets. However, porcine islet isolation procedures have been shown to be notoriously difficult. Morphological characteristics of the porcine pancreata could also be responsible for the highly variable islet yields. Similar to our findings in human donor pancreata, a remarkably high number of HIs was encountered when studying histological characteristics of the porcine pancreas in relation to islet isolation, as described in **chapter 6**. HIs were found in 48% of the pancreata in purebred pigs and in 68% of the pancreata in crossbred pigs. Similar to our results in human pancreata, significantly lower yields in the HI+ pancreata were found compared to the HI- pancreata in both purebred and crossbred pigs. No evidence for an ongoing chronic process was found, so it can be speculated that the HIs arose shortly before the exsanguination and death of the animal and that for instance a sudden rise in blood pressure could be responsible for the formation of HIs.

Since HIs were found in both human and porcine pancreata and have a similar effect on islet isolation outcome in both species, HIs are potentially an important factor in islet isolation outcome.

Given that collagen is the major target in the enzymatic dissociation of the pancreas, the collagen substrate within the pancreas is another variable that could account for the unpredictable, highly variable islet yields. In **chapter 7** we have described our findings in pancreata of 64 juvenile and 76 adult pigs. Islet isolation procedures in adult porcine pancreata are known to result in large islet yields (8, 9), whereas these procedures have been shown to be more difficult in juvenile porcine pancreata,

possibly as a result of the relative fragility of the islets of juvenile pigs (10, 11). Even though we found a difference in total amount of collagen between adult and juvenile pigs, this difference in collagen could not be explained by the age-difference, and should thus be explained by another (unknown) difference between adult and juvenile pigs. A collagen capsule surrounding the islet could potentially provide protection against enzymatic disintegration of islets and consequently their fragmentation. It has been suggested that a factor in the differing islet isolation outcomes is a more extensive capsule surrounding the islets of the adult pig pancreas as compared to the young pig pancreas (12, 13). However, in our study, we did not find a difference in islet encapsulation between adult and juvenile pigs. Both adult and juvenile pancreata had no or only a very limited collagen capsule. Furthermore, previous studies have shown that the amount of collagen in porcine pancreata was related to the isolation outcome (12). However, we did not observe a relation between islet isolation outcome and total amount of collagen, islet encapsulation or intra-islet collagen in 58 adult pigs.

It can be speculated that the composition of the islet capsule, and the relative concentration of the components could influence islet isolation outcome and possibly confound the observed relation between islet isolation outcome and the complete islet capsule. However, since we found no or only a very limited collagen capsule in our study population when we stained tissue samples for all types of collagen, we expect that collagen subtypes play no or only a minor role. Other matrix elements on the other hand could play a role and should be further investigated.

Ductal injection of collagenase has been shown to be the technique to produce the highest isolation yields. However, even when collagenase is delivered to the pancreas in this way, there is still a considerable loss of endocrine tissue. We showed in **chapter 8** that islets undergo a morphological change during most porcine and human islet isolation procedures. To quantify the morphological change of the islets, the mean beta cell/endocrine content ratios of the infused and not-infused tissue samples were compared. In a second experiment, 20 pancreata were similarly assessed after intraductal injection with Hank's Balanced Salt Solution (HBSS). The observed decline in the beta cell/endocrine content ratio was shown to be not specific for collagenase, but was also shown for HBSS, so that the morphological changes most likely seem to be due to volume expansion. This could potentially lead to islet fragmentation, resulting in reduced islet isolation outcome and impaired function.

In conclusion, this thesis has added evidence that the focus in pancreas transplantation should be on optimizing recipients to improve graft survival and on improving quality of pancreata procured by centers not performing pancreas transplantation (for example, by training the procurement surgeons to optimize pancreas procurement thus resulting in more transplantable organs.

In islet transplantations, it is recommended that the reporting of donor, pancreas and isolation factors should become more standardized, which would enable us to determine more accurately which factors are important predictors for islet isolation outcome. Furthermore, if more biomedical factors (e.g. the presence of hyperemic islets) would be reported in addition to the other factors, we would be able to assess the independent effect of these biomedical factors on islet isolation outcome and eventually the effect on islet transplantation in the clinical setting.

FUTURE PERSPECTIVES

Pancreas transplantation

In December 2010, more than 36,000 pancreas transplantations have been reported to the International Pancreas Transplant Registry (IPTR): more than 24,000 transplantations were performed in the US and more than 12,000 outside the US (14). Recipient age at transplantation increased over the course of 24 years of pancreas transplantation as well as transplantations in type 2 diabetes mellitus patients. Donor criteria have become more strict over time, with a concentration on younger donors, preferably trauma victims, with short preservation time. Surgical techniques for drainage of the pancreatic duct also changed over time. In the US, enteric drainage is the predominantly used technique in combination with systemic drainage of the venous effluent of the pancreas graft. In the Leiden University Medical Center, a two-step approach is routinely performed in most patients. Pancreas transplants are bladder drained initially, with patients undergoing elective pancreas conversion to enteric drainage 6 – 12 months after transplantation (15). Immunosuppressive protocols developed towards antibody induction therapy with Tacrolimus and Mycophenolate Mofetil (MMF) as maintenance therapy. The rate of transplantations with steroid avoidance increased over time. All of these changes together have resulted in improved patient and graft survival. Patient survival is now 95% at one year and over 83% 5 years after transplantation. Because donors are already highly selected prior to pancreas transplantation, future improvements in patient and pancreas graft survival may be realized primarily by optimizing recipient factors (e.g. BMI). Alternatively, living donor segmental pancreatectomy has been reported as a therapy in selected patients (16). An initial technical failure rate of more than 33% has been reported; nearly twice the rate in deceased donors, but this has declined to less than 1% since the start of this therapy. Living donor and deceased donor graft survival rates are more or less equivalent. If the use of living donors would increase, it is likely that eventually not only highly selected donors will be considered to be suitable, but criteria will become less strict over time, leading to more variation in donor characteristics that may influence survival. In this way, the use of living donors could potentially increase the

importance of donor factors in explaining survival differences. The potential downside of this therapy is that the donor must face a major surgical intervention, and even if a minimally invasive technique is used, the donor faces risks of surgical diabetes and potential risks for complications such as pancreatic fistula or infection.

Human islet transplantation

Clinical islet transplantation is currently being offered to a subset of approximately 15% of patients with type 1 diabetes with refractory hypoglycemia or marked hypoglycemic episodes. With over 750 islets transplants performed in over 30 international centers yearly, this therapy has been transferred from research to becoming a standard recognized clinical therapy (5). In the Leiden University Medical Center, 19 transplantations have been performed in 13 patients since the start in 2007. Currently, islet transplantation offers a means of endogenous, regulated insulin secretion, thereby stabilizing glycemic control, preventing hypoglycemia, and correcting glycosylated hemoglobin (HbA1C) to a level predicted to prevent and reverse secondary complications of diabetes. However, patients require immunosuppressive therapy for the rest of their lives. Islet transplantation is also being offered after kidney transplantation, where the choice is simpler as these patients already require lifelong immunosuppressive therapy, and the intraportal islets implantation procedure is a simple nonsurgical intervention with a relatively low risk.

Islet transplantation is thus likely to become a standard therapy once islet transplantation becomes more readily available. This means that the islet supply should be expanded (e.g. through expansion of existing islets, stem cell approaches or when xenotransplantation sources become available). The remaining challenges of inducing immunological tolerance, preventing islet destruction due to autoimmunity or alloimmune rejection and avoiding all potential side-effects due to immunosuppressive therapies, will all need to be addressed to facilitate this transition towards becoming a standard therapy. Furthermore, routine attainment of single-donor islet transplants success remains an important goal in islet transplantation. This would allow for many more subjects to be treated with islets, and would reduce the potential risk of donor HLA-sensitization by avoiding exposure to multiple donors. Islet allograft transplantation has also been performed with islets from three living donors, the last one successfully, showing the potential for further application (17-19).

Moving from multiple-donor to single-donor success will require a multimodal approach, including optimization of the pre-procurement condition of donor pancreas organs, protection of islets from cold and warm ischemic injury and the process of islet isolation, access to effective, stable and consistent human compatible collagenase enzyme blends for digestion, and several multimodal strategies for treatment of the recipient to suppress immunological, inflammatory and thrombosis pathways, while at the same time stimulating neovascularization and metabolic function of the islet graft.

Such a multimodal approach will transform short- and long-term islet transplantation success and will continue to facilitate the rapid transition from research to routine clinical care. This emphasizes the importance of optimizing islet isolation outcome.

In contribution to optimization of islet transplantation outcome, research in the Leiden University Medical Center is focused on the development of devices (in collaboration with the Technical University Twente) to create an optimal microenvironment for transplanted islets and alternative cell sources (e.g. precursor cells).

Porcine islet transplantation

Porcine islets could be an alternative to human islet transplantation, particularly if delivered in a way that evades the host immune system rejection (20). This can be achieved by protecting xenogeneic islets from immune rejection by selective semi-permeable barriers. Designated pathogen-free herds (21, 22) could provide a supply of wild-type porcine islets that are well tolerated when administered in a suitable protective delivery vehicle. Such barrier systems have enabled amelioration of diabetes in a variety of animal models and preliminary evidence suggests that similar results could be obtained in humans. Ongoing trials using encapsulated islets, without immunosuppressive therapy, are sponsored by Living Cell Technologies (LCT), either in Russia or New Zealand, as well as trials that are still in the planning phase in the US. The trial in New Zealand started in 1995 with six type 1 diabetes patients receiving either encapsulated or non-encapsulated neonatal porcine islets. One individual, receiving encapsulated islets, showed a reduction in average monthly insulin dose, a reduction in glycosylated hemoglobin and the detection of porcine C-peptide in urine. A biopsy from this patient 9,5 years after transplantation showed encapsulated islets expressing insulin (23). These improvements in diabetic state, although temporary, were encouraging and prompted further expansion. However, trials were temporarily put on hold because of concerns raised by the documentation of in vitro pig-to-human transmission of porcine endogenous retroviruses. This resulted in a long process of communication with the New Zealand regulatory authorities to fulfill requirements associated with the health status of the source pigs, pancreas processing and islet encapsulation, nationwide public consultation and implementation of a safety strategy. In the Russian trial, commenced in 2007, eight patients were transplanted with 5000-10000 IEQ/kg. Presenting the three- and six-month post-transplant data, evidence for efficacy was demonstrated in some, but not in all, patients. Five patients manifested a reduced insulin need and two patients (one at three months and one at six months after transplantation) temporarily did not need any insulin. Six patients showed a reduction in circulating glycosylated hemoglobin, average levels being 8.9% before transplantation, 6.9% at three months after transplantation and 7.3% at six months after transplantation. The procedure was found to be safe and could be repeated safely.

The trial in New Zealand continued in October 2009. It is an open-label dose-range study for one implant with 10000-15000 IEQ/kg in eight patients with unstable diabetes and severe hypoglycemic episodes. All patients, with a follow-up of 20-36 weeks, showed a clear reduction in hypoglycemia score. At the XXIII international congress of the Transplantation Society (24) one patient was presented in more detail, showing a 20% insulin dose reduction at 4 weeks after transplantation

These data show that clinical porcine islet transplantation is a safe procedure that might benefit patients with hypoglycemia unawareness and gives a modest improvement in diabetes control. In upcoming studies, the dose and timing of possible repeat doses will be addressed. Optimization of porcine islet isolation outcome would become of even greater importance once porcine islet transplantation becomes a more standard recognized clinical therapy.

This thesis is a first step in the direction of these future developments. By combining several research methods (registry-based comparative effectiveness research, systematic review techniques and biomedical research) we have accomplished a profitable interaction. On one hand we have assessed the general overview (by determining the relative importance of factors on outcomes of transplantation) and on the other hand we have unraveled some of the mechanisms through which potential factors may influence the outcomes of transplantation. The advantage of such a combination is that we do not only investigate how certain factors may influence transplantation outcomes, but at the same time try to quantify which part of the variation in outcomes may be explained by this factor, and thereby determine the room for improvement in those outcomes.

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