Pancreas Transplantation: associations with graft failure and measures of function

Thesis submitted to the Examination Schools for the degree of

Doctor of Philosophy

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Hilary Term 2015

ABSTRACT

Pancreas transplantation, either as a simultaneous pancreas kidney (SPK) or an isolated pancreas (IP), is a successful treatment option for some people with diabetes; however, grafts at the highest risk of failing are difficult to identify, and a means of monitoring graft function effectively post-transplant is lacking. The work in this thesis aimed to identify factors associated with pancreas graft failure and explore metabolic features of early graft dysfunction. First, the published Pancreas Donor Risk Index tool was predictive using UK National data in SPK but not IP transplantation, suggesting greater influence of recipient and post-transplant factors in determining IP graft survival. HLA antibody monitoring showed that the development of de novo DSA post-transplant was associated with poorer graft outcomes, particularly in IP transplant; and assessment of ICA and GADA autoantibodies also showed a deleterious effect of pre-transplant autoantibody positivity in IP transplantation; suggesting IP recipients may be immunological disparate to those receiving SPK. A retrospective analysis of early post-transplant oral glucose tolerance tests showed impaired glucose tolerance to be associated with later graft failure, and led to prospective studies examining glucose homeostasis in the early post-transplant period. Early impaired glucose tolerance was correlated to hyperglycaemia detected on continuous glucose monitoring. Analysis of patients pre- and post-transplant confirmed complete normalisation of glucagon concentrations early post-transplant and early impairment in insulin secretion. A comparison of the response to oral and intravenous glucose demonstrated that insulin secretion may be affected by the absence of the incretin effect early after pancreas transplantation, associated with reduced GLP-1 but normal GIP concentrations evident post-transplant. The incretin effect became established by 3 months post-transplant, and was associated with an increased GLP-1 response. Together these studies demonstrated novel features associated with high risk of graft failure which could help to identify patients who may benefit from therapeutic interventions aimed at improving graft outcomes.

ACKNOWLEDGMENTS

I would like to thank my supervisors Professor Peter J Friend, Professor Stephen CL Gough and Dr Edward Sharples for their support, encouragement and wisdom. Always accessible, insightful and inspirational, their influence has fuelled an academic interest that I expect to remain throughout my career. My unending gratitude to Rachel Franklin, a talented and dedicated research nurse, who has worked tirelessly to help make these projects happen. Thanks also to the NIHR Biomedical Research Centre Oxford, which funded much of this work.

I am grateful to Professor Susan Fuggle, whose sage advice has been invaluable, and to Suzanne Page and the clinical scientists in Transplant Immunology and Immunogenetics Laboratory, who made the analyses of chapter 4 possible. Gratitude must also be extended to Myura Nagendran and Michiel Voskuil, medical students from Oxford and Gröningen respectively, who aided with data collection. I am grateful also to Dr Gaya Thanabalasingham and Dr Agata Juszczak for their help in completing the intravenous glucose infusions, and to Professor Jens Holst and Filip Knop for advice and assays involved in chapter 7. Thank you also to Tim James and staff in OUH Clinical biochemistry, Sandy Humphries in the OCDEM laboratory and Prof Berne Ferry in OUH clinical immunology for their help and guidance with the processing of samples. Thank you also to the excellent research nurses in the OCDEM Clinical Research Unit, who were always quick to help when many hands were needed.

Recognition must go to the Consultants, nurses and clinical staff at the Oxford Transplant Centre, whose hard work made these studies possible, and who have made me feel so welcome. Last and of course not least, the greatest thanks must go to all the transplant recipients and healthy controls, who took part in these studies, and to their families for making it possible.

DECLARATION

All work presented in this thesis is my own, unless otherwise stated. I was involved in the design and conduct of all studies, with the support of my supervisors and a research nurse. Data was collated, analysed and interpreted by myself. Clinical work was conducted by the clinical team, including some tests analysed within this thesis. Assays undertaken as part of the prospective metabolic studies described were undertaken by clinical scientists and laboratory technicians.

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GLOSSARY OF COMMON ABBREVIATIONS

ANOVA	analysis of variance
AUC	area under the curve
BMI	body mass index
BP	blood pressure
CIT	cold ischaemic time
CGM	continuous glucose monitoring
cRF	calculated reaction frequency
DBD	donor after brain death
DCD	donor after circulatory death
DGF	delayed graft function
DSA	donor-specific antibodies
ESRF	end-stage renal failure
GADA	glutamic acid decarboxylase autoantibodies
GIGD	gastrointestinal glucose disposal
GIT	gastrointestinal tract
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide 1
HbA1c	glycated haemoglobin
HLA	human leukocyte antigen
HOMA	homeostatic model assessment
HR	hazard ratio
IAPP	islet amyloid polypeptide
ICA	islet cell antibodies
IDF	Internationsl Diabetes Foundation
IE	incretin effect
IGT	impaired glucose tolerance
IIGI	isoglycaemic intravenous glucose infusion
IP	isolated pancreas
IU	international units
Kg	kilograms
MFI	mean fluorescent intensity

MMF	mycophenolate mofetil
mmol	millimoles
NICE	National Institute for Clinical Excellence
NGT	normal glucose tolerance
OGTT	oral glucose tolerance test
PAK	pancreas after kidney
PDRI	pancreas donor risk index
PMH	past medical history
pmol	picomoles
PTA	pancreas transplant alone
QOL	quality of life
SD	standard deviation
SPK	simultaneous pancreas kidney transplant
SRTR	Scientific Registry of Transplant Recipients
T1D	type 1 diabetes
T2D	type 2 diabetes
UK	United Kingdom
US	United States of America

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Chapter 1

Introduction

1 INTRODUCTION

1.1 Diabetes in context

Diabetes is one of the most challenging health problems of the 21st century, and represents a growing global problem with significant socioeconomic implications. 382 million people are affected by diabetes worldwide, and while an increasing proportion is being diagnosed in developing countries, 56 million people with diabetes (8.5% of the adult population) are living in Europe (International Diabetes Federation 2013). In the UK, 3.2 million people are diagnosed with diabetes, and an estimated 630,000 people have the condition but have not been diagnosed (Diabetes UK 2014). Diabetes is not only common, but is also in the top five causes of death in most high-income countries and accounts for 11% ($548billion) of global health spending. This is particularly true in North America and Europe where $263billion (48% of global expenditure on diabetes) and $147billion (25% of global expenditure) is spent respectively, each greater than all other regions combined (International Diabetes Federation 2013).

Diabetes is a common life-long condition characterised by hyperglycaemia. In health, glucose is needed for energy and, when in excess, insulin is rapidly released from pancreatic beta-cells to encourage glucose metabolism, uptake in peripheral tissues, storage as glycogen or fat, and glucose utilisation for protein synthesis. When glucose is scarce, glucagon is the opposing hormone and is released from pancreatic alpha-cells triggering gluconeogenesis and the breakdown of glycogen and fat. Diabetes is a heterogeneous condition and broadly categorised as 2 main types: type 1 diabetes (T1D) and type 2 diabetes (T2D); although there are other well recognised forms of diabetes

including Maturity Onset Diabetes of the Young (MODY), Gestational diabetes and Neonatal diabetes, and rare genetic causes of diabetes including Wolfram Syndrome and Alström Syndrome, which fall in between. Both T1D and T2D are bi-hormonal disorders involving dysregulation of insulin and glucagon. This dysregulation may precede a diagnosis of diabetes by many years, and the patterns of beta and alpha cell dysfunction leading to the development of diabetes have been the subject of much investigation (Sosenko et al. 2012b), with T2D typically due to a combination of insulin resistance and an insulin secretory defect, and T1D is characterized by a near-absolute deficiency of insulin secretion.

T2D accounts for the vast majority of diabetes worldwide, and affects 85-95% of all people with diabetes. Usually, diagnosis occurs in people over the age of 40, although onset may appear much younger in some communities. Importantly, in recent years there is a trend to younger age of diagnosis, and T2D is becoming increasingly common in children of all ethnicities (International Diabetes Federation 2013). In T2D, insulin may be secreted but the ability of the pancreatic beta cell to release adequate hormone in phase with rising glycaemia is profoundly compromised (Ferrannini et al. 2005). T2D is heterogeneous in nature, in so far as there may be a variable degree of remaining beta cell function and the degree insulin resistance may be reversible. With the increasing frequency of T2D in children, and greater knowledge of rarer forms of diabetes, accurately distinguishing T2D from other causes has become more challenging (Tuomi et al. 2014). Unfortunately, there are often delays in diagnosis- it is estimated that 46% of people worldwide with T2D are undiagnosed (630 000 people in the UK), meaning many are progressing towards complications unawares, and many will have at least one chronic complication at the time of diagnosis.

In general, the management of T2D must therefore be tailored to individual situations and needs. Treatment involves a personalised combination of lifestyle modification and medication aimed at reducing insulin resistance and risk factors for complications, in particular those related to cardiovascular disease. A healthy diet (low fat, high fibre, moderation of salt) combined with increased physical activity (150min moderate exercise/week) can result in weight loss sufficient to provide clinical benefit (Look et al. 2007).

Regular clinical assessment then guides medication strategy, which primarily involves oral hypoglycaemics such as metformin, with the addition of a 2nd agent as needed. Many people with T2D will eventually require insulin therapy, which can be problematic. Insulin therapy is associated with weight gain, which may add to insulin insensitivity, and is also associated with a risk of hypoglycaemia, which may be limiting or dangerous. Most people with T2D maintain some endogenous insulin secretion even in late stages of disease, and management can be complex with the need to balance combinations of oral and subcutaneous therapy.

Over recent decades, alternative treatment options have emerged with a rise in the popularity of bariatric surgery for those who meet the criteria. Surgery has been shown to offer benefits in terms of weight loss outcomes and weight-associated comorbidities, as well as being associated with favourable changes in incretin hormones profiles.

Incretin hormones are released from the gastrointestinal tract (GIT) in response to food to potentiate insulin secretion, and this is referred to as the incretin effect. It has recently

been discovered that T2D is associated with a diminished incretin effect, and novel therapies have been developed in the last decade based on an improved understanding of insulin resistance and secretory responses, including incretin therapies which have been used with encouraging outcomes (Nauck 2009).

T1D differs from T2D, in that it develops due to autoimmune beta cell destruction, which results in insulin deficiency. The absence of an endogenous source of insulin means that injected subcutaneous insulin therapy must be administered regularly and religiously, since in its absence life-threatening acute complications will develop rapidly, and so making insulin therapy essential for life. Although T1D accounts for about 10% of all adults with diabetes, it is the most common type of diabetes found in childhood, and is particularly common in Europe (figure 1).

It can develop at any age, but usually appears before the age of 40 years, and often presents with acute complications (International Diabetes Federation 2013). Insulin therapy must be instituted immediately, however glycaemic control is often poor and, in 2012, only 20% of people with T1D had an HbA1c<7.5% (Gordon-Dseagu et al. 2013). In these young people, inadequacies in therapies mean the damaging effects, caused by hyperglycaemia and variability in glucose concentrations, accrue over many years even after diagnosis and often lead to several chronic complications, which confer significant morbidity and increased risk of death.

1.2 Type 1 diabetes

T1D is one of the most common endocrine and metabolic conditions in childhood. The incidence is increasing, particularly in under 15 year-olds with 79 000 children worldwide developing T1D in 2013, and with an estimated overall 3% increase in incidence per year. 497 100 children live with T1D, with Europe having the highest prevalence (approx. 129 300) and the highest incidence rates (20 000 new cases each year); the UK, the Russian Federation and Germany contributing largest numbers overall (International Diabetes Federation 2013) (Figure 1). The number of adults living with T1D is also increasing, and this is thought to be due the rising number of new-onset cases of T1D in adults and due to individuals with childhood-onset diabetes living longer (Chiang et al. 2014).

T1D is an autoimmune disorder leading to beta-cell destruction with onset attributed to both an inherited risk and external triggers, as shown from studies in monozygotic twins (Barnett et al. 1981). There is a 15-fold increase in risk of T1D in first-degree relatives of people with T1D and this has been linked to 18 regions of the genome (L. M. Dean, J. 2004). The Human-Leukocyte Antigen (HLA) genes encoding major histocompatibility complex (MHC) proteins on the surface of most cells have been widely studied for an association. Autoimmune disease occurs when a self-MHC is recognised as foreign and attacked. The genes encoding class II MHC proteins (HLA-DR, HLA-DQ and HLA-DP) are most strongly linked with diabetes, with 95% of Caucasian people with T1D having at least one allele of DR3 or DR4 (Hey et al. 1998). Mutations in the insulin gene also can lead to abnormal insulin production, and several other diabetes susceptibility genes have also been identified. Certainly, genetic factors are important, however complex external

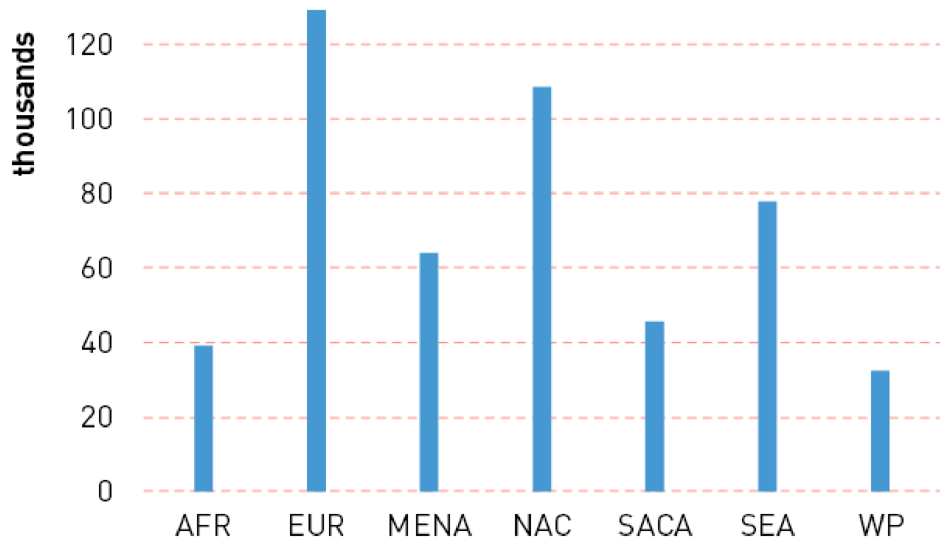
triggers are also thought to play a role. Changes in environmental factors, early prenatal events, early life diet or viral infections may have implications (Majeed et al. 2011), and combinations of these factors may account for regional variations and increased incidence over time.

T1D has traditionally been diagnosed in children over the age of 6 months (below which the diagnosis is most likely to be neonatal diabetes and treated with sulphonylureas (Gloyn et al. 2004)) based on acute clinical catabolic symptoms suggestive of insulin deficiency: polyuria, polydipsia and weight loss, and in these cases insulin therapy is initiated immediately. However, diagnosis can be more complex. In patients aged 10–17 years with phenotypic T2D, oral hypoglycaemic medications may be initiated first, and changed to insulin after a failure of response. In these cases, 10% have been shown to have evidence of islet autoimmunity, so suggesting T1D to be the more likely diagnosis (Klingensmith et al. 2010). Conversely, in T1D residual C-peptide (a surrogate marker for insulin secretion) may be detected over 40 years after initial diagnosis, and this may lead to confusion, making distinguishing T1D and T2D more difficult (L. Wang et al. 2012).

The American Diabetes Association's (ADA's) diagnostic criteria for T1D and T2D are the same, and are based on the presence of one of: an HbA1c >6.5%, a fasting plasma glucose of ≥7mmol/l, 2 hour plasma glucose ≥11.1mmol/l, or a random plasma glucose of ≥11.1mmol/l. However, the presence of pancreatic autoantibodies is characteristic of T1D with ~98% of individuals with autoantibodies at diagnosis. Five autoantibodies are commonly seen, including GAD antibodies (GADA), islet cell antibodies (ICA), insulin autoantibodies (IAA), protein tyrosine phosphatase antibodies (ICA512 or IA2A), and zinc transporter protein (ZnT8). Studies evaluating children at risk for developing T1D

have also shown that the presence of more than two autoantibodies was associated with a nearly 70% risk for disease development within 10 years and 84% within 15 years (A. G. Ziegler et al. 2013).

Figure 1 Estimated number of children (0-14 years) with type 1 diabetes by IDF Region, 2013. Reproduced from International Diabetes Federation Atlas



IDF region: Africa (AFR); Europe (EUR); Middle-East and North Africa (MENA); North America and Caribbean (NAC); South and Central America (SACA); South-East Asia (SEA); Western Pacific (WP)

1.3 Complications and aims of management

The recommended management of T1D involves an individually tailored multidisciplinary approach including patient education, support and self-management, particularly with regard to psychosocial issues, nutritional therapy and interactions with physical activity. Lifelong insulin therapy is necessary and many people are managed on basal bolus regimes alongside careful monitoring, with some people with T1D needing insulin pump therapy or adjunctive therapies (American Diabetes Association 2014).

The aim of diabetes treatment is to avoid the development of chronic or acute complications. Most chronic complications relate to either microvascular or macrovascular changes caused by poor glycaemic control. Microvascular complications can be debilitating and have a significantly deleterious effect on quality of life. In turn, they can also contribute to macrovascular risk, which ultimately is the principle cause of death for people with diabetes. There is a strong correlation between reduction of hyperglycaemia (as measured by glycated haemoglobin, HbA1c) and control of diabetic complications (Stratton et al. 2000). For this reason, it is essential to improve therapies available in the treatment of diabetes to decrease the burden of complications and improve outcomes in these groups.

Debilitating microvascular complications of diabetes come in the form of retinopathy and neuropathy. Microvascular changes result in retinopathy in a third of people with T1D, the leading cause of blindness in working-age adults and accounting for 1280 new cases of blindness in the UK each year (International Diabetes Federation 2013). Although active

screening has reduced rates of severe retinopathy in recent years, it still limits independence with impact on individuals, families and communities. Therapies for retinopathy are limited and so prevention and preservation of vision is the mainstay of treatment. Additionally, peripheral neuropathy, which also develops due to microvascular damage, leads to problems with mobility, pain or unrecognised injuries that could necessitate amputations, and autonomic neuropathy can cause debilitating gastrointestinal problems that are extremely difficult to manage.

One of the most important complications that people with diabetes face is nephropathy, and in the USA diabetic nephropathy accounts for 40% of new cases of end-stage renal failure (ESRF) (Molitch et al. 2004). ESRF is not only restrictive due to the need for regular dialysis, but it is also inherently associated with additional cardiac risk factors in this already high risk group (Baber et al. 2007). Renal dysfunction will occur in 20-30% of all people with either T1D or T2D, and an initial presentation of microalbuminaemia will advance to overt nephropathy in 20-40% of cases (Molitch et al. 2004). Progression to ESRF is more common in T1D, and although genetic factors play a role, good glycaemic control has been shown to reduce the decline in renal function (Goel and Perkins 2012; Smail et al. 2012).

Perhaps the most devastating complication of diabetes is the much increased risk of cardiac disease due macrovascular changes. It represents the leading cause of death in people with diabetes and, in 2013, 48% of deaths due to diabetes were in those aged under 60 and attributable to advanced cardiac disease (International Diabetes Federation 2013). Reducing deaths involves the management of many risk factors, and ultimately, is the principle aim of diabetes management.

The challenges in diabetes management were highlighted in a landmark prospective longitudinal study investigating the effect glycaemic control on the development and progression of diabetic complications in T1D. In this multicentre randomised clinical trial, intensive therapy with three or more insulin injections per day, or pump therapy, titrated for strict glucose control was compared to standard insulin therapy with 2-3 injections per day in two separate primary prevention and secondary intervention cohorts (The Diabetes Control and Complications Trial Research Group 1993). The intensive therapy group achieved significantly lower HbA1c and plasma glucose levels compared to the conventional therapy arm, however <5% maintained an average HbA1c value of 6.5% or less.

The intensive therapy arm showed a reduction in adjusted mean risk of developing retinopathy of 76%, and had a reduction in the average risk of progression of 54%, following an initial period of deterioration and then stabilisation in the first 18 months. The mean adjusted risk of microalbuminuria was also reduced by 43% for primary-prevention and by 56% for secondary-intervention. Similarly the appearance of neuropathy was reduced by 69%, and reduced by 57% in the intervention cohort. The small number of events made significant differences in macrovascular outcomes difficult to detect in this study, however in a 10 year follow-up of the same cohorts, the EDIC study, showed a 42% reduction in risk of a cardiovascular event despite convergence of HbA1c between the groups (Nathan et al. 2005). Importantly, however, in the original DCCT trial it was also noted that severe hypoglycaemic events were three times more common in the intensive-therapy arm, and included events requiring third party intervention, hospitalisations and fatalities potentially associated with hypoglycaemia.

These studies were seminal, both in confirming the dramatic potential benefits of tight glucose control for patient outcomes, and in highlighting the barrier to effective diabetes management of T1D – that insulin therapy was inadequate for achieving optimal glucose control without an unacceptably high risk of dangerous hypoglycaemic events.

1.4 Insulin therapy

The first step towards the understanding the pathophysiology of diabetes came in 1889 when German physicians Joseph von Mering and Oskar Minkowski surgically removed the pancreas from dogs rendering them diabetic (Brar. 1998). Isolation of a pancreas extract then became the focus of much interest, and it was Dr. Frederick Banting, a surgeon who joined the laboratory of Professor John Macloed in Toronto, and Charles Best, a medical student working as his assistant, who were first successful in isolating what they called ‘isletin’.

Banting tied the pancreatic ducts of dogs causing the degeneration of the pancreas exocrine component to leave a residue of insulin-producing cells, from which insulin could be extracted. In July 1921, experiments were started injecting this extract into diabetic dogs, with resulting improvements in blood glucose, reduction in glucosuria and improvements in clinical condition, such that a pancreatectomised dog could be kept alive for eight days by regular injections, or until supplies of the extract were exhausted. This exciting discovery lead to exploration of other sources for an alternative insulin extract, and a bovine source was found to sustain a pancreatectomised dog survival for 70 days (Brar. 1998). Dr. J. Collip, a biochemist, continued improving the purity of the pancreas extract, and later, Best carried on this work.

Bovine insulin was first used as human therapy when Leonard Thompson, then aged 14, was treated on January 11, 1922. However, only a slight lowering of blood sugar was achieved and abscesses developed at the injection site. Further refinement of the extract

was swiftly undertaken and, just weeks later, Leonard was treated again and showed a remarkable recovery. Astoundingly, these simple injections enabled him to live for a further 13 years, until he succumbed to pneumonia. In 1923, Banting and Macloed were jointly awarded the Nobel Prize for the discovery of insulin, which they each shared with their colleagues, Best and Collip. In his Nobel Lecture, Banting concluded the following about their discovery: *"Insulin is not a cure for diabetes; it is a treatment. It enables the diabetic to burn sufficient carbohydrates, so that proteins and fats may be added to the diet in sufficient quantities to provide energy for the economic burdens of life."*

Although the discovery of insulin by Banting was the first step, several further important milestones (and Nobel prizes) were achieved prior to the development of modern day therapy. First the insulin-making cells of the body were identified as beta cells, found in the pancreas gland as "islets of Langerhans", named after the German medical student who described them. In 1958, Frederick Sanger, a British biochemist, was awarded the Nobel Prize for determining the sequence of the amino acids that make up insulin, and indeed discovering that every protein has a unique sequence. Dorothy Hodgkin, also a British biochemist, furthered this work using X-ray crystallography to determine the three-dimensional structure of molecules. Having deciphered penicillin and vitamin B12, for which she awarded the Nobel Prize in 1965, she went on to identify the structure of insulin in 1969.

Until this time, when it became apparent that the structure of animal and human insulin differed, purified bovine insulin had been used in clinical therapies, but treatment was often complicated by immune-mediated side effects. For a time, human insulin was trialled for treatment but supply of human tissue was insufficient to make this option

feasible. In 1978 a revolution came with the production of recombinant human DNA insulin, which virtually eliminated problems with immune-mediated side-effects. There was large-scale uptake of human recombinant insulin, which forms the basis of most modern day preparations.

Early insulin preparations aimed to deliver insulin therapy in a single convenient injection, with modifying agents added to slow its release. However, absorption could be erratic and insulin requirements varied with meals. This lead to the development of a number of short-acting preparations designed to be used in combination with long- or intermediate-acting depots, and administered 15-20 minutes before a meal. These combinations of preparations remain the basis of current insulin therapy, with patients educated in how to match prandial insulin dose to carbohydrate intake, pre-meal blood glucose, and anticipated activity.

We can see, therefore, that although the discovery and development of injectable insulin has been fundamental to diabetes treatment, and although it has contributed to improvements in quality and quantity of life for many people with diabetes, it is not without its inadequacies. Basal bolus insulin injections can be difficult to manage even with patient education and frequent self-monitoring, as timings and doses of short-acting preparations are difficult to anticipate. Over-zealous treatment can quickly lead to potentially dangerous hypoglycaemic events. Severe hypoglycaemia rates increase with antecedent episodes of hypoglycaemia, age, and duration of diabetes and, if left untreated, will progress to coma or death. Hypoglycaemia is clearly dangerous in itself, and if frequent, can result in loss of normal sympathoadrenal counter-regulation resulting in unawareness that hypoglycaemia is occurring. This leads to a vicious cycle where

hypoglycaemic episodes become more severe and frequent, and there is increased risk of an event occurring suddenly and unrecognised, where there is reliance on third party intervention for the hypoglycaemia to be reversed (Seaquist et al. 2013b). For this reason, patients with hypoglycaemia unawareness or severe hypoglycaemia are often advised to raise their glycaemic targets for at least several weeks, in order to partially reverse unawareness. People in whom this occurs often may choose to run their HbA1c higher than recommended for fear of inducing a hypoglycaemic event, and this must again be balanced with the difficulty of avoiding complications. Additionally inadequate usage, due to failure to diagnose or effectively manage diabetes, can lead to diabetic ketoacidosis or hyperglycaemic hyperosmolar non-ketotic coma, and can be equally life-threatening. Young people often struggle to administer insulin correctly and desire freedom from the restrictions that go along with effective diabetes control. As a result, missed or inadequate doses can be as dangerous as administering too much.

Overall, the aim of injectable insulin therapy is to titrate doses to individualised glycaemic targets, in order to achieve the best possible control while minimizing the risk of severe hyperglycaemia and hypoglycaemia in the acute setting, and avoiding the development of chronic complications. Traditionally, an HbA1c goal has been recommended of <8.5% those under 6 years of age, <8% for 6–12 years old, and <7.5% for 13–19 years old. The reason for these generous targets being driven by concerns about the risk of hypoglycaemia and the consequences of events in children (Chiang et al. 2014). However, there has been growing awareness that raised HbA1c levels prior to puberty may confer risk for both micro- and macrovascular complications, as well as produce adverse effects on neurocognitive function (Barnea-Goraly et al. 2014), and this has led to a change in policy to use a single HbA1c goal of <7.5% across all paediatric age-groups (Chiang et al.

2014). Similarly to the management of children, the care of older adults with diabetes is complicated by their clinical and functional heterogeneity. Life expectancy is highly variable, and should be defined by comorbidity and functional status more than it is by age. The potential benefits of stringent glycaemic control for the development and progression of complications, must be balanced carefully with the risk of hypoglycaemia in a group that may be frail, particularly susceptible and may have difficulty coping (Kirkman et al. 2012). Therefore, although the ideal of 'as close to normal as possible' has been set, it is rarely achieved. In the realistic management of diabetes, for most adults a target of HbA1c of 7% or less is considered achievable and desirable, with a goal of <6.5% utilised only for select individual patients where the risk of significant hypoglycaemia or other adverse effects of treatment is considered minimal.

Injectable insulin preparations struggle to mimic the normal physiology of insulin release, and this has led to the development of continuous insulin delivery systems. Continuous subcutaneous insulin infusion pumps can be programmed to deliver a basal rate of insulin, with higher rates triggered at mealtimes with a push of a button. Further, sensor-augmented pump therapy has been developed to detect hypoglycaemia, and automatically suspend insulin delivery so resulting in lower 1 year HbA1c compared to insulin injections in T1D, and with less nocturnal hypoglycaemia (Bergenstal et al. 2010). Nevertheless, pump therapy is expensive, and the greatest benefits are only seen in those with poorer control or higher HbA1c. This has resulted in the development of NICE guidelines recommending pump therapy for people with T1D with disabling hypoglycaemia with multiple daily injections, or HbA1c levels ≥8.5% on multiple daily injection therapy (National Institute of Clinical Excellence 2013b).

The desire to avoid hypoglycaemia, and new knowledge in glucose homeostasis, has led to the development of alternatives to insulin, aimed at reducing insulin resistance and suppressing glucagon secretion with varied success. Biguanides and thiazolidinediones have been commonly used in the treatment of T2D, and improve insulin sensitivity. Indeed, metformin is first-line therapy in T2D, but has been shown to have some benefit in reducing insulin doses and weight in a meta-analysis of small studies in patients with T1D (Vella et al. 2010). Alpha-glucosidase inhibitors reduce glucose absorption from the gut independent of the pancreatic islet, and have in one small study been shown to result in a lower HbA1c, although this clearly needs validation in larger studies (Nagai et al. 2011). Sodium-glucose co-transporter 2 (SGLT2) inhibitors inhibit renal glucose reabsorption providing insulin-independent weight loss and HbA1C reduction in individuals with T2D. However, currently insufficient data exist to recommend clinical use of these agents in T1D (Chiang et al. 2014).

Islet amyloid polypeptide (IAPP) is co-secreted with insulin, and incretin hormones are secreted by the gastrointestinal tract to stimulate insulin secretion (the incretin effect). IAPP analogues and incretin-based treatment have also been used to reduce prandial hyperglycaemia. Both delay gastric emptying, blunt pancreatic secretion of glucagon, and enhance satiety, and can be used alongside insulin, albeit with some gastrointestinal side-effects. New and novel therapies are also being investigated. Leptin is an adipokine with central and peripheral roles affecting insulin sensitivity and suppressing glucagon, and is being investigated in a Phase I trial in T1D.

Therapy trials in children likely to develop T1D, aimed at preventing diabetes development and preserving remaining beta-cells, are ongoing. However despite initially

exciting results, longer term studies have been disappointing with treatment with rituximab showing no benefit at 2 years (Pescovitz et al. 2014), and alum-conjugated GAD immunisation (GAD-Alum) failing to show preserved c-peptide secretion at 15 months (Wherrett et al. 2011). Although current medical treatment strategies have revolutionised management of a previously fatal condition, there is much left to do to achieve normal glucose homeostasis and improve quality and quantity of life for people with T1D.

1.5 Beta-cell replacement

The concept of replacing lost beta-cell mass is clearly attractive, as it would enable normoglycaemia in a physiological manner, and avoid many of the existing problems of insulin therapy, namely the attainment of good glucose control without the risk of hypoglycaemia. Beta-cell replacement may be achieved through either whole organ pancreas or islet transplantation in select candidates, and has been a reality in the UK since the 1970s (Sutherland 1981).

Although scientists experimented with pancreas and islet cell transplantation prior to the discovery of insulin, it was not until the 1960s and the advancements in immunosuppression that clinical organ transplantation became a reality. In 1966, William Kelly and Richard Lillehei performed the first human pancreas transplant at the University of Minnesota, when they implanted a segmental pancreas graft simultaneously with a kidney transplant from a deceased donor into a 28 year old woman (Kelly et al. 1967). Unfortunately, the operation was followed by a number of complications resulting in the recipient's death two months later. The same team performed the next recorded transplant in 1969, hailed as a success with both graft and patient survival at 1 year. Nevertheless, the morbidity and mortality associated with these early transplants was high - a publication in 1970 showed only two patients out of a series of 10 survived (Lillehei et al. 1970), and Sutherland reported that, although by 1977 fifty-seven pancreas transplants had taken place, graft survival for greater than 12 months had only been achieved in just two cases and only 14 recipients were alive at the time of publication (Sutherland 1981). In the UK, the first pancreas transplant was performed at Guy's Hospital in 1972, followed shortly

thereafter by Cambridge in 1974. Unfortunately all the recipients at Guy's died soon after transplant due to complications related to the duodenal segment, while at Cambridge there were 2 deaths and 3 further graft failures, leaving 4 with functioning grafts at the time of reporting in 1981; a success attributed to the use of the then new immunosuppressant drug, cyclosporin A (Sutherland 1981). Fortunately, in the decades that followed, with further improvements in immunosuppressive therapies, perioperative care and antimicrobials, the morbidity and mortality of solid organ transplantation decreased, leading to increasing success and popularity. Despite this, inflammatory reactions to the implanted pancreas tissue remain a challenge today, and still result in horrific graft pancreatitis, systemic sepsis and death. For this reason, the risks of pancreas transplantation are still considered to be significant and, while the metabolic benefits of transplantation are most likely to be realised at an early time-point before the emergence of complications, pancreas transplantation is reserved for people with advanced diabetes, where the presence of existing and severe complications justify the intervention.

Ironically, it is the transplanted exocrine tissue that is the cause for the severe inflammatory reaction that leads to so much trouble, and there is definite appeal in the transplantation of only the cells of importance. In 1974, Paul Lacy performed the first clinical islet transplant. Although initially insulin independent, here too the recipient subsequently died from sepsis (Karl et al. 1977). Improvements in isolation and transplantation techniques were later shown to be consistently successful in a report from Edmonton (Shapiro et al. 2000) and since then islet cell transplantation has become widespread for select patients. Outcomes from islet transplantation have been reported to have improved over the last few years, with insulin independence rates of 70%, 55%, 45% and 36% at 1-, 2-, 3- and 4-years post-procedure respectively in 2009, now improved due

to identification of risk factors associated with graft outcomes, and increasing use of repeated islet infusions (CITR 2014). However, current indications for, and outcomes after, islet transplantation differ to those for whole organ pancreas transplantation.

The choice between islet transplantation and whole organ pancreas transplantation must be considered on an individualised basis. Currently, the large majority of patients undergoing solid organ pancreas transplantation are those with chronic renal failure secondary to diabetes. These patients, on or approaching the need for dialysis, would be candidates for kidney transplantation even in the absence of a pancreas transplant. However, kidney transplantation alone for diabetic renal failure has a relatively poor prognosis compared to other indications (Cosio et al. 2008; H. T. Kuo et al. 2010; Taber et al. 2013). For these patients, lifelong immunosuppression would be required for either transplant and there is increasing evidence that, in patients suitably assessed for the larger procedure, simultaneous pancreas and kidney (SPK) transplantation is associated with best outcome in terms of survival, graft survival and quality of life (Speight et al. 2010; van Dellen et al. 2013). Where a living donor option for kidney transplantation is available, it may be preferred to achieve earlier independence from dialysis by first undergoing kidney transplantation alone before subsequent pancreas after kidney (PAK) transplantation. In 2012, SPK accounted for 84% of all pancreas transplants world-wide compared to PAK which accounted for 9% (R. W. Gruessner and Gruessner 2013). In light of the inferior pancreas graft survival outcomes following pancreas after kidney transplants compared to those following simultaneous pancreas and kidney transplants (Kandaswamy et al. 2013), the decision must be made with careful consideration of the individual patient's circumstances and their likely waiting time on the combined pancreas–kidney list. The national median waiting time on the pancreas transplant list is 407 days, ranging from 199

– 614 days by transplant centre, and recipients with pre-formed HLA antibodies may wait considerably longer (Organ Donation and Transplantation 2014). For those with a living donor option, freedom from dialysis at the earliest time-point may be beneficial.

For people suffering frequent severe hypoglycaemia despite best medical therapy, in the absence of renal failure and other diabetes-related complications, pancreas transplant alone (PTA) or islet cell transplantation may be considered. The choice for these patients is a difficult one: to continue on insulin therapy maintaining relative hyperglycaemia, in order to avoid hypoglycaemia but risking complications, or to undergo a transplant procedure. Both whole organ and islet transplant procedures require lifelong immunosuppression with the associated increased risk of opportunistic infection and malignancy, and potential adverse effects on renal function of calcineurin-inhibitor immunotherapy (Smail et al. 2012). PTA accounted for 7% of pancreas transplants in 2012 (Trial 2002) and involves a major operative procedure (and associated morbidity) and so is more suitable for younger, fitter patients. The goal of pancreas transplantation alone is insulin independence, and this is generally achieved with associated improvements in quality of life. However, islet transplantation has the advantage of being less invasive, and therefore may be appealing, but insulin independence is less frequently achieved (National Institute of Clinical Excellence 2013a). Outcomes have improved over the past decade, and with repeated infusions insulin-independence can be maintained. However, the principle goal remains improvement in the frequency, severity and symptoms of hypoglycaemia, and to provide residual insulin secretory function such that, when insulin treatment is reinstituted, regimes are less complicated and normoglycaemia is more readily achievable (Chiang et al. 2014). The choice between pancreas and islet transplantation will, in part, therefore rest in the desired end-outcome, and requires careful and individualised decision-making.

1.6 Pancreas transplantation

Approximately 1600 pancreas transplants are now performed annually, with over 42 000 having been performed to date world-wide (A. C. Gruessner and Gruessner 2012). In the UK, pancreas transplantation has been performed since 1972, and is currently undertaken in eight UK transplant centres: Oxford, Manchester, Guy's, Cambridge, Cardiff, West London, Edinburgh and Newcastle. Between April 2013 and March 2014, 188 SPK transplants and 26 isolated pancreas (IP) transplants were performed (including 13 PTA and 13 PAK) across all UK centres (NHS Blood and Transplant 2012). Although surgical techniques may vary slightly between units, there is a relatively standard procedure (Han and Sutherland 2010). The arterial supply to the donor pancreas via the superior mesenteric and splenic arteries is attached to a Y graft usually from the bifurcation of the donor common iliac artery in order to provide a single arterial inflow. Most units transplant the pancreas with venous drainage to the common iliac vein or inferior vena cava and arterial inflow from the common iliac artery. A small proportion of units advocate venous drainage into the portal venous system, since the technique is associated with more physiological systemic concentrations of insulin, however this is not supported by evidence of substantial benefit with respect to graft or patient survival or other parameters (Bazerbachi et al. 2012). Although there has been concern that the hyperinsulinaemia associated with systemic venous drainage may be associated with adverse events such as an increased risk of atherosclerosis, there is no convincing evidence that systemic venous drainage places pancreas recipients at a disadvantage (Stadler et al. 2010).

In the UK, NHS Blood and Transplant (NHSBT) coordinate transplants nationally via a central registry. Data show that patient survival rates have continued to improve, reaching 96% at one year and 85-90% at 5 years post-transplantation in line with international data (R. W. Gruessner and Gruessner 2013). Pancreas graft survival has also improved progressively but remains highest with SPK transplantation. One year graft survival reached 85% in SPK transplantation, now close to those of kidney, liver and heart transplantation, compared to 65% in IP transplants, with 5 year graft survival at 77% and 44% respectively (NHS Blood and Transplant 2014a). However, pancreas transplantation is associated with high procedure-specific morbidity with many complications requiring reoperation, including haemorrhage, thrombosis, pancreatitis and sepsis. Indeed, the ischaemia-reperfusion injury that follows transplantation can be fatal, and often leads to graft failure within the first three months post-transplant. This accounts for a considerable proportion of graft losses occurring within the first year, when the risk of graft failure is greatest.

Pancreas transplantation is performed in a patient group already considered to be high-risk. People with diabetes and end-stage renal failure have a high chance of mortality and death rates on the waiting list are common (van Dellen et al. 2013). It is established that kidney transplant confers survival benefit over dialysis (Ojo et al. 2001; Port et al. 1993), and there is increasing evidence that survival after SPK is superior to that after cadaveric kidney transplant (P. Mohan et al. 2003; Ojo et al. 2001; Tyden et al. 1999), even considering the added surgical risk (Smets et al. 1999). Survival after living kidney transplantation was thought to be equivalent to that after SPK (Rayhill et al. 2000), however, there is now considerable evidence that after the first 18 months, successful pancreas transplantation increases life expectancy with a mortality hazard ratio of 0.55

(Morath et al. 2008) or 0.86 (Reddy et al. 2003). Further, although it is likely that stabilisation of renal function does contribute significantly to improved life expectancy after SPK, it has also been shown that the increased metabolic control of a functioning pancreas graft confers significant additional benefit beyond that offered by the kidney transplant alone (Norman et al. 2011; Salvalaggio et al. 2009; Weiss et al. 2009).

Pancreas transplantation has been shown to lead to improved quality of life (QOL) for people with diabetes (Speight et al. 2010; van Dellen et al. 2013; Ziaja et al. 2009). For recipients, freedom from insulin is exchanged for the complications of immunosuppression, and the short-term difficulties of post-operative recovery are balanced against the long-term benefits. Although individual experiences differ, some studies have shown increases in quality of life even in the face of pancreas graft failure (Gross et al. 1998; Speight et al. 2010), although most studies have been limited not only by size but also by the use of generic and heterogeneous QOL measures (Dew et al. 2000; Gross and Zehrer 1992; Hathaway et al. 1994; Speight et al. 2010).

It has been suggested that pancreas transplantation is beneficial with respect to the development and further progression of diabetes-related complications (R. W. Gruessner and Gruessner 2013; White et al. 2009). Studies have shown that pancreas transplantation can stabilise renal function over many years with the potential for tubulointerstitial remodelling (Fioretto et al. 1998; Mauer and Fioretto 2013), and that this functional improvement also translated into graft survival benefit in PAK over kidney alone (Browne et al. 2011). Positive effects of pancreas transplantation have also been observed on diabetic retinopathy, although the greatest benefit is likely to be seen in early disease (Chow et al. 1999; Giannarelli et al. 2006; Sosna et al. 1998). There is less evidence for

improvement in diabetic neuropathy, although some studies have some evidence of improvement (Allen et al. 1997; Kennedy et al. 1990; Martinenghi et al. 1997; Navarro et al. 1990). Data suggest that SPK transplantation reduces cardiovascular death (La Rocca et al. 2001) with improvement in dyslipidaemia (Luan et al. 2007), improvements in systolic and diastolic ventricular function (Fiorina et al. 2000; La Rocca et al. 2001) and beneficial effects on endothelial dysfunction (Fiorina et al. 2003; Stadler et al. 2009), as well as reduction in the progression of atherosclerosis.

Although a large-scale longitudinal observational study with objective measures at serial time-points is needed, in order to definitively demonstrate the role of pancreas transplantation in ameliorating the chronic complications of diabetes, the existing evidence strongly suggest that pancreas transplantation enables tight glycaemic control not achievable with insulin therapy. In turn, benefits in terms of complication progression have been demonstrated, even when complications were advanced at the time of transplantation. Clear and valuable benefits are available with successful pancreas transplantation, and the challenge is in maintaining insulin independence after transplantation.

1.7 Causes of graft failure

Transplant failure is commonly defined as a return to organ support, and this being the case, pancreas transplant failure is commonly defined as a return to exogenous insulin therapy. However, the need for exogenous insulin may not necessarily represent total beta-cell destruction as the pancreas graft may retain residual function, or may be functioning against greater insulin insensitivity, so rendering it unable to respond to extremes of demand (P. G. Dean et al. 2008). Therefore, clearer accepted definitions are needed to describe partial function and graft failure (Organ Procurment and Transplantation Network 2014a). Classification of the causes of pancreas graft failure lacks definition and clarity, and graft loss may represent immunological damage (acute/ chronic) or metabolic insufficiency (beta-cell depletion/ insulin insensitivity), or a combination of the two.

Separating these causes can present a diagnostic challenge, especially since investigations, such as biopsies, may pose a clinical risk that cannot be justified without clear potential benefit. Surgical technical complications are the most common reasons for graft loss in the first three months, followed closely by the sequelae of ischaemia-reperfusion injury. Graft losses between 3 and 12 months are thought largely to be the result of acute rejection, assumed in centres who do not perform biopsies and based on evidence in the literature of antibody-mediated rejection (C4d positive staining) detected in a large proportion of ‘for-cause’ biopsies performed within 1 year post-transplant (Niederhaus et al. 2013). Thereafter, it is presumed that graft loss is immunological, with chronic rejection gradually increasing from the time of surgery. However, with graft failure

appearing to occur suddenly and often without objective evidence of a cause, this is difficult to prove. The pathway from graft function, to dysfunction and failure has not been rigorously studied and factors influencing progressive dysfunction are poorly understood. It is likely that the mechanisms that lead to failure result from a combination of immunological and metabolic changes, and consideration of the multiple factors involved in deteriorating graft function is important for the assessment and optimisation of the failing pancreas graft.

The process of removing, re-implanting and re-perfusing organs leads injury and stress responses in the donor tissue, which in turn can profoundly influence immune responses in the recipient. Innate immune responses initiate injury and trigger adaptive immune responses. Recognition of mismatched MHC molecules by direct (particularly early after transplant) then indirect pathways (throughout the life of the graft) promotes T-cell differentiation, leukocyte recruitment and cellular injury contributing to rejection processes. Mismatched MHC (and non-MHC) molecules also provide antigenic targets for alloantibodies, leading to complement-mediated damage, most aggressively where preformed antibodies are present and as acute or chronic rejection where reactive antibodies arise (Wood and Goto 2012). Further, in addition to alloimmune injury, the impact of autoimmunity must be considered. Many pancreas transplant recipients may display autoantibodies at the time of transplantation, and it is likely that resurgence of immune responses will occur following transplantation and re-exposure to islet cell antigens. Autoimmune-mediated injury may therefore result in the recurrence of T1D, as has been detected in biopsies from pancreas transplants with high levels of circulating autoantibodies (Vendrame et al. 2010). It is likely that monitoring of autoantibody

emergence may be important in identification of grafts at risk of immune injury, however the current evidence is equivocal.

It is also possible that concurrently, and possibly accelerated by immunological injury, beta-cell dysfunction and exhaustion may occur. After a variable period of compensation, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress, dysfunctional triglyceride and fatty acid cycling, and glucolipotoxicity may contribute to islet dysfunction. These stresses may change over time and changes in weight and drug therapies may contribute. Once hyperglycaemia develops, as is seen in islet transplantation, glucotoxicity can lead to islet inflammation, amyloid deposition and accelerate beta-cell demise (Prentki and Nolan 2006). Some of these processes may have initiated in the donor before or after the diagnosis of death, and this may contribute to a reduced reserve of the transplanted organ to survive the trauma of the retrieval and implantation process. A post-mortem study showed that cadavers with pre-mortem impaired or diabetic glucose tolerance had reduced beta-cell volume, thought to be apoptosis-mediated (Butler et al. 2003). Reliable functional measures may not be available prior to acceptance of a donor, and donor beta-cells that are of poor quality at the time of transplantation may have less resistance to subsequent stress. Additionally, recipient genetic or phenotypic characteristics may contribute to the degree of immunological injury, and abnormalities in insulin or glucose sensitivity or metabolism may pose further challenges. Understanding how these many complex factors interact after pancreas transplantation, and which are important in determining graft survival will aid identification of grafts at the highest risk of failure.

1.8 Identifying grafts at risk of failure

In general, attempts to identify pancreas grafts at the greatest risk of later failure start pre-transplant, at the time of donor selection. The potential of a pancreas graft may be to some extent determined prior to transplant, however in the absence of a method of assessing function in an explanted pancreas, clinical features relating to the donor are frequently used to allocate risk. As a result, donor selection criteria for pancreas transplantation remain more stringent than for other organs, and of 1212 deceased donors in the UK between April 2012 and March 2013, only 715 (59%) were offered as meeting suitability criteria for pancreas transplantation. This resulted in retrieval of 439 donor pancreases (61% of those offered) and conversion to transplant in 236 (33% of those offered and 54% of those retrieved) (Organ Donation and Transplantation 2013); a poor conversion rate when compared to other abdominal organ transplant.

Once a donor is accepted, the pancreas may not be transplanted for many reasons, such as due to recipient-related, HLA crossmatch-related or logistical reasons. Pancreas graft injury, related to the cause of death, caused during retrieval or at the implanting centre may occur, or inspection of the pancreas graft may influence decisions on the safety of progressing to transplantation (NHS Blood and Transplant 2014b). Pancreas grafts with extensive parenchymal fat, or with evidence of oedema or inflammation, are likely to result in severe reperfusion pancreatitis, and although they may function, are likely to result in poor outcomes.

Given that a pancreas donor is considered suitable and other factors fall into place such that transplantation proceeds, analysis of outcomes and correlation to donor characteristics may help to provide guidance on practice and help to identify features, previously considered acceptable, that may result in poor graft survival. Several studies have examined donor factors for predictors of graft outcome, and characteristics frequently implicated are older donor age, donors after circulatory death (DCD) and prolonged cold ischaemia time (CIT). An objective approach to donor risk factors has been carried out by Axelrod with the construction of a pancreas donor risk index (Axelrod et al. 2010). Independent factors with the greatest risk for 1 year graft failure were identified (table 1), and then combined in a model that allows calculation of a measure of donor risk (relative to the median donor in the model-building dataset). To date, this remains the best guide for assimilating donor risk, but it does not address the very substantial effect of recipient risk factors. Risk relative to the median recipient may be useful to some extent, however it is not informative for individual patients. It has been argued that a composite risk index incorporating both donor and recipient risk factors would be of greatest benefit in order to optimise the usage of the limited supply of donor pancreases, as well as clinical outcomes (Berney and Johnson 2010). However, such an index has not yet been developed.

Table 1 Axelrod Pancreas Donor Risk Index - factors included in the index and associated risk factors

Variable	**Hazard Ratio**
Donor age	1.56
Donor after Circulatory Death	1.39
Black race	1.27
Cerebrovascular cause of death	1.23
Serum creatinine >2.5mg/dl	1.22

Adapted from Axelrod et al(Axelrod et al. 2010).

Several studies have sought to identify immunological markers associated with poorer graft outcome (rejection and graft failure) or conferring protection or tolerance. Some novel biomarkers have shown promise, including anti-endothelial cell antibodies, soluble CD30, CXC chemokine ligand 9and CXC chemokine ligand 10 in the case of rejection (Heidt et al. 2011), and T regulatory cells in the case of tolerance (Edozie et al. 2014). However, they have been criticised as correlating poorly with outcomes, and have not been thought sufficiently beneficial to justify incorporation into routine transplant monitoring undertaken in a clinical transplant setting.

It has been recognised that high degrees of HLA mismatch are associated with poorer long term graft survival in kidney transplantation (Opelz et al. 1999). HLA typing is now performed routinely in pancreas transplantation, and degrees of mismatch at HLA-A, -B and –DR loci are described and used in national allocation algorithms. Improvements in HLA antibody detection techniques have enabled enhanced identification of HLA antibody specificities (Tait et al. 2013), and has led to the recognition of donor-specific antibodies (DSA), which have been widely demonstrated as associated with inferior graft outcomes in kidney transplantation (O'Leary et al. 2014; Wiebe and Nickerson 2013). Currently monitoring of DSA following pancreas transplantation is not routinely undertaken in most clinical transplant centres, and associations with graft outcomes have not been analysed.

In the context of pancreas transplantation for T1D, autoantibodies may also be of significance and interest, although they are not incorporated into routine monitoring protocols in many centres. The development of autoantibodies may precede the development of diabetes and as such prove predictive. This was observed in a recent

study in three paediatric cohorts from Finland, Germany, and the U.S, which showed that nearly 70% of children who developed more than two autoantibodies developed T1D within 10 years and 84% within 15 years (Sosenko et al. 2013; A. G. Ziegler et al. 2013). In patients who have already had T1D of long duration, it is not known what significance circulating autoantibodies may have on a donor pancreas graft post-transplantation, or if re-emergence of autoantibodies may affect graft survival. Some centres have tried to address the question, however small numbers have made associations inconclusive.

1.9 Measures of graft function

Unlike kidney transplantation, pancreas transplantation suffers from a lack of a validated, simple and reproducible functional measure, with which to assess graft function post-transplant. In most centres, monitoring protocols follow a combination of laboratory and radiological testing. Serum amylase, lipase, glucose, c-peptide and insulin are checked, alongside periodic HbA1c and glucose tolerance testing (Margreiter et al. 2013). Radiological imaging can assess for adequate graft perfusion, thrombosis and intra-abdominal collections, and are usually prompted by clinical signs (Low et al. 2013). In most cases, clinical signs or symptoms of pancreas rejection are subtle or non-existent and, by the time abnormal glucose is apparent, damage is usually irreversible. Also, it is not known whether intervention would be more effective if carried out at an earlier stage, although logically this is likely to be the case.

The exocrine component of the transplant pancreas is often used as a surrogate for endocrine function. Amylase and lipase are monitored sequentially, however although rises in serum amylase and lipase are commonly associated with pancreatitis, their relevance in the context of graft pancreatitis or graft rejection is less clear. In clinical practice, a rise in serum amylase or serum lipase often prompts further investigation with radiological images or biopsies, and studies have shown raises amylase (3.6 fold) and lipase (8.3 fold) to occur concurrently and to be associated with biopsy-proven pancreas rejection with approximately 80% specificity (Klassen et al. 1996). However, both amylase and lipase may also be raised in non-immunological injury, and concentration levels are not associated with severity of rejection, nor response to treatment, and thus do

not correlate with graft outcomes (P. C. Kuo et al. 1997) or glucose levels (Papadimitriou et al. 1998).

The majority of transplant units around the world now transplant the solid pancreas together with a segment of duodenum. In the 1990s the anastomosis of the donor duodenum was usually to the bladder for drainage of exocrine secretions, with the advantage of enabling urinary amylase to be measured and fewer complications with regard to contamination from enterotomy or duodenal leaks (Sollinger et al. 2009). Urinary amylase, or other novel markers (Margreiter et al. 2013), would be monitored in 12- or 24- hour urine collections or as spot tests (Voskuil et al. 2014) since rejection of the exocrine pancreas can precede rejection of the endocrine pancreas by 5-7 days. Although inter-patient comparisons cannot be made, a value decrease greater than 50% would prompt further imaging or trans-cystoscopic biopsy of the donor duodenum (Prieto et al. 1987) enabling detection of rejection potentially otherwise missed. However, bladder-drainage does have disadvantages and is associated with metabolic and urological complications including dehydration, metabolic acidosis and irritation from cystitis. This can lead to multiple hospital admissions for recipients, with associated morbidity and reduction in quality of life. For this reason, bladder drainage has been largely supplanted by enteric drainage, in which the donor duodenum is anastomosed to the proximal jejunum. This is a more physiological technique, but one which renders the pancreas less easy to monitor. Despite this, outcomes after pancreas transplantation with enteric drainage are equivalent to those after bladder drainage, although some centres still employ bladder-drainage for closer pancreas monitoring (Van der Werf et al. 1998).

Endocrine function can be assessed more directly using measures commonly used in diabetes. Finger-prick monitoring of capillary glucose is used in many centres for monitoring, with high values triggering further investigation, usually radiological. However, abnormal capillary glucose when detected on a twice-daily measurement is unfortunately a late sign of rejection and/ or failure. Self-monitoring of blood glucose (SMBG) has been recommended for people with diabetes for assessment and to guide management (American Diabetes Association 2014), with more frequent SMBG correlated to lower HbA1C levels. Frequent testing daily was recommended, although individual needs were recognised to vary (R. Ziegler et al. 2011), and in the UK self-testing 8-12 times a week is considered sufficient. Frequent self-testing may have utility in pancreas transplantation to identify deviations from good control earlier, however, may be laborious and impractical for patients. Real-time continuous glucose monitoring (CGM) through the measurement of interstitial glucose still requires calibration with SMBG, but does offer the additional benefit over SMBG of alarms triggered by hypo- and hyperglycaemic excursions, and was also associated with a reduction in HbA1C compared with SMBG (Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study et al. 2008). CGM is often used in islet transplant groups to provide effective and convenient monitoring, but has been less widely used in solid organ pancreas transplantation.

HbA1C reflects average glycaemia over 2–3 months and strongly predicts diabetes complications (The Diabetes Control and Complications Trial Research Group 1993), and is therefore performed routinely in diabetes monitoring and indeed defines treatment goals. However, this simple test is not without drawbacks since glycosylation rates may vary with patients' race/ethnicity, as well as with anaemias, haemoglobinopathies, and

situations of abnormal red cell turnover (American Diabetes Association 2014). HbA1c also does not distinguish glucose variability or measure hypoglycaemia, which are also important outcomes in diabetes and pancreas transplantation. Interestingly, HbA1c has been shown to predict diabetes development, such that those with an HbA1C of 5.5 - 6.0% had a substantially increased risk of diabetes (5-year incidences from 9 to 25%) (American Diabetes Association 2014), and consequently monitoring HbA1c in pancreas transplant recipients is likely to be important for assessing graft function and potentially predicting graft failure. However, the infrequency of HbA1c sampling may limit utility in identifying grafts at risk of rapid functional decline and opportunities for intervention may be missed.

Measures of insulin and c-peptide can also be used to assess graft function, and have been manipulated into a number of useful metabolic scores. In diabetes, the HOMA score is used to objectively assess both beta-cell function and insulin sensitivity, and uses fasting glucose and insulin values in a calculation based on validated mathematical modelling of glucose metabolism. By providing a percentage function in reference to the normal population, the HOMA model can be used to monitor pancreatic function longitudinally (HOMA Calculator). Although variability in insulin assays between laboratories mean values cannot be compared between centres, it provides a useful assessment tool for intra-individual monitoring (Wallace et al. 2004). Used in islet transplantation, the beta-score is a composite of fasting glucose, HbA1c, stimulated C-peptide, and insulin requirement (Ryan et al. 2005), and the HYPO score primarily assesses resolution of hypoglycaemia, and the lability index (Ryan et al. 2004). These have proven useful in islet transplantation for measuring outcomes; however have limited utility in pancreas transplantation.

The principle problem with using models developed in health, diabetes or islet transplantation for measuring pancreatic function, and applying them to pancreas transplantation, is that they are designed and developed based on complex experimental and mathematical study of physiological glucose homeostasis. Secondly, they are often calculated using c-peptide levels. These two things mean the scores are difficult or impossible to interpret and to compare, since the systemic drainage of pancreas transplants means that glucose homeostasis is achieved in a non-physiological manner with loss of first pass metabolism of insulin, and altered dynamics on the hepatic glucose production, thus would require specific and extensive remodeling of existing scores. Second, c-peptide is used as a surrogate for insulin production in patients where insulin production cannot be measured. However, in the case of pancreas transplantation, where renal function may be impaired and likely to change in between monitoring time-points, renal excretion of c-peptide will be affected and results skewed.

Biochemical measures of function remain elusive in pancreas transplantation, and although measures established in diabetes are commonly used, they are not validated for the altered physiology of transplantation. In health, and in diabetes, it is known that glucose homeostasis involves the complicated interplay between multiple factors. Pancreas transplantation involves the implantation of a heterotopic denervated pancreas graft, leaving in situ the beta-cell defective native pancreas. As a result, the physiology of glucose homeostasis after pancreas transplantation is not fully understood. The incretin effect (the effect of the gut on insulin secretion) is important in the regulation of insulin and glucagon, and is important in mealtime insulin secretion in health. These effects are hypothesised to be mediated via a neuroendocrine axis and it is not known if this mechanism remains following implantation of a denervated pancreas. The effect of

systemically drained insulin on blood glucose and the regulation of native and transplanted alpha-cells is unclear (Diem et al. 1990). Loss of first pass metabolism in the liver and high peripheral insulin may have profound effects on glucagon, glucose levels and rates of hypoglycaemia (Choudhary and Amiel 2011). The relevance of this and the effect on measures such as glucose tolerance and HbA1c is unknown.

Glucose tolerance tests (OGTTs) are commonly performed in the diagnosis of diabetes. Gradual ratchet-fashion deterioration in glucose tolerance has been noted to precede a diagnosis of T1D in the Diabetes Prevention Trial (Sosenko et al. 2012a) and increased excursions on continuous glucose monitoring has been seen prior to the development of T2D (Madhu et al. 2013). Acute insulin response to intravenous glucose has been found to be diagnostic of suboptimal engraftment after islet transplantation and has been used to predict later graft failure (Hirsch et al. 2012). More sophisticated dynamic tests, such as arginine or glucagon stimulation, can also be performed to assess maximal beta-cell function, but can be expensive and labour intensive. Metabolic measures and stress tests have commonly been used in pancreas transplant recipients, both in the context of clinical and research protocols, however have not as yet been validated as predictors or risk factors for a return to insulin dependence after pancreas transplantation.

It may be that markers identified as predictive for the development of diabetes may be predictive of a return to insulin-dependence after pancreas transplantation, or it may be that a combination of measures is required (Krischer and the Type 1 Diabetes TrialNet Study 2013). Since these measures were not developed for use in the context of systemic insulin drainage and the altered physiology of transplantation, modification of the calculations is likely to be needed.

1.10 Potential for graft salvage

In transplantation of other organs, functional markers exist (for example, creatinine in the case of kidney transplantation) and when dysfunction is detected, radiological imaging and/ or tissue biopsy are initiated. This provides the opportunity for early identification of pathology and implementation of appropriate treatment. In pancreas transplantation, glucose derangement often occurs late, at which time damage may be irreversible, and the key to improving graft outcomes will be in improving monitoring. It is essential to find a way to monitor grafts more closely (and simply), so that potential injury can be identified early at a time-point where graft salvage would be possible, and targeted intervention employed.

Although, pancreas biopsies have been performed in several centres, the risk of serious complications has been reported to range from 2.8 to 11% (Atwell et al. 2004; Gaber 2007; Klassen et al. 2002) and, as such the risk must be balanced against potential benefits. Most centres do not perform pancreas biopsies and little data has been available to build experience and develop standardised protocols, particularly with regard to histological interpretation. Duodenal biopsies, or kidney biopsies in the case of SPK, have been used as surrogates for direct pancreas histology. However, in one study disconcordance was found in 38% of kidney and pancreas biopsy pairs (Troxell et al. 2010) so significantly challenging the validity of this approach. Correlation between duodenal and pancreatic rejection has been good in a number of studies, however, disconcordant rejection can occur such that a negative duodenal biopsy does not preclude rejection in the pancreas (Carpenter et al. 1990; Gaber 2007; R. W. Gruessner et al. 1994a;

Kuhr et al. 1995; Troxell et al. 2010). Until such a time that pancreas biopsies can be performed more safely and greater experience is acquired in the interpretation of biopsy samples, identification of effective, less invasive markers of graft rejection is of particular importance in pancreas transplantation. These markers may highlight those at highest risk, where undertaking biopsy may be justified, or may be sufficient to warrant immediate intervention.

Targeted therapies for those at risk of pancreas transplantation, are likely to involve modification of immunosuppression regimes, or specific intervention for metabolic function, such as insulin or therapies modulating the enteroinsular axis. Immunosuppression may involve short-course therapies or modification of long-term regimes. The complex interaction of allo- and auto-immunity will need to be considered and caution exercised with regard to the risk of complications from over-immunosuppression in this already significantly immunosuppressed group. In islet transplantation, therapies have been trialled in the pre-transplant setting to improve beta-cell function, with particular success using GLP-1 in vitro to activate signalling pathways to protect against apoptosis (Egan et al. 2003; Trumper et al. 2002). Preclinical studies in rodents have also provided evidence that GLP-1 may be able to halt the progressive decline in beta-cell mass and perhaps even promote the growth of new beta-cells in vivo (Stoffers et al. 2003; Xu et al. 1999). It is hypothesised in both islet and pancreas transplantation, that the pathogenesis of graft failure may follow the natural history of the development of T2D (Potter et al. 2014). Although, increases in beta-cell mass have not been directly demonstrated in people with T2D treated with GLP-1 analogues, there is evidence from human islets that GLP-1 inhibits apoptosis and increases intracellular insulin content (Farilla et al. 2003), and that treatment has clinical benefit having shown

promotion of insulin secretory capacity (Kjems et al. 2003) and delay in need for insulin treatment (Klonoff et al. 2008). It is not known if these therapies may have the potential to improve pancreas graft function and survival, and research is needed to guide intervention choice.

1.11 Study Rationale

Whole organ pancreas transplantation is a successful treatment for the management of insulin-dependent diabetes, but still presents many challenges. Clearly, pancreas transplantation offers many benefits for people with diabetes, however current indications are restrictive. There is a clear need to expand the utilisation of potential donor organs and increase pancreas graft survival rates, at least to the level currently achieved in kidney and liver transplantation. Certainly, achieving this would have important implications for individuals waiting for and undergoing pancreas transplantation, including personal, economic and social advantages through a longer period of insulin independence. Equally there would be similar benefits for healthcare and society, in terms of the resource use and the cost-utility of avoiding advancing complications, and in enabling people with diabetes to be independent and potentially return to employment. Further, by improving graft survival and so lowering the perceived risk, expansion of current listing criteria could be considered for patients with advanced complications but preserved renal function.

Current methods for assigning risk and monitoring pancreas grafts are insufficient and novel methods are needed to identify which grafts should be transplanted. Following pancreas transplantation, there is little known about the relevance of immunological markers in predicting graft survival, nor if early functional measures correlate to later graft outcomes. Pancreas transplantation results in a non-physiological state and better understanding of the mechanisms of graft function, and dysfunction, will enable appropriate targeted intervention therapies for pancreas grafts showing suboptimal function.

1.12 Aims

This thesis describes a number of clinical studies carried out in a UK pancreas transplant centre. Current clinical monitoring markers are investigated for correlation with graft failure, in order to identify those of the greatest utility. Detailed analyses of post-transplant metabolic mechanisms are described, in order to inform targeted intervention therapies. The specific aims of the thesis are as follows:

i. To assess the utility of donor factors in predicting graft survival in a UK cohort (chapter 3)

ii. To assess the role of donor-specific antibodies in predicting pancreas graft outcome (chapter 4)

iii. To assess the role of autoantibody positivity in predicting pancreas graft outcome (chapter 5)

iv. To investigate the role of the oral glucose tolerance test and continuous glucose monitoring post-transplant (chapter 6)

v. To investigate the role of glucagon and incretin hormones in glucose homeostasis early after pancreas transplantation (chapter 7)

Chapter 2

Methods and Study Population

2 METHODS AND STUDY POPULATION

2.1 Oxford Cohort Study

All recipients of pancreas transplants performed at the Oxford Transplant Centre were invited to take part in the study, having gained approval from National Research Ethics Service Committee South Central – Oxford B and the Oxford University Hospitals Research and Development department. Participants received information leaflets and had the opportunity to ask questions before signing and returning informed consent forms for the collection of their clinical data into an anonymised research database.

The patient cohort included all recipients of deceased donor pancreas transplants performed at the Oxford Transplant Centre from 2002 to November 2011, at which time the study began. Follow-up data was collected for all patients included in the database until November 2014, at which time the database was closed. Recipients of simultaneous pancreas kidney (SPK), pancreas transplant alone (PTA) and pancreas after kidney (PAK) were included. PTA and PAK were considered together as isolated pancreas (IP) transplants.

2.2 Clinical protocols

2.2.1 Indications and listing procedures

Potential recipients for pancreas transplantation were referred to the Oxford Transplant Centre for pre-assessment by a multi-disciplinary team for SPK, PTA or PAK transplantation according to centre criteria. Patients were assessed for appropriateness for listing according to clinical protocol, having ensured the absence of contraindications to

surgery or immunosuppression, as shown in table 2.1. Following counselling, patients considered suitable for pancreas transplantation underwent further investigation. Metabolic work-up was performed and consisted of eliciting diabetic history, the presence and extent of diabetic complications and medication history, as well as blood tests including serum c-peptide levels, HbA1c and autoantibody levels. All patients were discussed in a multidisciplinary listing meeting to determine the likelihood that pancreas transplantation would offer clinical benefit in terms of diabetes management, symptom relief and avoidance of further progression of diabetic complications. Fitness for surgery was confirmed with cardiac assessment, including stress echocardiography, myocardial perfusion scanning and/ or angiography as appropriate. In the presence of diabetes complications, cardiovascular comorbidities were common and all abnormal results were reviewed and discussed in a multidisciplinary meeting, including transplant surgeons, transplant physicians, cardiologists and anaesthetists. Potential recipients were considered to gain a potential benefit from, and be fit enough to tolerate, the additional stress of the pancreas transplant above a kidney transplant alone.

All potential recipients underwent routine pre-transplant immunogenetic analysis comprising ABO grouping, HLA genotyping and alloantibody detection. These data were used to inform potential waiting times to transplantation, and were used to enable national allocation of deceased donor organs. Alloantibody detection was routinely repeated periodically for every recipient on the waiting list, in order to identify any new or re-emerging antibodies that may influence clinical decisions. Following full assessment, patients deemed suitable for transplantation were placed on the SPK or pancreas alone deceased donor waiting list, with comprehensive annual reassessment to confirm ongoing suitability, until invited for transplantation.

Table 2.1 Inclusion and exclusion criteria for pancreas transplantation at the Oxford Transplant Centre

SPK	PAK	PTA
Insulin dependent diabetes Age <65 years BMI <30kg/m^2 Limited cardiovascular disease		
eGFR <20ml/min/1.73m^2 or on dialysis	Kidney transplant for diabetic nephropathy	Life-threatening hypoglycaemia awareness where all other therapies have failed Poor quality of life Minimal renal impairment
Contraindications: Relative: Myocardial dysfunction or severe non-correctable coronary artery disease Hepatitis B or C; HIV seropositivity Active infection Significant history of non-compliance Absolute: Ongoing substance abuse; major psychiatric illness Active HIV infection BMI >35kg/m^2 Active malignancy (excluding localised skin)		

Simultaneous pancreas kidney transplantation (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK), body mass index (BMI), estimated glomerular filtration rate (eGFR), human immunodeficiency virus (HIV)

2.2.2 Pancreas donor selection and allocation procedures

Deceased donors were categorised as donors after brainstem death (DBD), where brainstem death criteria were satisfied and assessed by two doctors who had been registered for more than five years and were competent in the procedure; or donors after circulatory death (DCD), where non-survivable injuries existed and brain death criteria were not met due to residual brain stem function.

Allocation of suitable pancreas donors was approached through a nationally- agreed allocation policy. Prior to 2010, donor pancreases were allocated to the pancreas transplant centre local to the donor hospital. The transplant centre would then select a suitable recipient from their waiting list. This decision was often based on appropriate ABO and HLA matching, and on the time the potential recipient had been on the waiting list. After 2010, pancreas allocation was centralised and determined using a points-based algorithm, whereby donor pancreases were offered to named recipients, instead of to a transplant centre (table 2.2).

Recipient coordinators received offers and relayed these to consultant transplant surgeons at the Oxford Transplant Centre, who accepted or declined the offer based on knowledge of the donor and recipient clinical history (table 2.3). When a potential recipient had been identified, the recipient co-ordinator would telephone the patient and request that they travel to the Oxford Transplant Centre as soon as possible. Once at the transplant centre, they would be assessed to confirm suitability to proceed.

Table 2.2 National allocation points system[137]

RECIPIENT FACTORS	
Waiting time	Patients accrue points with time on the waiting list
Sensitisation	Highly sensitised patients are prioritised for a given match
Dialysis	Dialysis-dependent patients are prioritised
DONOR RELATED FACTORS	
Travel time	Points awarded to transplant centres close to donor hospital (especially for donors after circulatory death)
Total HLA A, B & DR mismatch	Preference to better matched recipients
Donor BMI	Donors with high BMI preferred for islet transplant; low BMI preferred for whole organ
Donor to recipient age matching	Points awarded for better age match

Body mass index (BMI), human leukocyte antigen(HLA)

Table 2.3 Criteria for donor pancreas acceptance at Oxford Transplant Centre

DBD age <65
DCD age <60
Donor BMI <32
No history of diabetes
No history of pancreatitis
Negative for Hepatitis C, HIV and CJD

Donor after circulatory death (DCD), donor after brainstem death (DBD), body mass index (BMI), human immunodeficiency virus (HIV), Creutzfeldt-Jakob disease (CJD)

2.2.3 Transplant operation

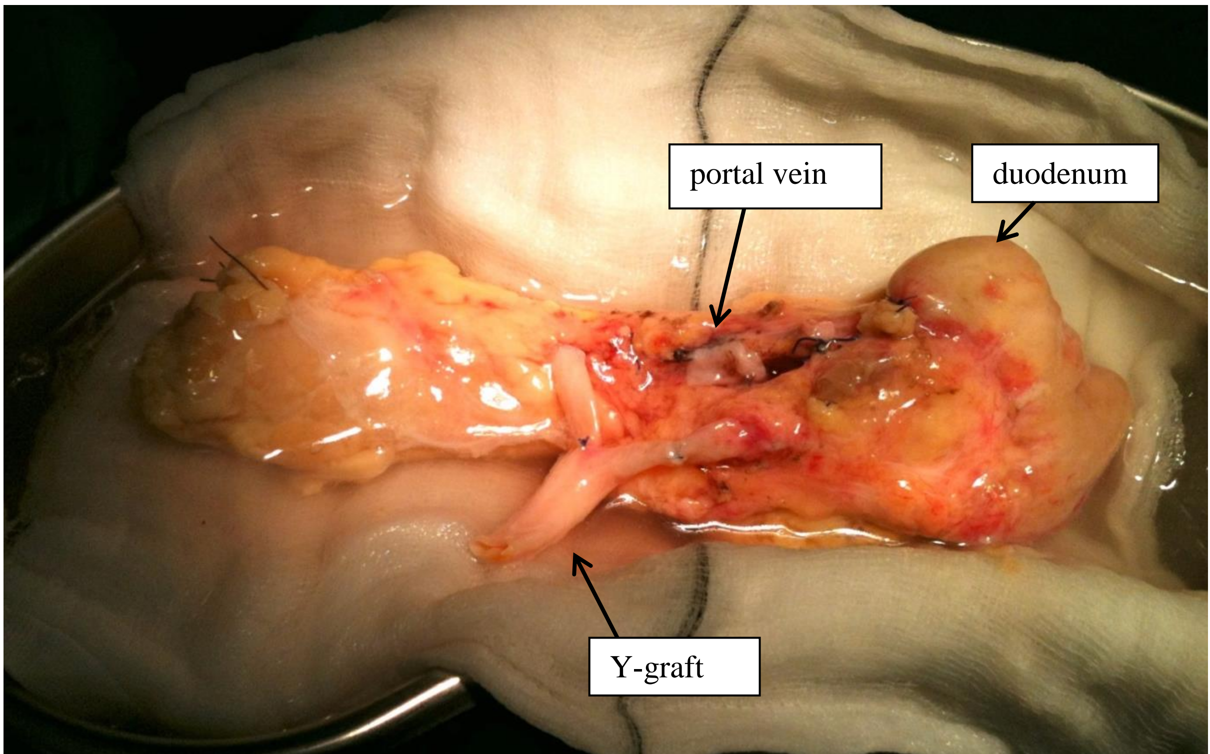
Potential transplant recipients were admitted to the Transplant Unit, at which time clinical assessment was made and blood tests were taken for cross-matching to ensure appropriateness to proceed to transplantation.

Immediately following retrieval, preservation of the donor pancreas was by static cold storage, with University of Wisconsin solution to minimise preservation injury. At the recipient centre, the donor pancreas would be removed from static cold storage in a sterile manner and inspected to identify steatosis, fibrosis or injury that would preclude transplantation. Once assessed as suitable for transplantation, the pancreas graft was prepared on the back-table with removal of the spleen and non-pancreatic attached tissue, ligation of potential points of bleeding and preparation of the arterial supply via the superior mesenteric and splenic arteries with a Y graft (usually from the bifurcation of the donor common iliac artery) in order to provide a single arterial inflow (figure 2.1).

During this time, providing a safe cross-match had been confirmed, the recipient was anaesthetised in order to minimise cold ischaemic time. The transplant operation involved implantation of the donor pancreas with arterial inflow from the recipient right common iliac artery and venous drainage systemically into either the recipient inferior vena cava or, much less commonly, the right common iliac vein. Enteric drainage of the exocrine secretions with anastomosis of the donor duodenum to recipient jejunum was used in all, except 16 recipients who received isolated pancreas transplants (PTA or PAK) with exocrine drainage into the bladder due to a change in centre protocol in January 2011. This change in practice for IP transplants only was due to observed inferior outcomes in this group (figure 2.3), and in order to allow monitoring of urinary amylase. In the case of

SPK transplantation, the donor kidney was usually implanted intra- or extra- peritoneally onto the left external iliac artery and vein. In all cases, the native pancreas was left in situ.

Figure 2.1 Y graft anastomosed to pancreas graft prior to implantation



2.2.4 Peri- and post-operative care protocol

Post-operatively, all SPK recipients were managed in the Intensive Care Unit for 24 – 48 hours, prior to discharge to the Transplant Ward. Isolated pancreas (IP) recipients were returned directly to the Transplant Ward from the operating department recovery suite.

2.2.4.1 Immunosuppression protocol and monitoring

Immunosuppression regimen followed a standard protocol. All recipients received alemtuzumab induction immunosuppression (Campath 30mg, day 1 and day 2). Maintenance immunosuppression therapy consisted of tacrolimus, initially at 0.5mg/kg bd and titrated to maintain trough levels between 8 and 10ng/ml throughout the follow-up and mycophenolate mofetil, at 750mg bd, with dose adjustments as clinically indicated. To guide dose changes, trough serum tacrolimus levels and absolute neutrophil counts were monitored regularly on an outpatient basis, initially at least twice weekly for the first postoperative month, then with reducing frequency to monthly according to clinical status and stability. Mycophenolate levels were not monitored. No steroids were used in maintenance immunotherapy.

HLA antibody analysis was requested routinely at set time-points post-operatively, and at the time of clinical events. Samples were analysed by clinical scientists at the Transplant Immunology and Immunogenetics Laboratory at the Oxford Transplant Centre, Churchill Hospital, Oxford. Results were used with other available clinical information in an individualised manner to guide management decisions.

2.2.4.2 Prophylactic medications and assessment

Anticoagulation was routinely prescribed. Heparin was administered intra-operatively (5000 units subcutaneously), and post-operatively patients were maintained on aspirin and subcutaneous heparin 2500 units twice daily continued for 6 weeks. Immediately post-operatively and continuing until the reestablishment of diet, recipients also received an infusion of dextran 40 in 0.9% sodium chloride, which was titrated according to daily thromboelastogram results. Hypercoagulable states were assessed with reference to the clotting index, as reported by the thromboelastogram, and responded to by incremental increases in the rate of the dextran infusion.

All recipients received prophylactic medications as per protocol including, intraoperatively broad-spectrum antibiotics, antifungals and methyl prednisolone, and postoperatively prophylaxis for pneumocystis carinii, tuberculosis and CMV as necessary. Following discharge, recipients were regularly monitored for CMV infection for 6 months post-operatively. Evidence of CMV infection prompted reduction in immunosuppression and/ or treatment with valganciclovir.

In the case of SPK transplantation, the kidney graft was assessed post-operatively with Doppler ultrasound, in order to check that good perfusion of the graft was present. If graft perfusion was compromised, and considered due to technical causes, a return to theatre was initiated. The pancreas graft was not routinely imaged. Urinary catheters were maintained for 5 days post-operatively, in order to protect the cystoureteric anastomosis, prior to the patient leaving the ward for home. J-J ureteric stents were routinely placed intra-operatively and removed at 6 weeks post-transplant under local anaesthetic, for the same reason.

2.2.4.3 Functional assessment of pancreas graft

Intra-operatively, recipients were maintained on an intravenous sliding scale titrated to capillary glucose levels. When blood levels normalised, the sliding scale was discontinued. Capillary blood sugar levels were thereafter monitored with 2- hourly finger prick testing, with a value above 8mmol/l prompting medical review. If the abnormal value was related to consumption of food or drink within the preceding hour, observation was continued. If the abnormal value was not related to food or drink within the preceding hour, imaging of the pancreas graft with computerised tomography or magnetic resonance imaging with intravenous contrast was organised. This was to assess for arterial or venous thromboses and/ or compromise of perfusion to the pancreas graft. If perfusion was normal and no thromboses were identified, no specific treatment was initiated. If thromboses were identified (with or without compromise of graft perfusion), anticoagulation was initiated with therapeutic dose subcutaneous heparin, and continued for 3 months. At this time, repeat imaging was undertaken and anticoagulation stopped if there was resolution of the thrombus.

Prior to discharge from the ward, recipients underwent a standard 75g oral glucose tolerance test (OGTT) with glucose testing at 0 and 2 hours, in order to establish baseline function. If abnormal glucose tolerance was detected, pancreas imaging was performed and acted on as above.

Following discharge, recipients self-monitored capillary glucose levels twice daily, and alerted the unit in the event of raised glucose levels, which would prompt full clinical assessment and pancreas graft imaging. Recipients attended regular out-patient clinics for follow-up care including clinical assessment of diabetic symptoms and complications, and

3 monthly metabolic assessments with HbA1c and OGTT until 1 year post-transplant and annually thereafter. Recipients also had post-operative testing for development of autoantibodies.

Pancreas graft biopsies were not performed as part of the protocol, and therefore pancreas rejection could not be confirmed definitively. Pancreas graft rejection was suspected in cases of abnormal blood glucose, raised serum amylase or, in the case of bladder-drained PTA, reduced urinary amylase and treated with pulse methyl prednisolone where considered clinically appropriate on a case-by-case basis. In this study, pancreas graft failure and kidney graft failure were defined as a return to exogenous insulin (Organ Procurment and Transplantation Network 2014b) and dialysis respectively. Clinically evident and biopsy-proven kidney rejection episodes were treated with pulsed methyl prednisolone in the SPK group.

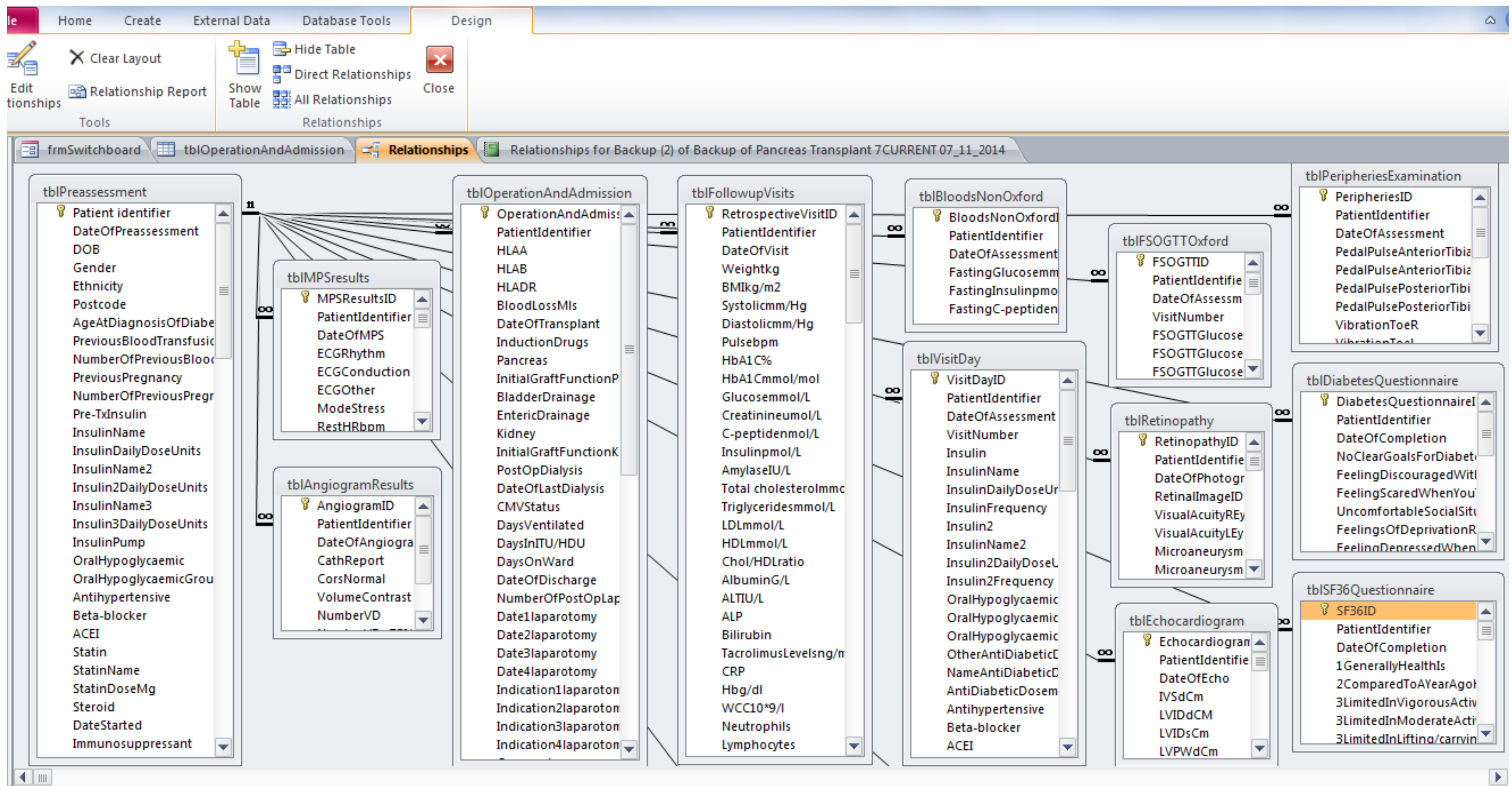
2.2.4.4 Post-discharge follow-up arrangements

Following discharge, pancreas transplant recipients were reviewed regularly in a consultant-led out-patient follow-up clinic. After one month post-transplant, or when recipients were clinically stable, those living significant geographical distances from the transplant centre were referred for more regular appointments at their local transplant or nephrology centre. All recipients were invited for follow-up at the Oxford Transplant Centre every 3 months until one year post-transplant, and annually thereafter. The follow-up care of recipients referred from Bristol was undertaken at Southmead Hospital by Dr Richard Smith, and these patients did not receive any routine follow-up at the Oxford Transplant Centre.

2.3 Research database design

A bespoke research database was designed in Microsoft Access® to collate clinical data for analysis. Tables were created for time-points relating to stages in the transplantation process: pre-transplant assessment, operation and inpatient stay, post-transplant clinical follow-up and research-related visits (figure 2.2). Within each table, fields were created with specified field properties as appropriate. Data were retrieved manually from clinical notes and hospital electronic records and databases, and entered into the research database. Data were associated with a unique identifier and entered into a password-secured anonymised research database held on a University of Oxford network drive accessible only to registered members of the research team. Where recipients had continuing follow-up care at local hospitals, data were collected through communication with local clinical teams. Data were censored at time of death or duration of follow-up.

Figure 2.2 Screen-print of research database relationships



2.3.1 Study queries

For each hypothesis-driven study, a query was created to extract data for analysis. Relevant fields were thereby collated and exported into a Microsoft Excel® format. Missing data were defined using the value 9999 and the data was transferred into IBM SPSS Statistics (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) for all statistical analyses.

2.3.1.1 Cohort characteristics: donor and recipient factors

485 pancreas transplants were included in the transplant database, including 364 SPK, 68 PTA, 39 PAK and 14 re-transplants (or 2nd transplants). The mean recipient was 43 years of age, with a BMI of 25.3 kg/m^2. The mean donor was 36.4 years of age with a BMI of 23.9 kg/m^2. The mean CIT was 689 minutes. Recipient and donor factors were comparable between SPK, PTA, PAK and re-transplants, although there was a higher proportion of donors after circulatory death (DCD) in the PTA and PAK groups, due to biases in the kidney allocation schemes. This imbalance occurred because DBD kidneys were offered under a national allocation, whereby preference was given to SPK transplants after paediatric and highly sensitised recipients. However, DCD kidneys were allocated locally and, in circumstances where the local centre was not a pancreas transplant centre, the kidneys were often used in kidney only transplants and a pancreas alone transplant was performed by the closest appropriate pancreas transplant centre. Table 2.4.

Table 2.4 Oxford Cohort demographics: donor and recipient characteristics

	Overall N=485	**SPK N=364**	**PTA N=68**	**PAK N=39**	**2nd N=14**
Donor type (% DCD)	12.9%	6.3% ^#	33.8% *+	41.0% *+	7.1% ^#
Donor gender (%male)	52.2%	50.8%	52.9%	64.1%	0%
Donor age (years)	36.4 ± 13.3	36.9 ± 13.6	35.0 ± 13.2	33.7 ± 10.6	39.0 ± 12.4
Donor BMI (kg/m^2)	23.9 ± 4.1	24.2 ± 4.4	23.3 ± 3.2	23.0 ± 3.1	23.6 ± 2.3
Recipient gender (% male)	59.8%	62.6% ^	42.6% *	61.5%	44.5%
Recipient age (years)	43.3 ± 7.9	43.5 ± 7.9	42.1 ± 9.3	43.3 ± 6.7	43.4 ± 5.9
Recipient BMI (kg/m^2)	25.3 ± 3.9	25.4 ± 4.0	24.9 ± 3.1	25.8 ± 4.3	24.8 ± 4.5
Cold ischaemia time (mins)	689 ± 172.4	685 ± 176.8	679 ± 143.5	729 ± 173.5	740.1 ± 189.5

Data represented as mean ± SD for continuous variables and percentage of the cohort for categorical variables.
* $p<0.05$ vs SPK; ^ $p<0.05$ vs PTA; # $p<0.05$ vs PAK; + $p< 0.05$ vs 2nd transplant
Simultaneous pancreas kidney transplant (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK). Donor after circulatory death (DCD), body mass index (BMI)

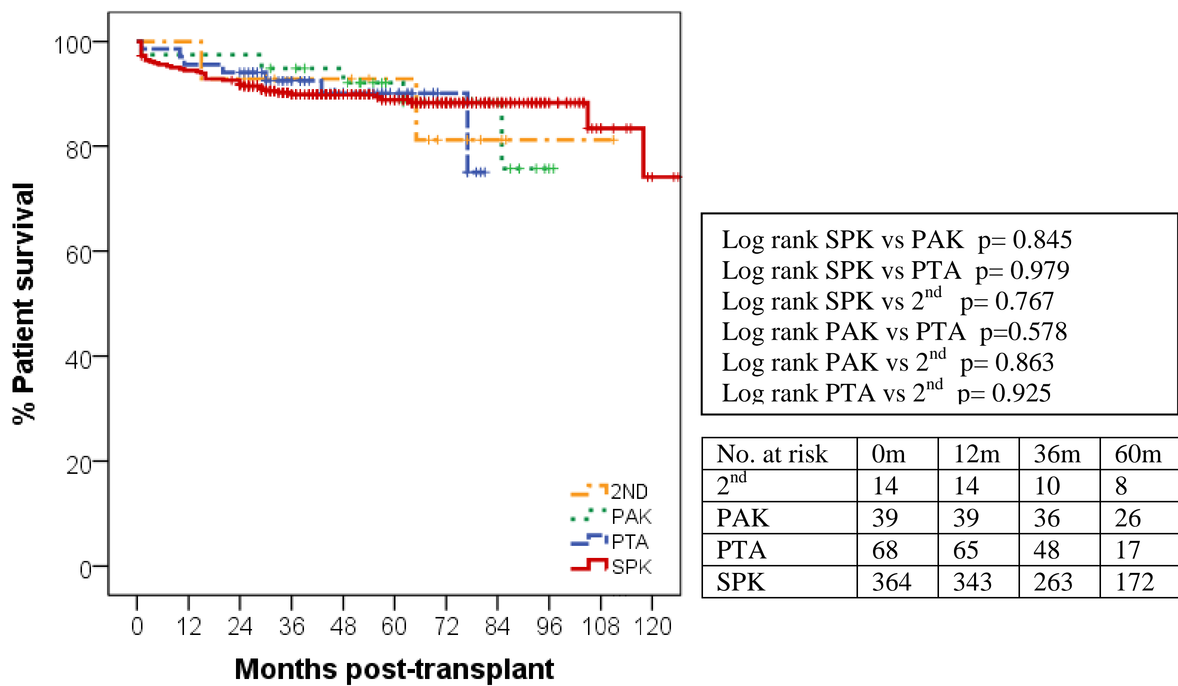
2.3.1.2 Outcome analysis: patient and graft survival

In this cohort, patient survival was comparable for all types of transplantation. In the SPK group, there were 41 deaths, giving a 1-, 3- and 5-year patient survival of 97.3%, 89.9% and 88.9% respectively. In the PTA group, there were 7 deaths, giving a 1-, 3- and 5-year patient survival of 95.6%, 92.5% and 90.1% respectively. In the PAK group, there were 5 deaths, giving a 1-, 3- and 5-year patient survival of 97.4%, 94.9% and 92.1% respectively. In the re-transplant group, there were 2 deaths, giving a 1-, 3- and 5-year patient survival of 92.9%. Figure 2.3.

The cohort was examined for pancreas graft survival by transplant type. In the SPK group, there were 54 graft failures, giving a 1-, 3- and 5-year graft survival of 88.3%, 85.5% and 84.1% respectively. In the PTA group, there were 9 graft failures, giving a 1-, 3- and 5-year graft survival of 85.2%, 70.3% and 70.3% respectively. In the PAK group, there were 11 graft failures, giving a 1-, 3- and 5-year graft survival of 84.5%, 73.6% and 73.6% respectively. Graft survival in the re-transplant group was poor, with 7 graft failures, giving a 1-, 3- and 5-year patient survival of 57.1%, 50.0% and 50.0%. Figure 2.4.

These observations confirm patient survival and graft survival outcomes in this cohort are comparable to those in the international literature, and therefore comprise a representative cohort for further analysis. Graft survival after PTA and PAK are confirmed to be equivalent, as are donor and recipient demographic variables, and thus these groups can be justifiably combined in further analyses. This has the advantage of providing a larger sample size, in which to investigate associations.

Figure 2.3 Oxford Cohort Patient Survival: by transplant type

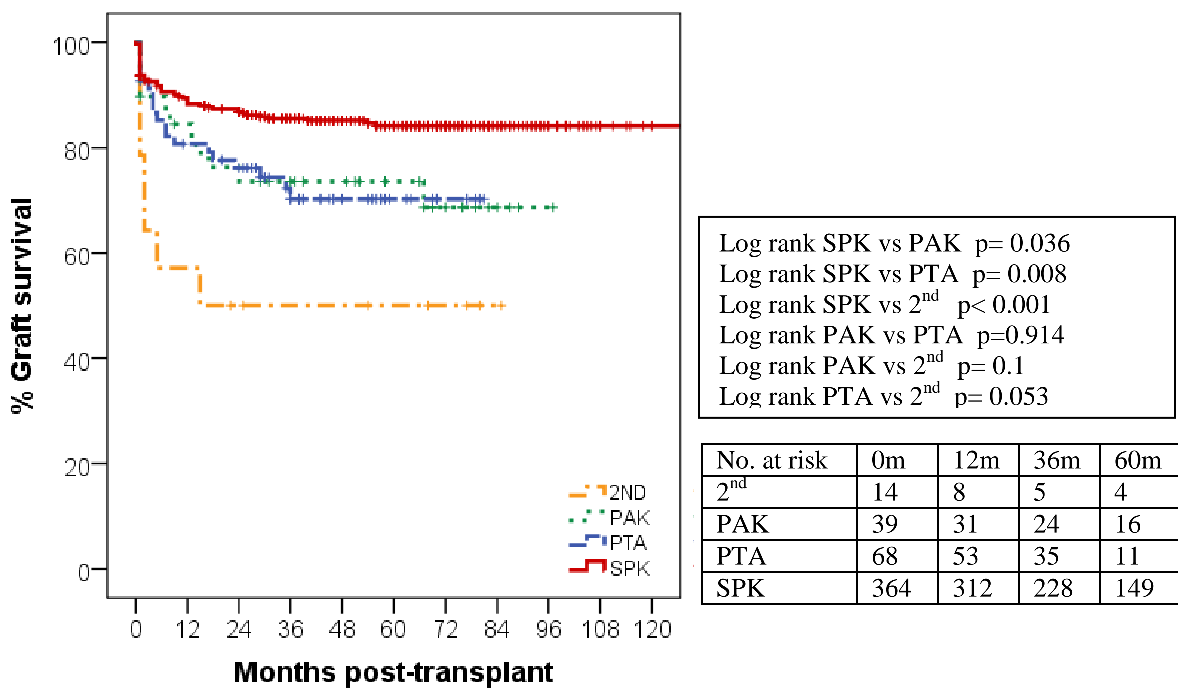


Log rank SPK vs PAK p= 0.845
Log rank SPK vs PTA p= 0.979
Log rank SPK vs 2nd p= 0.767
Log rank PAK vs PTA p=0.578
Log rank PAK vs 2nd p= 0.863
Log rank PTA vs 2nd p= 0.925

No. at risk	0m	12m	36m	60m
2nd	14	14	10	8
PAK	39	39	36	26
PTA	68	65	48	17
SPK	364	343	263	172

simultaneous pancreas kidney (SPK); pancreas transplant alone (PTA), pancreas after kidney (PAK), retransplant (2nd)

Figure 2.4 Oxford Cohort Pancreas Graft Survival: by transplant type



Log rank SPK vs PAK p= 0.036
Log rank SPK vs PTA p= 0.008
Log rank SPK vs 2nd p< 0.001
Log rank PAK vs PTA p=0.914
Log rank PAK vs 2nd p= 0.1
Log rank PTA vs 2nd p= 0.053

No. at risk	0m	12m	36m	60m
2nd	14	8	5	4
PAK	39	31	24	16
PTA	68	53	35	11
SPK	364	312	228	149

simultaneous pancreas kidney (SPK); pancreas transplant alone (PTA), pancreas after kidney (PAK), retransplant (2nd)

2.3.1.3 Demographic factors predictive of patient mortality and pancreas graft failure

Patient mortality was considered the worst possible outcome after pancreas transplantation and demographic factors were analysed for associations. Donor age, BMI, gender and DCD status were considered, as were recipient age, BMI and gender. Cold ischaemia time, transplant type and kidney graft failure were also analysed. A univariate Cox regression analysis for patient survival found that although donor and recipient variables were not associated with patient mortality, both pancreas graft failure and kidney graft failure were significantly associated with risk of death. In a multivariate model, both pancreas failure and kidney failure were confirmed to be independently associated with mortality (pancreas graft failure: hazard ratio, HR 2.01 [1.12-3.60], p=0.019; kidney graft failure: HR 2.89 [1.35-6.19], p=0.006). This provided important justification for the focus of this thesis, and supported data reported in the literature suggesting pancreas transplantation offers a survival benefit independently of kidney transplantation and that loss of pancreas function led to significantly increased risk for patients.

A second Cox regression analysis was performed to identify if clinical factors can predict pancreas graft failure, and in univariate and multivariate analysis donor and recipient factors were not associated with pancreas graft survival. Factors associated with pancreas graft failure in a multivariate analysis were transplant type (HR 1.62 [1.25-2.09], p<0.001) and kidney failure (HR 4.54 [2.41-8.54], p<0.001). That is to say that having an isolated pancreas transplant was associated with poorer graft outcome, as has been widely observed and reported; and that if kidney failure occurs, there is a very good chance that pancreas failure will also occur. This finding was important in confirming that simple demographic features are insufficient for identifying grafts at risk in a large UK single centre cohort, and that more sensitive risk factors need to be identified. This is likely to be

beyond the scope of most registry databases, where data relies on accurate reporting and is not available in sufficient detail. These findings also allude to a potentially common mechanism affecting the pancreas and kidney graft, which is likely to be immunological in nature.

2.4 Prospective metabolic studies

Prospective metabolic studies were performed in cohorts independent to that included in the Oxford Cohort Study. Protocols and participant information leaflets were approved by the National Research Ethics Service Committee South Central- Oxford C and the Oxford University Hospital NHS Trust Research and Development department. Invitation letters were sent with participant information leaflets and reply slips to potential recipients on the pancreas transplant waiting list. Following an expression of interest, participants were given the opportunity to ask questions about the research study. If interested, potential participants were screened to ensure they could provide consent and tolerate an oral glucose tolerance test, before being invited to sign an informed consent form. Study participants provided consent for the research team to inform their General Practitioner of involvement in the research study. Relevant medical professionals were informed of any abnormal results discovered. All participants were reimbursed for travel expenses and inconvenience incurred due to participation.

For each study, procedures were performed as per the appropriate study protocol, detailed within the relevant results chapters. Assays were performed in clinical and research laboratories by clinical scientists. Patient confidentiality was maintained with anonymisation of data, which was stored in a password-protected secure database held at on a University of Oxford network drive.

2.5 Statistical analyses

Statistical calculations were made using SPSS for Windows software (IBM SPSS Statistics version 20, Chicago, IL, USA).

2.5.1 Large cohort study

Where the sample size is large (n>40), the sample can be assumed to have a normal distribution (symmetrical, bell-shaped distribution), and parametric tests can be applied.

2.5.1.1 Descriptive statistics

Descriptive statistics have been used to summarise cohort characteristics. Mean values were used to describe the centre of the distribution, and standard deviation (SD) as a measure of dispersion. The 95% confidence interval is given by ± 1.96 standard deviations of the mean.

Dependent variables were defined as continuous or categorical and compared. Continuous variables were compared using the independent sample t-test, or by one-way analysis of variance (ANOVA) in the case of more than 2 sample groups. Paired t-tests or repeated measures ANOVA were used for related groups. Categorical variables were compared using the chi-squared test. Repeated measures were compared with the Chi-squared or Nemar test.

2.5.1.2 Survival analyses

Patient and death-censored graft survival was assessed for associations using Kaplan-Meier curves. Survival distributions of two or more populations were compared with the

log-rank test, which is appropriate for non-parametric and censored data. The log-rank test statistic compares estimates of the hazard function of the two groups at each observed event time.

The Cox proportional hazard regression model, which allows for time-dependent variables, was utilized to estimate the impact of particular variables on patient and graft survival. Variables with a significance level of $p<0.15$ on univariate analysis were selected for inclusion in the multivariate model. Values of $p<0.05$ were considered statistically significant.

2.5.2 Small prospective studies

2.5.2.1 Sample size calculations

Power calculations were performed in collaboration with a statistician. Since there is no literature on the effect size (which is the difference between the means of two groups over the pooled standard deviation) of pancreas transplant on glucagon levels or the incretin effect, sample size calculations were based on hypotheses and values derived from the published literature of other populations. For changes in glucagon, we hypothesised that the glucagon levels will decline from those seen in T1D towards glucagon levels seen in healthy individuals. The null hypothesis is that the mean levels post- and pre-transplant are similar, and we estimated that 27 patients would be required for this study to reject the null hypothesis of no difference in mean glucagon levels between pre and post operation, allowing for a 25% drop-out rate and using an alpha level of significance of 5% for two-sided test and a power of 90%. For changes in the incretin effect, we hypothesised that a change of 15% would occur following post-transplant. Given an estimated standard deviation of 10%, with an alpha level of significance of 5% for two-sided test and a power of 90%, 10 participants would be needed in each group to allow for a drop-out rate of 20%.

2.5.2.2 Descriptive statistics

These studies contained small to medium sample sizes and therefore parametric conditions could not be assumed. Variables were assessed for normality using visual assessment of histograms and P-P plots, and through statistical testing with the Shapiro-Wilk test. Variables found to be parametrically distributed were summarised using the mean and standard deviation. Parametrically distributed continuous variables were compared using the independent sample t-test, or by one-way ANOVA in the case of more than 2 sample

groups. For related groups or repeated measures, paired t-tests or one-way repeated measure ANOVA was used. Non-parametrically distributed variables were summarised using the median and interquartile range. Non-parametric continuous variables were compared using the Mann-Whitney U test, or Kruskal-Wallis test in the case of more than 2 groups. The Wilcoxon signed ranks test was used for repeated or related measures. Categorical variables were compared using the chi-squared test, or Fisher's Exact test where the sample size was small. Related groups were compared with Chi-squared or Nemar test. Values of $p<0.05$ were considered statistically significant.

Chapter 3

Validation of the Pancreas Donor Risk Index for a UK cohort

3 VALIDATION OF THE PANCREAS DONOR RISK INDEX FOR A UK COHORT

3.1 Introduction

Identifying grafts at high risk of failure after pancreas transplantation has been the subject of previous research. Several transplant units have attempted to describe pre-transplant factors that may predict outcome pre-transplant through assessment of their cohort with multivariate regression analysis. There are variables, such as donor BMI, donor type (DBD or DCD) and donor age, that emerge commonly amongst studies (R. W. Gruessner et al. 1994b; Humar et al. 2004; Salvalaggio et al. 2007; Sousa et al. 2014). However, it is not clear from these studies which identifying factors are predictive of a poor outcome, or how these variables should be assimilated when multiple risk factors are present in a single donor.

Axelrod et al recognised this shortcoming, and developed a model that combined multiple variables and that could be used at the time of organ offering, to better assess which pancreases would result in good graft survival. Using data from the Scientific Registry of Transplant Recipients (SRTR), they performed a multivariate regression analysis to identify donor factors independently associated with pancreas graft failure at 1 year post-transplant. Factors found to be independently associated with graft survival were: donor age, type (DCD), BMI, gender, cause of death, race, height, raised serum creatinine and preservation time; with donor age and type (DCD) having the greatest impact (Table 3.1).

These variables were then combined using their associated logistic co-efficients to form a calculation, which forms the basis of the model. The model was adjusted to the median

donor from their cohort (not necessarily the ideal donor), to provide the calculation below, whereby variables in italics equalled 1 if ‘yes’ or 0 if ‘no’.

PDRI = exp { (-0.13792 x *[female donor]*)
+ (-0.034455 x *[donor age <20]* x *[donor age – 20]*)
+ (0.026149 x *[donor age -28]*)
+ (0.1949 x *[donor creatinine >2.5]*)
+ (0.23951 x *[donor black race]*) + (0.15711 x *[donor Asian race]*)
+ (-0.000986347 x *[donor BMI -24]*) + (0.033274 x *[donor BMI >25]* x *[donor BMI -25]*)
+ (-0.006073879 x *[donor height -173]*)
+ (0.21018 x *[donor cause of death CVA]*)
+ (-0.28137 x *[donor cause of death CVA for PAK]*)
+ (0.014678 x *[preservation time -12]*)
+ (0.33172 x *[DCD]*)

When the model was applied to any given donor, the result would provide the Pancreas Donor Risk Index, or PDRI, and was such that the median donor would give a PDRI of 1. Therefore, a donor with a PDRI <1 was then considered to carry less risk than the median donor, and a PDRI >1 carried more risk than the median donor (Axelrod et al. 2010). Figure 3.1

Figure 3.1 Pancreas Donor Risk Index, as described by Axelrod et al.

Donor characteristics	Reference donor (DRI = 1.00)	Change factor value to	DRI
Gender	Male	Female	0.87
Age	28	45	1.56
Black race	No	Yes	1.27
Asian race	No	Yes	1.17
BMI	24	30	1.17
Height (cm)	173	190	0.9
Cause of death: CVA/stroke	No	Yes	1.23
Cause of death: CVA/stroke in PAK	No	Yes	0.93
Pancreas preservation time (h)	12	20	1.13
DCD	No	Yes	1.39
SCr > 2.5	No	Yes	1.22

Donor risk index (DRI), body mass index (BMI), donor after circulatory death (DCD), cerebrovascular accident (CVA), creatinine (Cr)

This PDRI can be used, therefore, in clinical practice at the time of organ offering, and enables the recipient surgeon to combine donor factors and provide an objective measure of risk, which in turn enables prediction of one-year graft survival. This knowledge of the one-year graft survival associated with a given donor pancreas may influence decisions on organ allocation and acceptance.

The UK donor cohort has been noted to show demographic differences to the SRTR cohort, from which the PDRI was derived, with UK donors being older, more often DCD and with cardiovascular cause of death being more common (Mittal et al. 2013). It is, therefore, necessary to validate the use of the PDRI for this population. If validated for use in a UK population, the PDRI tool may be used to provide a valuable objective measure, potentially standardising practice, improving organ utilisation and ultimately overall pancreas graft outcome.

3.2 Aims of chapter 3

This study aimed to apply the PDRI to a UK cohort, in order to determine if it could be validated for use in this population. If validated, we aimed to establish an acceptable range, outside of which pancreas graft survival was unacceptable.

3.3 Methods

This was a retrospective registry analysis. Data was retrieved from a nationally maintained database held at NHS Blood and Transplant for all whole organ pancreas transplants performed between April 2004 and July 2011, following formal application and approval. Data relating to donor, recipient and transplant factors were requested. Graft survival was calculated, with graft failure defined as a return to exogenous insulin therapy or explant of organ.

Once received, data was checked for missing entries. Cases were excluded if pancreas graft outcomes were unknown, or if variables included in Axelrod PDRI calculation were missing. The UK dataset was described by transplant type (SPK, PTA and PAK) and compared to the published US dataset for recipient and donor characteristics. Cold ischaemia time (CIT) was defined as the time between cold perfusion in the donor and reperfusion in the recipient. HLA mismatch was described as total HLA-A, -B, -DR mismatch (0-6). Continuous variables were compared using Student's t-test and categorical variables were compared using the chi-squared test.

The PDRI was calculated for all transplanted pancreases using the published formula(Axelrod et al. 2010), as described above, with appropriate conversion of units as necessary. Cases were categorised according to PDRI quartile and compared for death-censored one-year graft survival by type of transplant using Kaplan Meier log-rank survival analysis. The association between the PDRI as a continuous variable and death-censored graft survival was determined using Cox Regression analysis. Statistical analyses were performed using SPSS 20.0.

3.4 Results

3.4.1 Demographics

Data for 1265 whole organ pancreas transplant programme was retrieved. 244 cases were excluded due to missing data. 1021 pancreas transplants were included in the analysis. Transplant centres were allocated an anonymised identifier within the national database. The demographics of the recipient and donor cohort are displayed in Table 3.1. The cohort was compared by transplant type and the PTA group was found to have more female recipients (p=0.005), more donors after circulatory death (p<0.001), shorter cold ischaemia time (p<0.001) and fewer HLA mismatches (p=0.018), reflecting biases in UK allocation procedures and attempts to minimise modifiable risk factors.

The demographic characteristics of the UK cohort were compared to those of the US cohort utilised in the development of the PDRI formula. Statistically significant differences were observed between the UK and US cohorts in all recipient, donor and transplant characteristics examined (table 3.2). However, these differences were numerically small and of little clinical consequence in most cases. However, the UK donor cohort was older (34.9 vs. 26.3 years, p<0.0001), with a more even gender distribution (49.1% vs. 67.3% male, p<0.0001), largely Caucasian (95.8% vs. 71.8%, p<0.0001) and consisted of a greater proportion of DCD transplants (10.8% vs. 1.4%, p<0.0001).

The calculated PDRI for the UK cohort ranged from 0.49- 3.40, and was comparable between transplant type (table 3.3) and transplant centre. The PDRI calculated in the

Axelrod cohort had a narrower range compared to the UK cohort, with the UK cohort being skewed towards higher PDRIs.

Table 3.1 Characteristics of the UK cohort: by transplant type

	All N=1021	**SPK** N=842 (82.5%)	**PTA** N= 63 (6.2%)	**PAK** N= 116 (11.4%)
RECIPIENT				
Age (years ± SD)	41.64 ± 8.23	41.58 ± 8.21	41.59 ± 8.67	42.10 ± 8.21
Gender (% male)	590 (57.8%)	497 (59.0%)	24 (38.1%)	69 (59.5%)
BMI (kg/m2 ± SD)	25.53 ± 3.54	25.54 ± 3.46	24.96 (3.23)	25.80 ± 4.22
Ethnicity				
Caucasian	948 (92.9%)	777 (92.3%)	62 (98.4%)	109 (94.0%)
Asian	61 (6.0%)	55 (6.5%)	0 (0%)	6 (5.2%)
Black	7 (0.7%)	5 (0.6%)	1 (0.1%)	1 (0.9%)
Chinese	1 (0.1%)	1 (0.1%)	0 (0%)	0 (0%)
Other	4 (0.4%)	4 (0.4%)	0 (0%)	0 (0%)
Waiting time (days)	379.3 (393.5)	379.1 (390.87)	314.7 (83.35)	383.6 (426.382)
DONOR				
Age (years ± SD)	34.86 ± 13.19	35.15 ± 13.13	33.33 ± 14.10	33.57 ± 13.11
Gender (% male)	501 (49.1%)	409 (48.6%)	27 (42.9%)	65 (56.0%)
BMI (kg/m2 ± SD)	23.63 ± 3.56	23.64 ± 3.53	23.23 ± 4.08	23.72 ± 3.52
Ethnicity				
Caucasian	978 (95.8%)	805 (95.6%)	61 (96.8%)	112 (96.6%)
Asian	26 (2.5%)	22 (2.6%)	1 (1.6%)	3 (2.6%)
Black	6 (0.6%)	6 (0.7%)	0 (0%)	0 (0%)
Chinese	1 (0.1%)	1 (0.1)	0 (0%)	0 (0%)
Mixed	8 (0.8%)	7 (0.8%)	0 (0%)	1 (0.9%)
Other	2 (0.2%)	1(0.1%)	1 (1.6%)	0 (0%)
DCD	110 (10.8%)	77 (9.1%)	18 (28.6%)	15 (12.9%)
Serum				
Sodium (mmol/l ± SD)	148.4 ± 8.52	148.51 ±8.54	147.10 ± 8.58	148.58 ±8.35
Creatinine (µmol/l ± SD)	80.05 ± 39.05	78.62 ± 36.18	84.38 ± 56.71	88.01 ±46.16
Amylase (IU/l ± SD)	115.38 ± 203.4	119.17 ±218.14	147.10 ± 8.58	99.84 ±116.91
ALT (IU/l ± SD)	72.02 ± 233.89	70.73 ±207.61	57.35 ± 71.92	88.95 ±403.79
GGT (IU/l ± SD)	72.1 ± 81.69	72.69 ±84.52	62.30 ± 44.96	73.39 ±76.63
PMH				
Hypertension	75 (7.3%)	62 (7.5%)	6 (9.7%)	7 (6.1%)
Smoking	500 (49%)	419 (50.6%)	23 (37.1%)	58 (50.9%)
Alcohol	74 (7.2%)	61 (7.4%)	4 (6.5%)	9 (7.9%)
Drugs	85 (8.3%)	73 (9.0%)	4 (6.6%)	8 (7.0%)

	All N=1021	**SPK** N=842 (82.5%)	**PTA** N= 63 (6.2%)	**PAK** N= 116 (11.4%)
TRANSPLANT				
No				
1	984 (91.4%)	837 (99.4%	60 (95.2%)	87 (75.0%)
2	35 (3.4%)	5 (0.6%)	3 (4.8%)	27 (23.3%)
3	2 (0.2%)	0 (0%)	0 (0%)	2 (1.7%)
CIT mins (SD)	779.6 (217.7)	772.0 (215.27)	726.03 (161.53)	863.61 (241.12)
HLA mismatch				
0-2	132 (12.9%)	99 (11.7%)	15 (23.8%)	22 (19.0%)
3-4	540 (52.9%)	450 (53.4%)	25 (39.7%)	61 (52.6%)
5-6	349 (34.2%)	293 (34.8%)	23 (36.5%)	33 (28.4%)
Transplant year				
2004	40	37	0	3
2005	92	80	0	12
2006	133	113	2	18
2007	178	145	9	24
2008	160	123	15	22
2009	172	137	16	19
2010	160	135	11	14
Centre				
A	148 (14.5%)	118 (14.0%)	11 (17.5%)	19 (16.4%)
B	110 (10.8%)	109 (12.9%)	0 (0%)	1 (0.9%)
C	79 (7.7%)	55 (6.5%)	3 (4.8%)	21 (18.1%)
D	86 (8.4%)	80 (95%)	1 (1.6%)	5 (4.3%)
E	136 (13.3%)	127 (15.1%)	2 (3.2%)	7 (6.0%)
F	50 (4.9%)	40 (4.8%)	2 (3.2%)	8 (6.9%)
G	324 (31.7%)	248 (29.5%)	42 (66.7%)	34 (29.3%)
H	75 (7.3%)	53 (6.3%)	2 (3.2%)	20 (17.2%)
I	13 (1.3%)	12 (1.4%)	0 (0%)	1 (0.9%)

Simultaneous pancreas kidney transplant (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK). Donor after circulatory death (DCD), body mass index (BMI), cold ischemia time (CIT), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), human leukocyte antigen (HLA)

Table 3.2 Comparison between UK cohort and Axelrod cohort demographics

	All n=1021	Axelrod n=9401	p-value
Recipient			
Age (years)	41.6 (8.2)	41.1 (8.2)	0.06
BMI (kg/m^2)	25.5 (3.5)	25.0 (4.2)	0.0003
%male	57.8%	58.1%	0.873
% Caucasian	92.9%	81%	<0.0001
Donor			
Age (years)	34.9 (13.2)	26.3 (10.8)	<0.0001
BMI (kg/m^2)	23.6 (3.56)	24.0 (4.2)	0.003
%male	49.1%	67.3%	<0.0001
% Caucasian	95.8%	71.8%	<0.0001
DCD	10.8%	1.4%	<0.0001
Serum Creatinine (umol/L)	80.05 (39.05)	92.8 (78.7)	<0.0001
Transplant			
CIT (mins)	779 (217.7)	816 (336)	0.0006

Data represented as mean (SD).
Donor after circulatory death (DCD), body mass index (BMI), cold ischemia time (CIT).

Table 3.3 Pancreas Donor Risk Index by centile: as calculated for UK cohort, by transplant type, and compared to Axelrod cohort

Centile	All N=1021	SPK N=842	PTA N= 63	PAK N= 116	Axelrod N= 3375
0	0.49	0.49	0.58	0.64	0.64
25	0.92	0.90	0.97	0.92	0.84
50	1.29	1.30	1.24	1.21	1.00
75	1.77	1.78	1.69	1.77	1.30
100	3.40	3.12	2.64	3.40	2.86

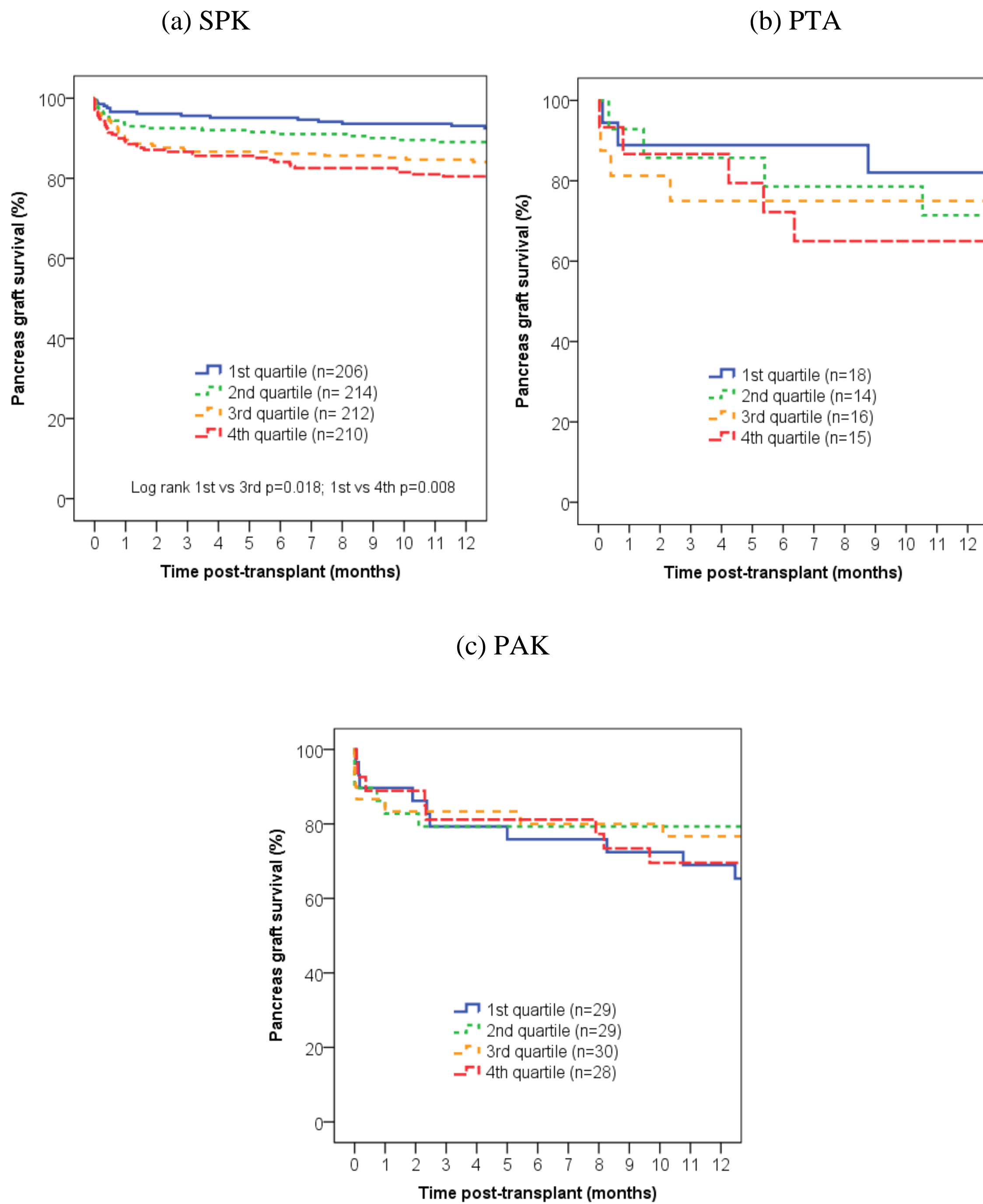
simultaneous pancreas kidney transplant (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK).

3.4.2 PDRI and graft survival

Comparison of pancreas graft survival by PDRI quartile showed PDRI to accurately discriminate graft survival for SPK with the lowest to highest risk quartiles achieving one year survival of 93.1%, 89.1%, 84.6% and 80.5% respectively (Figure 3.2a). The lowest risk group (1st quartile) achieved significantly better graft survival compared to the third quartile (p=0.018) and fourth quartile (p=0.008). The greatest discrimination in graft survival was evident in the first six months post-transplant, with the survival difference narrowing over time such that the 5-year graft survival was 78.3%, 76.5%, 72.2% and 71.7% respectively for the corresponding risk quartiles (Figure 3.3). In the SPK group, when analysed as a continuous variable, a multivariate Cox regression analysis adjusted for recipient variables confirmed the PDRI was associated with graft survival in the SPK group with a hazard ratio of 1.52 (p=0.009).

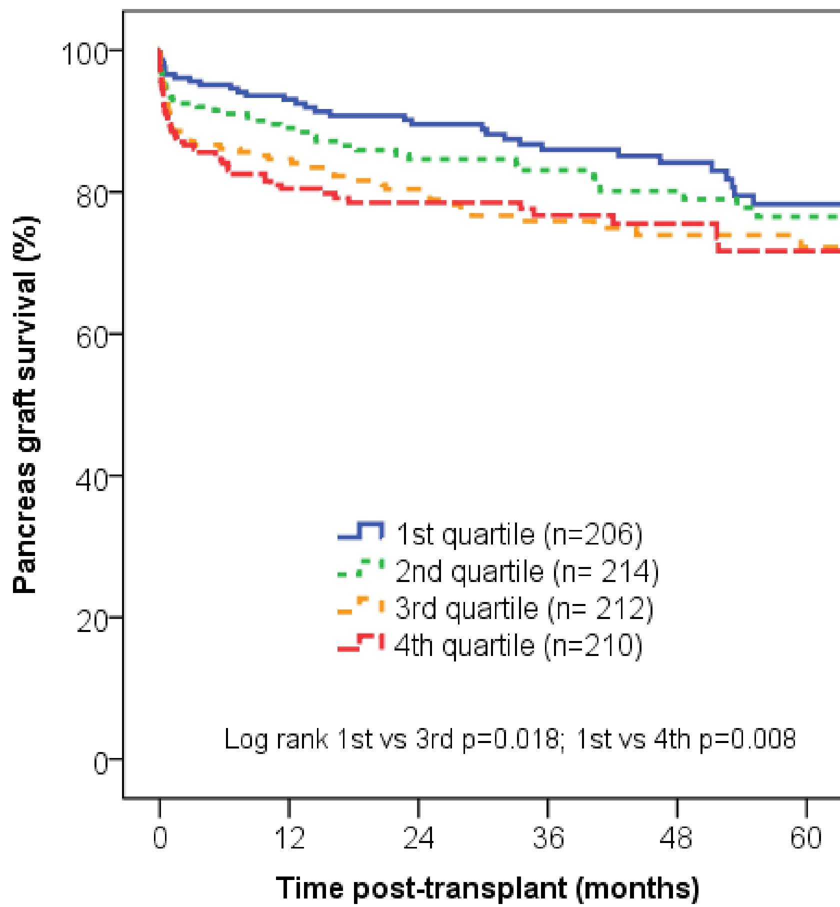
However, in the PTA and PAK groups, no association between PDRI quartile and graft survival was observed in Kaplan-Meier analysis (figure 3.2b,c). Multivariate Cox regression analysis with PDRI as a continuous variable also confirmed that PDRI was not significantly associated with graft outcome in these groups (PTA: HR 1.98, p=0.19; PAK: HR 0.95, p=0.86).

Figure 3.2 Kaplan Meier survival according to PDRI quartile



Where present, statistically significant differences between curves are shown.
Pancreas Donor Risk Index (PDRI); simultaneous pancreas kidney (SPK); pancreas transplant alone (PTA), pancreas after kidney (PAK);

Figure 3.3 Five year survival for SPK transplants by PDRI quartile



Pancreas Donor Risk Index (PDRI); simultaneous pancreas kidney (SPK);

3.5 Discussion

The PDRI was designed using SRTR data to provide a model to be used pre-transplant to inform risk and guide organ utilisation. This is the first study to validate the PDRI for use in a UK, or indeed European, cohort. This analysis highlights the significant demographic differences in the UK donor and recipient cohorts compared to the USA, with the UK using older, DCD donors and representing a more Caucasian population. Although the incidence of risk factors varies between the two cohorts, the calculated PDRI is similar implying that clinicians in both countries are combining risk factors effectively with no difference in risk aversion.

We have shown the PDRI to be significantly associated with early graft survival in SPK transplants. However the difference between the highest and lowest risk group was reduced to less than 7% at 5 years. Therefore, although the PDRI is a useful tool to estimate graft survival, good outcomes were achieved throughout the PDRI range and so the impact on graft utilisation may be small in numerical and practical terms.

The PDRI is further limited as a pre-implantation tool by the use of an estimated CIT and the lack of recipient variables. The true CIT is not known until the moment of implantation, at which time the decision has already been made. Using an estimated value in a complex calculation will hamper its accuracy and validity. Additionally, for the implanting surgeon, the principle question is: *is this donor suitable for my recipient?* Since the PDRI was constructed following adjustment for a median donor, and contains no

recipient variables in the calculation, there is no indication of whether a given donor would have a better outcome in patient A or patient B.

Another attempt at constructing a model that may guide acceptance of donor pancreas offers was the Preprocurement Pancreas Suitability Score (P-PASS). The P-PASS was developed by the Eurotransplant Pancreas Advisory Committee and is a score based on 9 clinical variables that estimates the likelihood that a given donor pancreas will be suitable for transplantation (Vinkers et al. 2008b). However, although some small studies have suggested the P-PASS may predict early surgical complications (Ziaja et al. 2011), larger studies have not shown the P-PASS to be associated with graft survival (Schenker et al. 2010; Woeste et al. 2010). It is, therefore, based on factors that were considered important by recipient surgeons when they decided whether or not to implant a donor pancreas, and so provides a measure of whether or not a transplant will take place, but is not related graft survival. The P-PASS was not validated within the current study, as only data relating to transplants that had taken place were available, and additionally, the P-PASS requires the concentration of lipase to be known (Vinkers et al. 2008a), which is frequently unavailable at donor centres. Although centres continue to explore variables of interest in univariate and multivariate analyses, a comprehensive risk index incorporating donor and recipient factors remains elusive.

This anonymised UK dataset may have provided the opportunity to devise such a model, and an attempt to do so was performed as part of this study. However insurmountable difficulties meant that this was not possible. In order to construct the model the cohort was divided into two datasets: one for model construction, and one for validation. Using the modelling dataset, significant recipient and donor variables were identified and

constructed into a risk model, in the same manner as described by Axelrod et al. However, when the new UK risk model (constructed using donor and recipient characteristics from the model construction dataset) was applied and tested in the validation dataset, the calculated risk score did not accurately predict graft outcome and failed to validate (appendix 4). This suggests that factors significantly predictive in the model dataset were not predictive in the validation dataset, and this is likely to be due to sample size. We can therefore conclude that the UK dataset is not large enough to enable construction of a robust risk model, which could also be validated, nor are there sufficient numbers of IP transplants to construct a model for this, more challenging group. In a future study, it may be possible to combine UK data with that collected by Eurotransplant (subject to appropriate approvals) to increase dataset size, in order to develop a European model, however, it is unlikely that this would provide much value beyond that offered by the existing Axelrod PDRI validated in this analysis.

We have also shown that the PDRI was not able to discriminate graft survival in the PTA and PAK groups, although this may be due to small numbers in this group, it may also be due to other contributing factors. We did observe the universally reported poorer outcomes in PTA and PAK transplantation, and like Axelrod, we observed poorer survival even for a given donor risk index score. Although donor factors may have some impact in PTA and PAK recipients, it is likely that there are other factors that have a larger, overwhelming effect that are masking the donor effect. These other influencing factors may relate to recipient characteristics as, since the indications for SPK and PTA are different, it follows that there will be differences in the characteristics of the recipients of these two operations beyond demographic factors. Alternatively, it may relate to differences in post-operative management, perhaps relating to immunosuppression

management. The superior outcomes observed in SPK transplants have been considered previously in part attributable to surrogate post-operative monitoring of rejection episode via the transplanted kidney (Margreiter et al. 2013). It may also be the case that more effective monitoring in the SPK group, led to detection of rejection events and initiation of appropriate treatment, whilst in the PTA and PAK group, this disadvantage may have had a significant impact on graft survival.

Inevitably, as this was a retrospective registry analysis, some cases had to be excluded due to missing data; nevertheless there is no evidence to suggest this has a negative effect on interpretation and this study remains the largest to date. This study has shown statistical validity of the PDRI for use in SPK transplant but not in PTA or PAK transplant, and this may be due to small numbers in the latter group, or related to a greater impact of other influencing factors. For the purpose of this study, graft failure has been defined as a return to exogenous insulin, and therefore further analysis into causes of graft failure, or insulin dosing analyses was not feasible in this registry analysis. It is not known whether separate analysis of those on small doses on insulin, compared to doses equivalent to a pre-transplant state, would have yielded more information or better discrimination.

This is the only study to validate the PDRI in a European cohort and confirms that the PDRI is equally valid in the UK as in a North American population, at least in SPK. However, it suffers from the same limitation that the survival difference between the lowest and highest risk groups is small, and as such may have limited clinical utility. Donor risk factors in PTA and PAK are similar to SPK and do not therefore explain the poor outcomes in this group. Better analysis of which donors are associated with superior

outcomes may contribute, however identification of post-transplant markers will be key for improving survival.

3.6 Conclusion

This study has successfully validated the Pancreas Donor Risk Index for use in the context of SPK transplantation, but not for IP transplantation. However, even for SPK transplantation, the difference in overall graft survival between the highest and lowest risk groups was small and the PDRI is unlikely to influence the decision to proceed to transplantation. The PDRI has not been validated for use in IP transplantation, and this may be due to the greater influence of non-donor factors in determining graft outcomes in this group.

Chapter 4

The role of HLA antibody monitoring

4 THE ROLE OF HLA ANTIBODY MONITORING

4.1 Introduction

Transplants from genetically different donors are recognised as foreign by recipient immune systems, largely due to mismatched donor HLA, resulting in immune responses and graft injury. Foreign HLA is presented on antigen-presenting cells causing T cell activation and recruitment of effector cells. This promotes the activation and differentiation of B cells into antibody producing plasma cells, leading to antibody-mediated graft injury.

HLA typing of donor and recipient is performed routinely in clinical transplantation in order to identify HLA mismatches and guide organ allocation. In the UK, an analysis of 6338 kidney transplants showed an incrementally deleterious effect of HLA mismatch on kidney graft survival (Morris et al. 1999), and this effect has been shown to remain significant both for graft survival (Sasaki and Idica 2010) and rejection rates (Opelz and Dohler 2007). Donor and recipient HLA typing using DNA technologies now allows more accurate matching, which is particularly important in recipients with high levels of pre-transplant antibodies (Opelz et al. 1999).

Patients can develop antibodies to foreign HLA through exposure to alloantigen during pregnancy, transfusion with blood products and previous transplantation, although HLA-specific antibodies have been observed in patients in the absence of such priming events (El-Awar et al. 2009). Patients with antibodies prior to transplant are considered sensitised, and may need to wait longer for a suitable donor.

Further, it has been seen that alloantibody levels and specificities may change over time and de novo HLA antibodies may develop post-transplant (McKenna et al. 2000). In kidney transplantation, emergence of HLA antibodies was seen to be evident prior to rejection episodes (Terasaki and Cai 2008) and strongly linked to allograft loss (Kimball et al. 2011; Mao et al. 2007), leading to recommendations for post-transplant HLA antibody monitoring (Tait et al. 2013). Advancements in solid-phase assay techniques have greatly improved detection and specification of HLA antibody resulting in greater sensitivity and precision (Tait et al. 2013; Zito et al. 2013). This has enabled the further discovery that circulating antibodies directed against donor HLA, or donor-specific HLA antibodies (DSA), confer the poorest allograft survival.

Several studies in renal transplantation have shown that DSA preformed before transplant, even at low levels, increase risk of antibody-mediated rejection and graft failure (Caro-Oleas et al. 2013; S. Mohan et al. 2012). Formation of de novo DSA post-transplant also confers poor graft outcome with a near 40% difference in graft survival at 10 years between those with de novo DSA and those without (Wiebe et al. 2012), and risk of graft loss within 3 years of DSA emergence at 24% (Everly et al. 2013). Similar findings of inferior outcomes in the presence of DSA have been shown in cardiac (Ho et al. 2011; Smith et al. 2011), and to a lesser extent, lung (Campbell 2013; Snyder et al. 2013) and liver (Kaneku et al. 2013; O'Leary and Klintmalm 2013) transplantation. However, research into the importance of HLA antibodies in pancreas transplantation has been lacking with only two such series described to date (Cantarovich et al. 2011; Mujtaba et al. 2012).

4.2 Aims of chapter 4

HLA antibody monitoring is not performed routinely after pancreas transplantation in many centres, and it is not known if there is a similar relationship between the presence of DSA and outcomes following pancreas transplantation. At the Oxford Transplant Centre, HLA antibody monitoring has been part of clinical protocol since 2006. This study aims to assess the role of serial post-transplant HLA antibody monitoring in identifying grafts at risk of failure after pancreas transplantation.

4.3 Methods

4.3.1 Patient cohort

The database was queried to retrospectively identify pancreas transplant recipients since the commencement of routine HLA post-transplant antibody monitoring. The patient cohort included all recipients between 2006 and 2011: 317 simultaneous pancreas kidney (SPK) and 116 isolated pancreas (IP) transplants, including 68 pancreas transplants alone (PTA), 35 pancreas after kidney (PAK) transplants and 13 second pancreas transplants. The PTA, PAK and second pancreas transplant groups had equivalent graft outcomes, and were therefore combined as the IP group. The SPK group had superior graft outcomes. All transplants were performed according to standard protocol, as described in Chapter 2.

Pre-transplant HLA antibody status was available for all patients. Clinical and HLA antibody data were requested at 1, 6, 12 months and annually for the duration of follow-up, as well as at the time of clinical events. Seventy-nine patients were excluded from the post-transplant HLA antibody analysis because serum samples were unavailable for analysis.

4.3.2 HLA antibody analysis

HLA antibody analysis was performed as part of routine patient care by clinical scientists in the Histocompatability and Immunogenetics laboratory, part of the Oxford Transplant Centre.

Pre-transplant

Pre-transplant, patients were typed for HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DQ and, where appropriate in sensitised patients, HLA-DP using polymerase chain reaction single-specific primer methods (Bunce et al. 1995) as part of routine clinical care. Recipient and donor HLA incompatibilities were expressed as an HLA A, B, DR mismatch grade. Pancreas transplant recipients were categorised by total HLA A, B, DR mismatch 0-4 or 5-6, and by DR mismatch 0, 1 or 2.

Sera were screened pre-transplant for the presence of HLA antibodies by Luminex technology using LABScreen® Mixed kits (One Lambda Inc., Canoga Park, CA, USA). In each kit, microspheres coated in known antigens, and uniquely identifiable by their colouration, each formed through the combination of two dyes in different proportions, enable antibody bound to them to be detected by a reporter dye on a secondary antibody. A dedicated flow cytometer was used with lasers that excite both the internal dye and the reporter on the secondary antibody indicating a reaction. Results were reported as units of mean fluorescent intensity (MFI) reflecting the level of antibody to individual HLA specificities present in recipient sera. Where the presence of HLA antibodies was detected in recipient sera, antibody specification was performed using LABScreenPRA® and Single Antigen beads as appropriate. Screening was repeated on a three monthly basis according to national and international standards (BSHI 2014) while potential recipients remained on the transplant waiting list.

HLA antibody status was described using the calculated HLA antibody Reaction Frequency (% cRF), defined as the percentage of HLA incompatible, blood group compatible, donors in a pool of 10,000 UK donors. Patients with low frequency

specificities were reviewed to identify genuine HLA antibodies against rare specificities in the donor pool. In order to differentiate these from spurious reactivity, recipients were considered sensitised if their % cRF was greater than 5%. All antibodies contributing to the recipient's pre-transplant sensitisation status were directed against non-donor HLA, and no transplants included in this analysis were performed in the presence of preformed DSA.

At the time of transplantation, all patients were crossmatched against their allocated donor by complement-dependent cytotoxicity (CDC) and flow cytometry crossmatch (FC) using recipient sera. Multiple recipient sera samples were tested, including the most recent, a sample from six months prior, a sample for each year the recipient has been on the transplant waiting list and samples relating to the highest period of alloantibody sensitisation and sensitising events. CDC crossmatching was performed with pre-incubation of patient serum, with and without dithiothreitol (DTT) to distinguish between IgG and IgM antibodies. CDC IgM positive results were not considered clinically relevant, and therefore not a contraindication to transplant (Bryan et al. 2001). However, to identify weak IgG antibodies, FC was performed. All transplants were CDC and FC IgG negative.

Post- transplant

Samples were requested for routine prospective serial HLA antibody screening for HLA class I and II antibodies by Luminex technology at 0, 6, 12 months post-operatively then annually thereafter, as well as at the time of clinical events, as described above. A mean fluorescent intensity (MFI) value of 1000 was considered positive (Wiebe and Nickerson

2013). Recipients were assessed for changes in their HLA antibody profile and development of DSA.

Recipients were categorised according to whether de novo HLA antibodies were formed. Recipients who had formed de novo antibodies were further categorised as having formed de novo HLA antibodies that were specifically directed against the donor (DSA), or having formed de novo HLA antibodies that were not donor specific.

4.3.3 Clinical data

The database was queried for corresponding demographic and graft outcome data, including donor, recipient and transplant-related variables. Outcome data included kidney rejection episodes, pancreas and kidney graft failures (as previously defined in chapter 2) and patient survival.

4.3.4 Statistical analysis

Quantitative parametric data were compared between groups using Student's t-test or the Mann-Whitney U test in the case of non-parametric distribution. Cross-tabulated data were analysed by the Chi squared test or by the Fisher's exact test when the expected count was less than 5. Patient and death-censored graft survival and incidence of HLA antibodies were assessed using Kaplan-Meier curves, and compared with the log-rank test. The Cox proportional hazard regression model, which allows for time-dependent variables, was utilised to estimate the impact of HLA antibodies, DSA and other covariates on graft survival. Variables with a significance level of $p<0.15$ on univariate analysis were selected for inclusion in the multivariate model. Values of $p<0.05$ were

considered statistically significant. Statistical calculations were made using SPSS for Windows software (IBM SPSS Statistics version 20, Chicago, IL, USA).

4.4 Results

4.4.1 Demographics

433 pancreas transplants had sufficient data and included in this analysis. 317 (73.2%) received a SPK transplant and 116 (26.8%) received an IP transplant. 293 (92.4%) of SPK recipients received pancreases from donors after brainstem death (DBD) compared to 79 (68.1%) of IP recipients. This difference was statistically significant (p<0.001) and was principally due to UK organ allocation procedures, as described previously. The groups were otherwise comparable in terms of donor and recipient characteristics, and cold ischemia time (CIT), Table 4.1.

4.4.1 Pre-transplant immunological assessment

Pre-transplant HLA antibody data was available for all recipients included in the analysis. 114/ 433 (26.3%) were found to have pre-transplant HLA antibodies and were considered to be sensitised (% cRF> 5%): 88/317 (27.8%) SPK, 26/116 (22.4%) IP. Sensitisation status between the SPK and IP group was not significantly different, and comparison of pancreas graft outcomes showed that patients who were sensitised pre-transplant had outcomes equivalent to unsensitised patients (log rank p=0.15; Figure 4.1a).

Analysis of donor and recipient HLA mismatching revealed that the SPK and IP transplant groups were comparable in terms of total HLA mismatch, however, the IP group had a lower level of DR mismatches compared to the SPK group (p=0.026; table 4.1). Neither degree of mismatch (0-4 vs. 5-6) nor degree of DR mismatch (0, 1 or 2) were associated with pancreas graft failure (log-rank p=0.34 and p=0.25 respectively; Figures 4.1b and 1c).

All donor and recipient CDC crossmatches were IgG negative but 100/317 (31.5%) SPK and 30/116 (25.9%) IP recipients had IgM positive results. This was not significantly different between groups (p=0.253). IgM positive results are likely to represent autologous antibodies and comparison of graft outcomes showed that IgM positivity in the CDC crossmatch was not associated with inferior pancreas graft outcome after SPK or IP transplant (log rank p=0.46; Figure 4.1d).

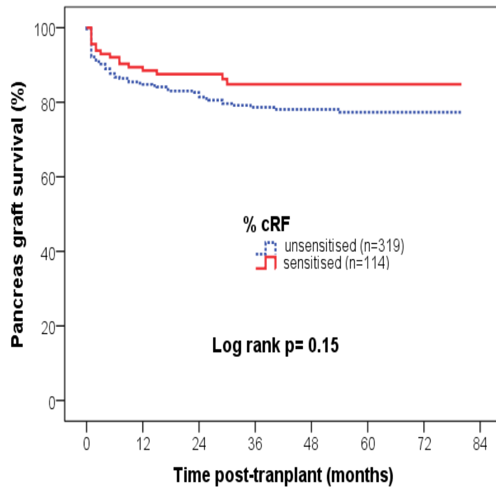
Table 4.1 Demographics and pre-transplant characteristics of study cohort by transplant type.

	SPK	**IP**	**p-value**
N	317 (73.2%)	116 (26.8%)	
Donor type (% DBD)	293 (92.4%)	79 (68.1%)	<0.001
Donor female (%)	155 (48.9%)	52 (44.8%)	0.453
Donor age (median years)	39.0	35.5	0.110
Donor BMI (kg/m2)	24.2	25.6	0.334
Recipient female (%)	121 (38.2%)	55 (47.4%)	0.083
Recipient age (median years)	44.0	42.0	0.545
Recipient BMI (kg/m2)	25.3	25.4	0.851
CIT (min)	684	705	0.285
% cRF (>5%)	88 (27.8%)	26 (22.4%)	0.263
HLA mismatch (5-6)	98 (30.9%)	34 (29.3%)	0.748
DR mismatch 0 (%) 1 (%) 2 (%)	32 (10.1%) 166 (52.4%) 119 (37.5%)	23 (19.8%) 55 (47.4%) 38 (32.8%)	0.026

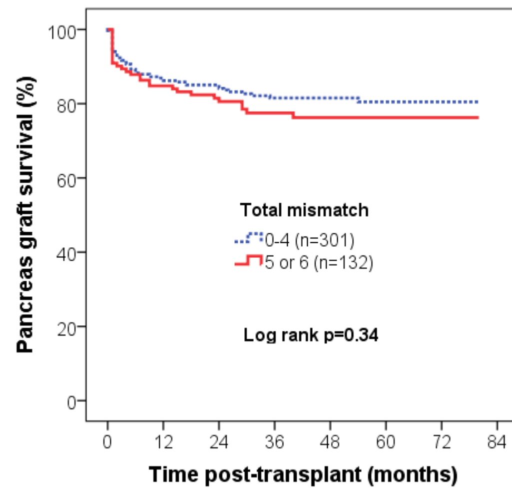
simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP). Donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT), calculated reaction frequency (% cRF)

Figure 4.1 Kaplan-Meier plots of death-censored pancreas graft survival according to pre-transplant status.

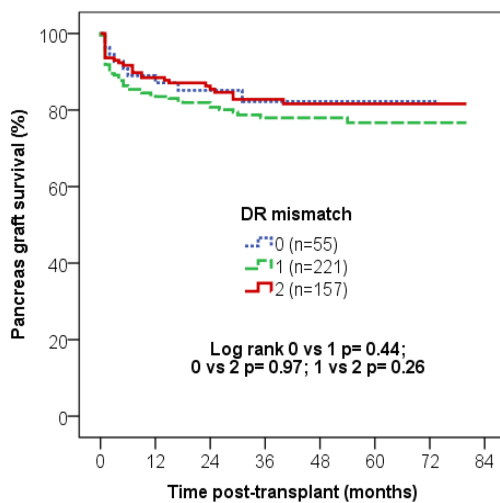
(a) Sensitisation status (cRF>5%)



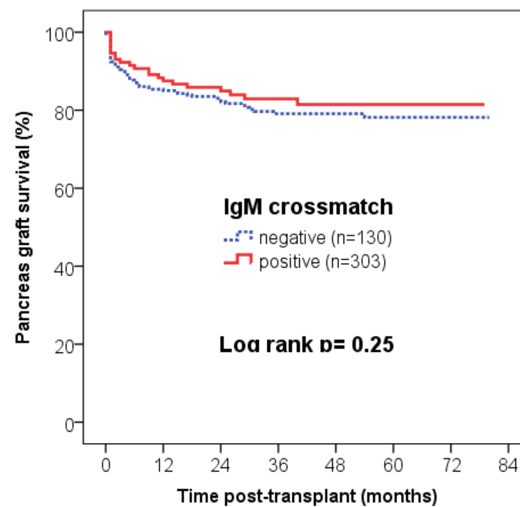
(b) Total mismatch



(c) DR mismatch



(d) CDC crossmatch IgM



Calculated reaction frequency (cRF); complement-dependent cytotoxicity (CDC)

4.4.3 Post-transplant de novo HLA antibody development

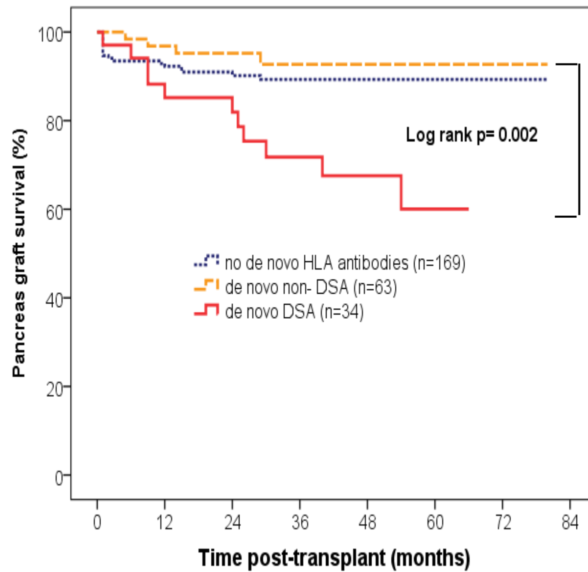
Post-transplant HLA antibody monitoring was available for 354 (81.4%) patients, including 266 SPK and 88 IP. 79 patients did not have post-transplant antibody monitoring because adequate serum samples were not available. Pancreas graft failure occurred in 59/354 (16.7%) pancreas transplant recipients with available post-transplant monitoring.

De novo HLA antibodies developed in 134/354 (37.9%) transplant recipients with no difference in incidence between transplant groups (97/266 or 36.5% SPK vs. 37/88 or 42.0% IP). Kaplan-Meier comparison showed poorer pancreas graft survival in patients who developed de novo HLA antibodies, and this was statistically significant in the IP group (log rank p=0.011).

Further analysis showed that de novo DSA developed in 52/354 (15.3%) patients, of which 34/266 (12.8%) were SPK and 18/88 (20.5%) IP transplants. Comparison for associations to graft failure revealed that the development of HLA antibodies that were not directed against the donor, showed no association between the development of non-donor specific HLA antibodies and graft failure. The previously observed negative effect of HLA antibodies was therefore attributable to the development of de novo DSA, which was significantly associated with poorer pancreas graft outcomes. Inferior one and three year graft survival rates were observed in SPK recipients who developed de novo DSA compared to those who did not (1 year graft survival, 85.2% vs. 93.5%; 3 year survival 71.8% vs. 90.3%; log rank p=0.002), and the differences were more pronounced in the IP group (1 year graft survival, 50.0% vs. 82.9%; 3 year survival 16.7% vs. 79.4%; log rank p=0.001), Figure 4.2.

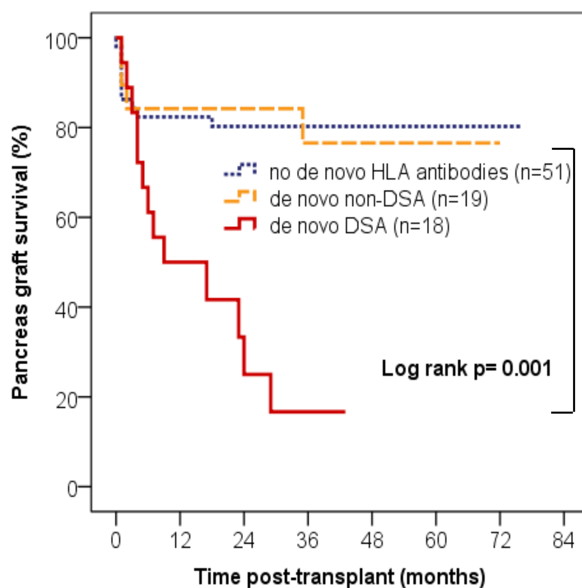
Figure 4.2 Death censored pancreas graft survival and development of de novo HLA antibodies after simultaneous pancreas kidney transplant (SPK) and isolated pancreas transplant (IP)

a) Simultaneous pancreas kidney transplant



de novo DSA vs de novo non-DSA, p=0.002
de novo DSA vs no de novo HLA, p=0.001
de novo non-DSA vs no de novo HLA, p=0.362

b) Isolated pancreas transplant



de novo DSA vs de novo non-DSA, p=0.001
de novo DSA vs no de novo HLA, p<0.001
de novo non-DSA vs no de novo HLA, p=0.904

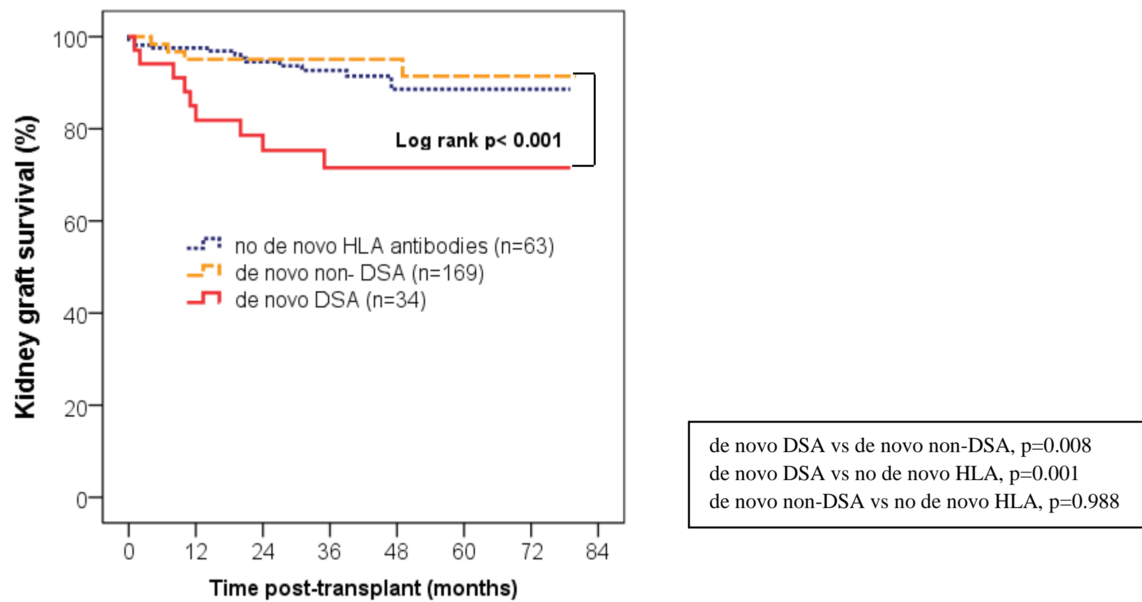
Human leukocyte antigen (HLA), donor-specific antibodies (DSA)

In SPK recipients who developed de novo DSA, biopsy-proven kidney rejection was significantly more common (11/34 or 32.4% vs. 17/226 or 7.5%; p<0.001) and kidney graft survival was also significantly poorer (log rank p>0.001; fig 4.3). Antibody mediated rejection was reported in two recipients with DSA. The remaining 26 cases showed acute cellular rejection, with evidence of arteritis more common in recipients who developed DSA (2/9 vs 2/17). Patients who developed DSA also had a significantly higher chance of losing both pancreas and kidney grafts (8/34 or 23.5% vs. 8/226 or 3.5%; p<0.001; fig 4.4). In the absence of pancreas biopsies, pancreas rejection was not easily characterized in our cohort and was not analysed.

Recipients who suffered late, insidious, presumed immunological, pancreas graft failure, were more likely to have de novo DSA (19/24 or 79.2% DSA+ve failures were immunological vs 18/35 or 51.4% of DSA –ve failures were immunological, p=0.05).

Multivariate analysis demonstrated that donor and recipient factors were not predictive of pancreas graft failure, perhaps reflecting the narrow acceptance and listing criteria at our centre. Recipients of IP transplants had poorer pancreas graft survival compared to those receiving SPK transplants (HR 2.51, p=0.007). However, the development of de novo DSA emerged as the most predictive independent risk factor for pancreas graft failure (HR 4.66, p<0.001), Table 4.2.

Figure 4.3 Kidney graft survival after simultaneous pancreas kidney transplant stratified by development of de novo DSA



Human leukocyte antigen (HLA), donor-specific antibodies (DSA)

Figure 4.4 Flow diagram proportions of patients suffering pancreas and kidney graft failure associated with the development of de novo DSA

Donor specific antibodies (DSA), simultaneous pancreas kidney transplant (SPK), pancreas transplant alone (PTA)

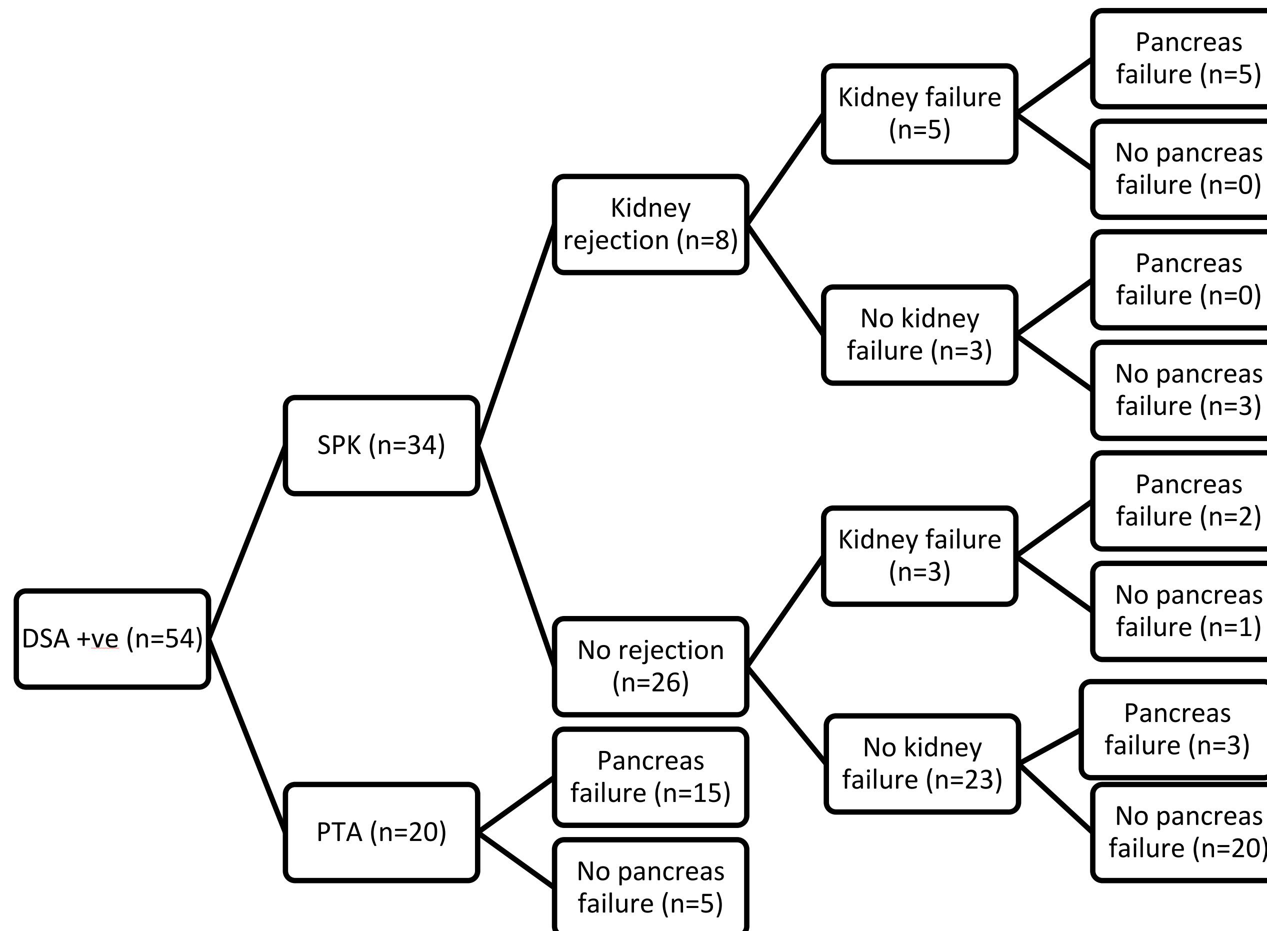


Table 4.2 Multivariate Cox regression survival analysis for predictors of pancreas graft survival.

	Reference	**HR**	**CI**	**p-value**
Transplant	SPK	2.51	1.28 – 4.90	0.007
Donor type	DBD	1.01	0.45 – 2.27	0.989
Donor gender	Female	0.71	0.37 – 1.36	0.301
Donor age (years)		1.02	1.00 – 1.04	0.125
Donor BMI (kg/m2)		0.99	0.96 – 1.02	0.592
Recipient gender	Female	1.66	0.83 – 3.35	0.154
Recipient age (years)		1.00	0.96 – 1.03	0.868
Recipient BMI (kg/m2)		1.07	0.98 – 1.17	0.134
CIT (mins)		1.00	1.00 – 1.00	0.869
%cRF	<5%	0.75	0.34 – 1.63	0.463
HLA mismatch	0-4	1.20	0.63 – 2.31	0.463
De novo DSA	No DSA	4.66	2.40 – 9.05	<0.001

Simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP), donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT), calculated reaction frequency (%cRF), human leukocyte antigen (HLA), donor specific antibodies (DSA); HR (hazard ratio), CI (confidence interval)

4.4.4 De novo donor specific antibodies

Analysis of DSA specificities showed all loci to be represented, although recipients who developed both class I and class II DSA were more likely to have pancreas or pancreas and kidney graft failure than recipients who developed either class I or class II only, Table 4.3.

Comparing those patients who developed DSA to those who did not, there were statistically significant differences between the groups for donor age and recipient BMI. Notably, patients who were sensitised pre-transplant were more likely to develop de novo HLA antibodies (sensitised 22/96, 22.9%; non sensitised 30/258, 11.6%; $p<0.001$). Table 4.4

Analysis of the timing of DSA emergence in relation to graft failure did not reveal any temporal relationship. DSA emerged between 1 and 35 months post-transplant in both the patients that went on to pancreatic graft failure and those that did not. However, where pancreas graft failure did occur this occurred within 9 months of the appearance of DSA in all but 2 cases.

Table 4.3 Class of donor specific HLA antibodies (DSA) formed after pancreas transplantation in the overall cohort, patients with pancreas graft failure and patients with failure of both transplant organs.

	Overall n=52	**Pancreas failed n=24**	**Both organs failed n=11**
Class I only	21 (40.4%)	2 (8.3%)	2 (18.2%)
Class II only	14 (26.9%)	3 (12.5%)	2 (18.2%)
Both	17 (32.7%)	19 (79.2%)	7 (63.6%)
HLA-A	22 (42.3%)	16 (66.6%)	7 (63.6%)
HLA-B	29 (55.8%)	15 (62.5%)	5 (45.5%)
HLA-C	7 (13.5%)	3 (12.5%)	2 (18.2%)
HLA-DR	18 (34.6%)	12 (50.0%)	4 (36.4%)
HLA-DQ	21 (40.4%)	13 (54.2%)	5 (45.5%)
HLA-DP	1 (1.9%)	0 (0%)	0 (0%)

Human leukocyte antigen (HLA)

Table 4.4 Demographics of groups according to post-transplant HLA antibody status.

	DSA positive n= 52	**Non- donor HLA antibodies n= 82**	**No de novo HLA antibodies n= 220**
Transplant (% SPK)	34 (65.4%)	63 (76.8%)	169 (76.8%)
Donor type (DBD)	47 (90.4%)	73 (89.0%)	189 (85.9%)
Donor female	19 (36.5%)	44 (53.7%)	102 (46.4%)
Donor age (years)	37.0	40.4 $^{+}$	35.5
Donor BMI (kg/m2)	23.5	24.9	24.9
Recipient female	22 (42.3%)	40 (48.8%)	81 (36.8%)
Recipient age (years)	42.4	45.3	43.4
Recipient BMI (kg/m2)	25.4	26.5 $^{+}$	25.1
CIT (mins)	725	659	694
cRF % (>5%)	22 (42.3%)^	29 (35.4%) $^{+}$	45 (20.5%)
HLA mismatch (5-6)	19 (36.5%)	21 (25.6%)	65 (29.5%)
Pancreas failures (%)	24 (46.1%)**^^	8 (9.8%)	27 (12.3%)
Kidney failures (%)	9 (17.3%)**^^	4 (4.9%)	13 (5.9%)

**DSA vs non-donor HLA p<0.01; ^^DSA vs no HLA p<0.01; $^{++}$non-donor vs no HLA p<0.01
*DSA vs non-donor HLA p<0.05; ^DSA vs no HLA p<0.05; $^{+}$non-donor vs no HLA p<0.05

Simultaneous pancreas kidney transplant (SPK), donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT), calculated reaction frequency (%cRF), human leukocyte antigen (HLA)

4.5 Discussion

The prognostic significance of de novo DSA on transplant outcomes has been demonstrated in solid organ transplantation including kidney, heart and lung; however evidence in pancreas transplantation has been limited. Prior to this analysis, there have only been two studies examining HLA antibodies after pancreas transplantation, each with conflicting results. Cantarovich et al examined 167 pancreas transplant recipients, of which 26 (15.6%) developed DSA, and found de novo DSA to be associated with more rejection episodes, more severe rejection, and poorer pancreas graft survival (Cantarovich et al. 2011). Following this, Mutjaba et al followed 35 pancreas transplant recipients, of which 8 developed de novo DSA. However, graft survival was equivalent regardless of post-transplant HLA antibody status (Mujtaba et al. 2012). The current study, described in this chapter, is the largest series of its kind to date and uniquely describes a cohort of pancreas transplants performed in the modern era with homogeneous pre-transplant assessment, immunosuppressive and operative management and post-operative monitoring. The much larger cohort involved enables, unlike its predecessors, outcomes in SPK and IP transplants to be examined separately, and greater generalisability of the findings.

The results of this study are consistent with data from kidney transplantation. De novo DSA developed in 14.7% of pancreas transplant recipients, similar to previous reports in kidney transplantation, where protocol Luminex screening identified DSA in 15- 25% of patients (Cooper et al. 2011a; Everly et al. 2013; Wiebe and Nickerson 2013). In this large homogenous cohort, the development of DSA conferred significantly poorer

pancreas and kidney graft survival, and de novo DSA was a significant independent predictor of pancreas graft failure in a multivariate model. This highlights the importance of post-transplant DSA monitoring for identifying pancreas grafts at risk of failure, above and beyond donor and recipient demographic factors.

Uniquely, this study has shown for the first time that the association between DSA and pancreas graft failure is more pronounced in the IP group. The reasons for this are not clear but may be important with respect to the known inferior long-term outcomes of IP compared to SPK transplants (Kandaswamy et al. 2013). The hypotheses proposed to explain this include: (i) the greater immunocompetence of IP recipients in the absence of uraemia(Vaziri et al. 2012), (ii) an immunological effect of transplanting both organs simultaneously; (iii) the advantages of using the kidney in an SPK recipient as an early surrogate indicator of rejection (de Kort et al. 2013). Although it is appreciated that kidney rejection is not a robust indicator of pancreas rejection (Troxell et al. 2010), the lack of a kidney graft in the IP group may result in the under-treatment of some subclinical rejection episodes, contributing to poorer longer term outcomes. In kidney transplantation, the development of DSA in association with acute rejection has been observed (Cooper et al. 2011b) and it is notable that, in our study, SPK patients who developed DSA also experienced significantly more kidney rejection episodes.

It was interesting to find that pre-transplant immunological factors including sensitisation status and degree of HLA mismatch were not predictive of pancreas graft outcome. To date, the impact of HLA mismatching on pancreas graft survival has not been proven- one study demonstrated a higher rate of acute kidney rejection in SPK transplants with 4-6 HLA mismatches, however showed no difference in 3 year kidney and pancreas graft

function or survival (Berney et al. 2005). The lack of evidence in the literature that HLA mismatch impacts on graft survival after pancreas transplantation may be due to a high percentage of pancreas transplant recipients receiving relatively poorly matched grafts (Kandaswamy et al. 2013) - in our study, only 30/433 (6.9%) recipients received a transplant with fewer than two mismatches. This study, therefore, is not powered to demonstrate a statistical difference between well and poorly matched recipients.

With optimised immunosuppression, HLA DR mismatches have been associated with a higher rejection rate and poorer functional outcome in renal transplantation (Taylor et al. 1993) and studies have suggested the greater importance of class II DSA in the pathological processes leading to graft failure (Wiebe and Nickerson 2013). In this study, HLA DR mismatches were not associated with poorer outcomes and HLA loci were equally represented. It appears that concurrent de novo DSA against both class I and class II HLA conferred worse graft survival, and this group appeared to be at very high risk and frequently lost both pancreas and kidney grafts.

From this analysis, preventable causes for emergence of de novo DSA cannot be easily discerned. Previous transplantation and pregnancy were rare and did not significantly predict for the development of HLA antibodies. 197/354 (55.6%) of recipients received a blood transfusion post-operatively, however this was not significantly different between those who developed HLA antibodies and those who did not, and was not predictive for the development of de novo DSA. It was observed that recipients developing HLA antibodies post-transplant were more likely to have been sensitised pre-transplant. This may represent a more immune-reactive recipient, the presence of transient HLA antibodies, or pre-transplant sensitisation status may be related to future DSA by epitope

spreading. Alemtuzumab induction has been associated with the emergence of DSA through its modifying effects on B cell phenotype (Todeschini et al. 2013). Phenotypic changes were not assessed in this study and detailed knowledge of cell subtypes may be useful in further delineating risk groups. Regimes containing MMF have been associated with a lower prevalence of DSA (Lederer et al. 2005), and MMF has been seen to specifically reduce the development of DSA antibodies during episodes of acute rejection (van der Mast et al. 2003). It is not known if HLA antibody emergence may have correlated to periods of reduced dosing according to clinical protocol for neutropenia in this group. Available data relating to drug levels does not suggest that compliance with immunosuppression was a significant problem in this cohort.

A commonly used MFI cut-off was used for the purposes of this analysis, although there is debate surrounding at what level this should be set for clinical relevance. Limitations of Luminex, including batch variation, denatured antigen, blocking of IgG antibody binding by IgM and bead saturation, may affect MFI values and pose challenges to interpretation. Loupy et al followed 1016 kidney transplant recipients and demonstrated that the presence of complement-binding DSA conferred the poorest kidney graft outcomes (Loupy et al. 2013). Such testing was not performed in this study, although this may be a valuable investigation and it is not known if this would delineate risk further.

In this study, sample acquisition was possibly not frequent enough to determine a temporal relationship between formation of DSA and graft loss, or to clearly define the persistent or transient nature of de novo antibodies, and in future studies, more frequent sampling may be informative. Additionally, further stratification of failure groups by cause and clinical

course would be useful; however the relatively small number of patients available would make drawing meaningful conclusions difficult.

The natural history of de novo DSA development has not been well defined and a causal relationship between DSA and graft dysfunction has not been proven. Several studies have shown DSA to develop prior to graft dysfunction (Wiebe and Nickerson 2013) and, it has been suggested that persistent or increasing antibody levels are associated with poorer graft outcome (Dieplinger et al. 2014). Wiebe et al found early histological evidence of injury at the time of DSA detection (Wiebe et al. 2012), and it is not yet clear if DSA and graft injury emerge contemporaneously as a result of an underlying process, or if antibody production precedes and mediates injury. Although pancreas biopsy studies have been scarce, the presence of C4d staining in combination with DSA has been correlated to poorer pancreas graft outcomes than DSA alone (de Kort et al. 2010; Rangel et al. 2010) and grade of C4d staining has been found to correlate to higher MFI of DSA (Niederhaus et al. 2013; Torrealba et al. 2008). In this study, pancreatic histology would have enabled analyses relating DSA emergence to findings of antibody mediated rejection (AMR) (Drachenberg et al. 2011). In addition to being of mechanistic interest, it is not known whether additional histological data would inform management decisions in the presence of DSA, with or without clinical dysfunction. This question should be addressed in future research.

Having defined a sub-group of patients at high risk of immunological graft loss, it is clearly essential to define an effective clinical response to mitigate this risk. Suggested treatment strategies have fallen into two camps: those aimed at antibody depletion, and those aimed at suppression of underlying cellular responses. Plasmapheresis and

immunoabsorption have been used to remove antibodies in AMR, with some success in graft salvage, although robust trials have been lacking and studies directly comparing the therapies are needed (Sandal and Zand 2015). Therapies modulating B cell activity have largely been extrapolated from other autoimmune diseases, and comprehensive clinical trials of therapies and treatment protocols for acute and chronic antibody mediated rejection have been rare. The best evidence to date suggests that intravenous immunoglobulin and rituximab are likely to be the most effective, plasmapheresis and bortezomib may be promising, and the remainder of therapies currently lack good evidence for efficacy (Sandal and Zand 2015). However, much of this evidence comes from potentially biased case series and, until a time when a clinical intervention of proven efficacy becomes evident, intense monitoring with antibody testing at 3-monthly intervals and at the time of clinical events is appropriate and practicable. Defining causation is difficult and, although we show that DSA are strongly associated with allograft failure, unknown variables may also have an impact on survival. Further work is needed to identify an appropriate intervention, and the correct time-point, at which it should be administered. If (and when) an effective means to remove DSA is developed then we will be in a position to investigate whether these antibodies are the cause or the result of graft damage.

4.6 Conclusion

In conclusion, this is the largest study to date examining DSA and pancreas graft outcomes, and clearly demonstrates a strong association between development of DSA and subsequent pancreas graft failure, so highlighting the potential for immunological surveillance alongside functional monitoring to prospectively assess graft status.

Chapter 5

The role of autoantibody monitoring

5 THE ROLE OF AUTOANTIBODY MONITORING

5.1 Introduction

Although it has been known for many years that T1D resulted from greatly diminished beta-cell mass that progressed rapidly to complete destruction (Gepts 1965), it was not until 1974 that an association between autoantibodies directed against islet cells and histological insulitis was suggested (Bottazzo et al. 1974). Several studies have now shown that autoantibodies precede the development of diabetes. A study of 105 children with recent onset diabetes showed that the presence of islet cell antibodies (ICA) was common within weeks of symptom onset, however titres were diminished by a year post diagnosis, probably due to the disappearance of islet antigens (Lendrum et al. 1975). Autoantibodies have also been shown to have significance in those at risk of diabetes. Further studies showed ICA were present prior to onset of symptoms and were observed in children with impaired glucose tolerance, who went on to require insulin therapy (Lernmark et al. 1978). These observations led to studies in the first-degree relatives of children with T1D, thought to be at high risk of developing diabetes, which confirmed ICA to be present many years prior the development of overt diabetes (Gorsuch et al. 1981) and, indeed, that the presence of ICA positivity in a family member conferred a relative risk of >75 for the development of diabetes (95% CI 7-106), when compared to family members that were ICA negative (Tarn et al. 1988).

More recently specific islet antigens have been identified, and assays developed, including those detecting glutamic acid decarboxylase autoantibodies (GADA), insulin autoantibodies, tyrosine phosphatase like insulinoma antigen 2 (IA-2) and islet cell antibody 512 (ICA-512) (Watkins et al. 2014). The presence of multiple antibodies

predicts more rapid progression to T1D, with GADA being the most common, appearing in 70-80% of people at diagnosis of T1D (Watkins et al. 2014). Additionally, in people initially diagnosed with T2D, positivity for multiple autoantibodies has been associated with greater risk of insulin-dependence. In this group, a Swedish study found that positivity for both ICA and GADA had a 100% positive predictive value for insulin therapy within 6 years of diagnosis (Littorin et al. 1999), while a UK study found ICA positivity alone to have an equivalent predictive value to a combination of ICA and GADA at 94% (Turner et al. 1997). As a result, the detection of autoantibodies has become key in identifying patients at risk of developing diabetes, and is usually an important inclusion criterion for intervention trials aimed at preventing T1D (Sosenko et al. 2013). In transplantation also, salvage of pancreas grafts has been described with intervention initiated by a rise in autoantibodies and biopsy evidence of insulitis and selective beta-cell loss (Vendrame et al. 2010).

Following pancreas transplantation, isletitis typical of T1D has been observed in non- or minimally immunosuppressed recipients of transplants from identical twin and HLA-identical sibling donors, possibly suggesting re-emergence of autoimmunity and graft failure due to recurrence of T1D (Sutherland et al. 1989). In the context of pancreas transplant graft failure following long-standing function and autoantibody positivity, selective beta cell destruction, in the absence of signs of rejection, has been noted in explanted deceased donor pancreases (Tyden et al. 1996), so showing autoimmunity may re-emerge even with immunosuppression therapy. In the literature, case reports can be found describing histological features of recurrent T1D in failed pancreas grafts, often preceded by sudden deterioration in function (Assalino et al. 2012; Ishida-Oku et al. 2010), and sometimes associated with rises in autoantibody titres (Braghi et al. 2000).

Although published cohort studies have not proven a link between autoantibody positivity and pancreas graft failure, associations with dysglycaemia have been identified (Martins et al. 2014). The lack of large cohort studies with serial autoantibody assessment has limited robust analysis of the role of autoantibodies to identifying pancreas grafts at risk of failure, however some groups maintain that autoimmunity is under-investigated and under-reported, and may account for a significant proportion of chronic pancreas graft loss (Martins 2014).

5.2 Aims of chapter 5

Autoantibody status is routinely assessed as part of unit clinical protocol. The aim of this chapter was to examine the study cohort to assess the association between autoantibody positivity, before and after transplantation, and pancreas graft failure.

5.3 Methods

5.3.1 Patient cohort

The database was interrogated to retrospectively identify primary pancreas transplant recipients with available autoantibody monitoring results. The patient cohort included 471 recipients between 2002 and 2011: 363 simultaneous pancreas kidney (SPK) and 108 isolated pancreas (IP) transplants, including 73 pancreas transplants alone (PTA) and 35 pancreas after kidney (PAK).

Pre-transplant autoantibody status was available for all patients. Post-transplant autoantibody assessment was requested at least annually for the duration of follow-up. A large proportion of transplant recipients had follow-up care organised at their local transplant centre and returned to Oxford infrequently. This resulted in a large proportion missing post-transplant autoantibody sampling, such that 240 patients were excluded from post-transplant autoantibody analysis because samples were unavailable.

5.3.2 Antibody analysis

Autoantibody analysis was performed as part of routine patient care by clinical scientists in the Oxford University Hospital NHS Trust Immunology Laboratories.

GADA were assessed with enzyme-linked immunosorbent assays (ElisaRSR™ GADAb; Cardiff, UK), with a lower detection limit of 0.57U/mL and specificity and sensitivity of 98% and 92% respectively. Results were considered positive if >5U/mL.
ICA were assessed using immunofluorescence techniques using primate pancreas tissue sections (NOVA Lite® ICA; San Diego, California). Slides were prepared with diluted

serum and treated with rhodamine and monkey absorbed fluorescin conjugate, and examined under an ultraviolet microscope. Results are reported, with reference to a standard serum established by the Juvenile Diabetes Foundation (JDF) which contains 80 JDF units of ICA antibody activity, as 5, 10, 20, 40 or >40 JDF units.

5.3.3 Clinical data

The database was queried for corresponding demographic data, including donor, recipient and transplant-related variables, and outcome data including pancreas graft failures and patient survival.

5.3.4 Statistical analysis

Quantitative parametric data were compared between groups using Student's t-test or the Mann-Whitney U test in the case of non-parametric distribution. Cross-tabulated data were analysed by the Chi squared test or by the Fisher's exact test when the expected count was less than 5. Patient and death-censored graft survival and incidence of autoantibodies were assessed using Kaplan-Meier curves, and compared with the log-rank test. Values of $p<0.05$ were considered statistically significant. Statistical calculations were made using SPSS for Windows software (IBM SPSS Statistics version 20, Chicago, IL, USA).

5.4 Results

5.4.1 Demographics

SPK and IP transplants included in the analysis were comparable for donor and recipient factors, and for degree of DR mismatch, although there were more DCD donors in IP group, p>0.001, as has been noted in previous chapters.

5.4.2 Pre-transplant autoantibody assessment

Pre-transplant GADA titres were available for 386/471 (82.0%) and were positive in 72/386 (18.7%). Samples of ICA analysis were available for 385/471 (81.7%) and were positive less frequently, 21/385 (5.5%). Table 5.1

There was no difference in GADA positivity between the SPK and IP group; 56/292 (19.2%) vs 16/94 (17.0%), p=0.719. GADA positivity was not associated with poorer graft survival in Kaplan-Meier analyses in the SPK (p=0.669). However, in the IP group recipients who were GADA positive pre-transplant had significantly poorer graft survival at 1-, 3-, and 5-years compared to those who were GADA negative (68.8%, 56.3% and 25.7% vs 81.3%, 72.6% and 64.9% respectively; log rank p=0.024, figure 5.1).

Pre-transplant ICA positivity was significantly more common in the IP group; 13/288 (6.1%) SPK vs 8/97 (8.2%) IP, p<0.001). ICA positivity was not associated with graft survival in the SPK group (p=0.584). However, in the IP group lower graft survival was observed at 1-, 3-, and 5-years post-transplant in those with ICA positivity pre-transplant compared to those who were ICA negative (80.0%, 72.3% and 59.9% vs 62.5%, 37.2% and 25.0%; log rank p=0.038, figure 5.2).

Recipients who were ICA positive pre-transplant were likely to also have GADA antibodies: 8/13 (61.5%) in SPK group, 6/8 (75.0%) in IP group. Recipients with presence of both autoantibodies had the lowest graft survival at 66.7%, 33.3% and 16.9% at 1-, 3-, and 5-years post-transplant, although this was not statistically different to GADA or ICA alone.

GADA titres ranged from 6- 1000 U/mL, with a median of 25 U/mL. ICA titres ranged from 5– 40 JDF units, with a median of 10 JDF units. GADA and ICA titre did not further discriminate graft survival beyond autoantibody positivity alone when considered either as a continuous or categorical variable.

Table 5.1 Comparison of groups by pre-transplant autoantibody status

a) Pre-transplant GADA status

	GADA +ve	**GADA -ve**	**p-value**
N	72	314	
Donor type (DBD %)	64 (86.5%)	272 (85.3%)	0.857
Donor age (mean years)	37.9	36.4	0.390
Donor BMI (kg/m2)	24.3	23.9	0.345
Recipient age (mean years)	44.6	42.9	0.303
Recipient BMI (kg/m2)	25.0	25.5	0.363
Duration of diabetes (years)	26.9	28.5	0.165
CIT (min)	719.0	684.9	0.144
Transplant (SPK %)	56 (75.7%)	236 (74.0%)	0.719

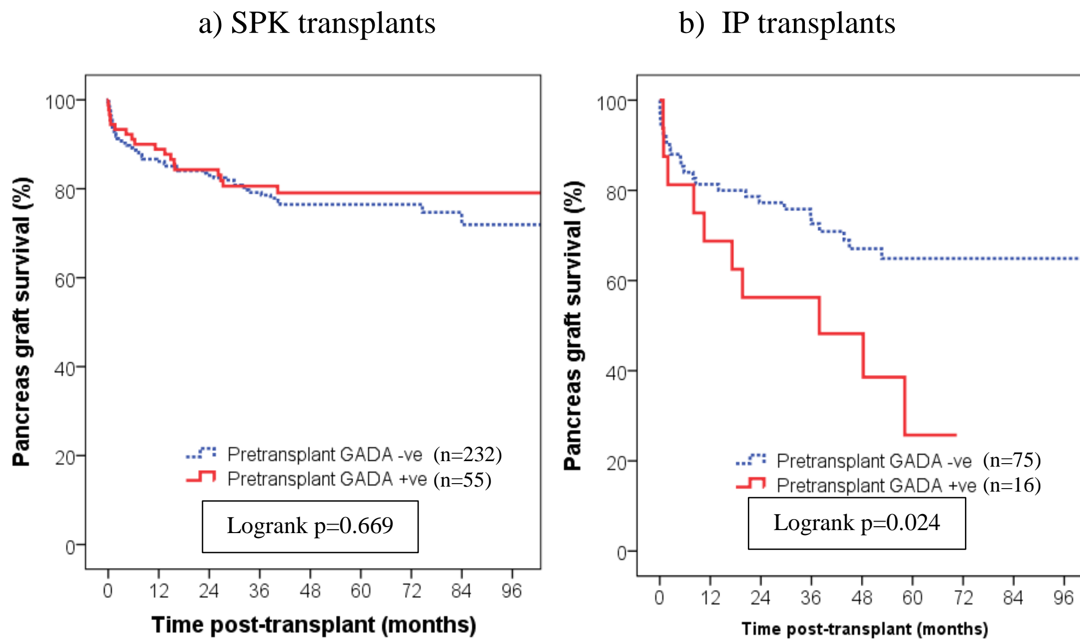
b) Pre-transplant ICA status

	ICA +ve	**ICA -ve**	**p-value**
N	21	364	
Donor type (DBD %)	18 (75.0%)	317 (86.1%)	0.138
Donor age (mean years)	37.0	36.6	0.911
Donor BMI (kg/m2)	23.5	23.9	0.611
Recipient age (mean years)	41.9	43.5	0.463
Recipient BMI (kg/m2)	24.2	25.5	0.122
Duration of diabetes (years)	24.3	28.4	0.002
CIT (min)	708.4	689.9	0.629
Transplant (SPK %)	13 (54.2%)	275 (74.7%)	<0.001

Glutamic acid decarboxylase (GADA), islet-cell antibodies (ICA), simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP). Donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT)

Figure 5.1 Kaplan- Meier plots of death-censored pancreas graft survival according to pre-transplant GADA status

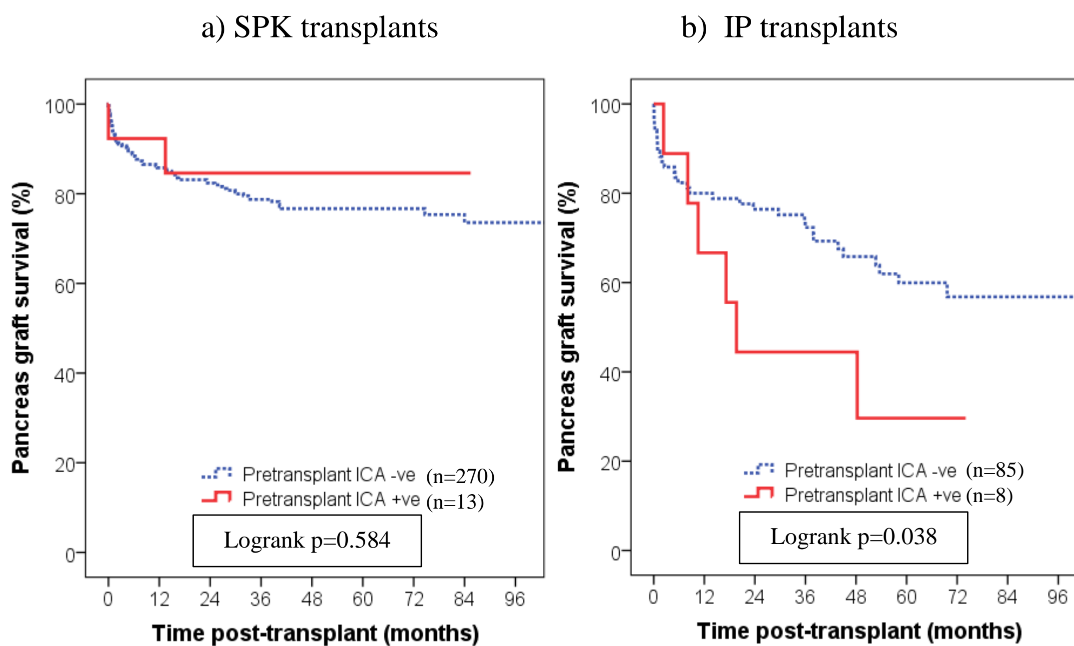
a) SPK transplants b) IP transplants



Glutamic acid decarboxylase (GADA), simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP).

Figure 5.2 Kaplan- Meier plots of death-censored pancreas graft survival according to pre-transplant ICA status

a) SPK transplants b) IP transplants



Islet-cell antibodies (ICA), simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP).

5.4.3 Post-transplant autoantibody assessment

Samples for post-transplant GADA status analysis were available for 188 SPK and 43 IP transplants, and were positive in 93/231 (40.3%). There was no significant difference between transplant types, with 72/ 188 (38.3%) SPK recipients and 21/ 43(48.8%) IP recipients identified as positive for GADA post-transplant, p=0.442 (table 5.2). There was no association between post-transplant GADA positivity and pancreas graft survival for either SPK (log rank p=0.536) or IP transplant (log rank p=0.689); figure 5.3.

Samples of ICA analysis were available for 187 SPK and 42 IP transplants, and were positive less frequently compared to GADA, and did not differ by transplant type, with 13/ 187 (7.0%) SPK recipients, and 4/ 42 (9.5%) IP recipients showing ICA positivity, p=0.638. There was no association between post-transplant ICA positivity and graft survival for either the SPK or IP groups, p=0.570 and p=0.872 respectively; figure 5.4.

GADA titres ranged from 6- 12500 U/mL, with a median of 27 U/mL. ICA titres ranged from 5– 40 JDF units, with a median of 10 JDF units. Increases in GADA titre, or change to autoantibody positivity from pre-transplant status, were not significantly associated with graft failure. 14/17 (82.4%) recipients who were ICA positive post-transplant, were also positive for GADA. Positivity for both GADA and ICA was also not associated with graft outcome.

Table 5.2 Comparison of groups by post-transplant autoantibody status

a) Post-transplant GADA status

	GADA +ve	**GADA -ve**	**p-value**
N	93	138	
Donor type (DBD %)	82 (88.2%)	125 (90.1%)	0.830
Donor age (mean years)	36.7	34.7	0.226
Donor BMI (kg/m2)	23.9	23.8	0.785
Recipient age (mean years)	44.0	43.2	0.605
Recipient BMI (kg/m2)	24.9	25.7	0.144
Duration of diabetes (years)	25.8	28.9	0.003
CIT (min)	684.5	713.2	0.279
Transplant (SPK %)	72 (77.4%)	116 (84.1%)	0.442

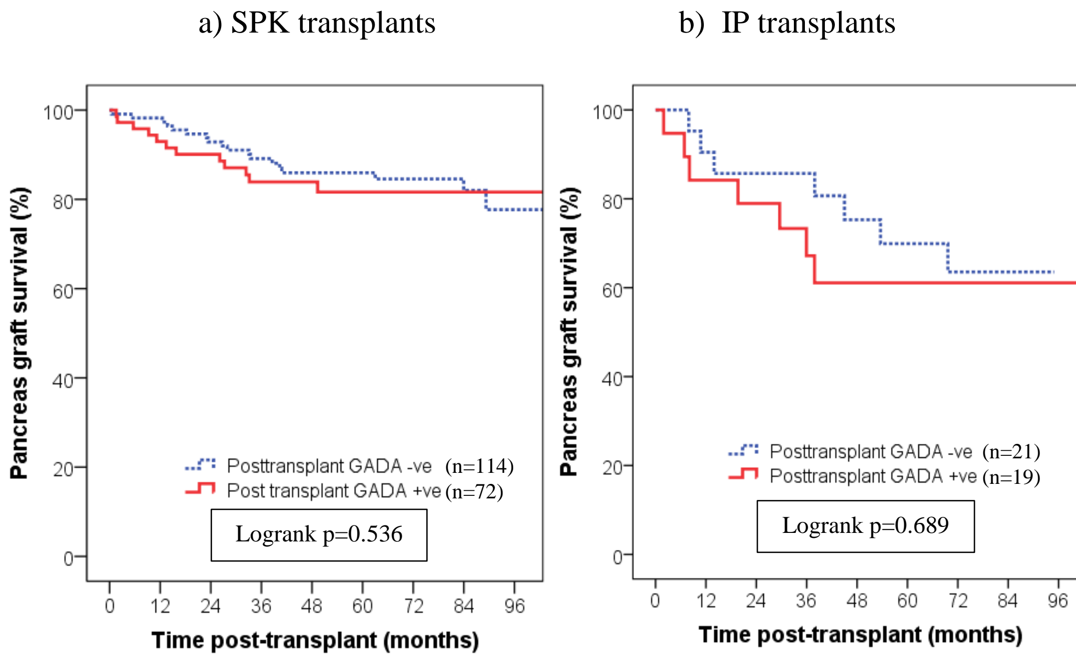
b) Post-transplant ICA status

	ICA +ve	**ICA -ve**	**p-value**
N	17	212	
Donor type (DBD %)	13 (76.5%)	192 (90.6%)	0.090
Donor age (mean years)	32.9	35.6	0.397
Donor BMI (kg/m2)	24.0	23.9	0.863
Recipient age (mean years)	42.4	43.5	0.696
Recipient BMI (kg/m2)	24.9	25.4	0.595
Duration of diabetes (years)	24.6	27.8	0.015
CIT (min)	696.2	701.8	0.910
Transplant (SPK %)	13 (76.5%)	174 (82.1%)	0.638

Glutamic acid decarboxylase (GADA), islet-cell antibodies (ICA), simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP). Donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT)

Figure 5.3 Kaplan- Meier plots of death-censored pancreas graft survival according to post-transplant GADA status

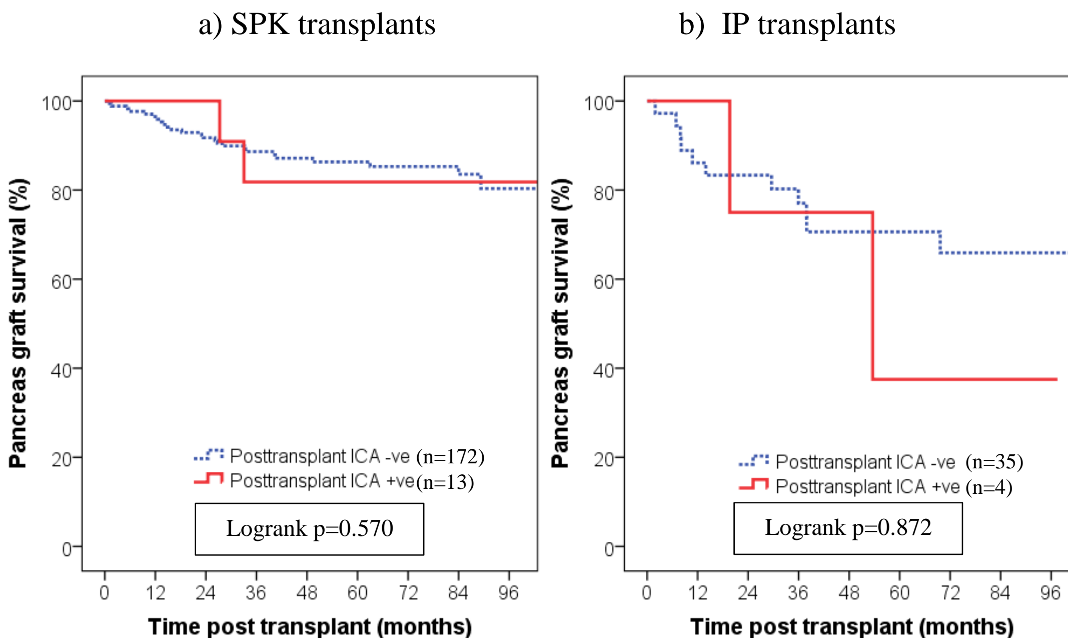
a) SPK transplants b) IP transplants



Glutamic acid decarboxylase (GADA), simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP).

Figure 5.4 Kaplan- Meier plots of death-censored pancreas graft survival according to post-transplant ICA status

a) SPK transplants b) IP transplants



Islet-cell antibodies (ICA), simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP).

5.4.4 Risk factors of autoantibody positivity

No differences were identified between pancreas transplant recipients who were positive for GADA pre-transplant and recipients who were GADA negative in terms of donor, recipient and transplant factors. Recipients who were ICA positive pre-transplant had a shorter duration of diabetes (24.3 years vs 28.4 years, p=0.002) and were more likely to have had IP transplantation (8/24, 33.3% vs 89/368, 24.2%; p<0.001) compared to recipients who were ICA negative (table 5.1).

Duration of diabetes was also associated with the presence of positive autoantibodies post-transplant; GADA positive recipients had shorter duration of diabetes (25.8 vs 28.9 years, p=0.003), as did ICA positive recipients (24.6 vs 27.8 years, p=0.015) compared to those without GADA and ICA respectively. Positivity for autoantibodies was not associated with positivity for donor-specific antibodies, and no other donor or recipient factors were associated with post-transplant autoantibody status.

5.5 Discussion

Although investigated previously, an association between autoantibody status and pancreas graft outcomes has not been clearly demonstrated. Reports in the current literature have involved small cohorts, with heterogeneity in the antibodies assessed and sampling time-points making meaningful interpretation difficult (Bosi et al. 1989; Dieterle et al. 2005; Esmatjes et al. 1998; Martins et al. 2014).

In concordance with our findings, other studies have reported GADA to be more common pre- and post-transplant when compared to ICA, with positivity for these antibodies frequently existing concurrently (Bosi et al. 1989; Esmatjes et al. 1998). As a result, GADA has been the most widely analysed. Our observation, that pre-transplant GADA is not associated with poorer pancreas graft survival in the SPK group, is supported by investigations by Martins et al in 135 SPK recipients and Thivolet et al in 68 SPK recipients[17,19]. However, there has been little reported in the literature regarding the effect of autoantibody positivity in the context of IP transplantation.

IP transplantation and shorter duration of diabetes were found to confer greater risk of pre-transplant ICA positivity, and shorter duration of diabetes was also implicated in the development of post-transplant autoantibody positivity. The reason for the greater impact of pre-transplant autoantibody positivity in the IP group on graft outcomes is not clear and may be multifactorial. Diabetes is associated with a proinflammatory state and impairment of peripheral tolerance (Pugliese 2014) that could be more active at an earlier stage in diabetes progression, before depletion of stimulating antigens. It may be that this

contributes to an immune environment where the emergence of autoantibodies occurs more readily and is more likely to have pathogenic consequences. In IP recipients, this is combined with the absence of defective T-cell responses associated with renal failure, which persist after kidney transplantation (Vaziri et al. 2012). This may result in a potentially more hostile immune environment for transplantation, compared to that in the SPK recipient. Alternatively, the greater importance of autoantibody positivity in IP compared to SPK transplantation may relate to differences in post-operative management, where IP recipients may receive less immunosuppression, and subclinical rejection episodes are less easily detected and treated.

The impact of post-transplant antibody status has been difficult to discern from the literature, since although most studies show no impact of autoantibody positivity on graft survival, this is in the context of overall autoantibody positivity, without the distinction between pre- and post-transplant, single or multiple antibodies and autoantibody specificity. Bosi et al reported the impact of post-transplant ICA positivity in 25 SPK recipients and found an association with poorer graft survival (Bosi et al. 1989). Estimates et al also found a detrimental influence of ICA positivity where, although there was no association with graft failure, ICA positivity was associated with abnormal glucose tolerance (Esmatjes et al. 1998). Associations between overall autoantibody positivity and abnormal HbA1c have also been observed in other cohort studies (Martins et al. 2014). It is not known if this is a harbinger of later graft failure, and analysis of functional outcomes was not possible within this analysis. In this study, antibody positivity for GADA and ICA were proportionately more common post-transplant, however an association between post-transplant autoantibody positivity and graft survival was not demonstrated. This may

be due to the smaller number in the analysis, and may become evident in a larger cohort, or due to the effects of immunosuppression.

Many studies describe heterogeneous immunosuppression induction and maintenance therapy, sometimes instigated in response to autoantibody positivity. This is in contrast to our cohort, which benefits from a consistent clinical and immunosuppression protocol Autoantibody assay results did not inform clinical decision-making or result in any changes in management. Repka et al reported persistence of autoantibodies to be common after basiliximab induction and tacrolimus and mycophenolate maintenance (Repka et al. 2006), and while Martins et al reported relative depletion after anti-thymoglobulin induction followed by ciclosporin, prednisolone and azathioprine (Martins 2014), Dieterle showed greater likelihood of autoantibody positivity if treated with ciclosporin when compared to tacrolimus (Dieterle et al. 2005). It is not known to what extent these variations in immunosuppression may influence autoantibody-related pathology, or if changes in immunosuppression therapy in response to autoantibody positivity could improve graft outcomes.

It has been shown that the presence of multiple autoantibodies confers greater risk of developing diabetes (Sosenko et al. 2013), and Braghi et al showed in 75 cases with pre and post-transplant assessment, that a marked increment in GADA or insulin antibody titre was associated with poorer outcomes of pancreas but not kidney grafts (Braghi et al. 2000). Thivolet et al also found an association with increase in GADA titre (Thivolet et al. 2000); however, in our study such an association was not evident. Underlying immune processes may lead to antigen spreading and therefore titre increases, or emergence of positivity to additional antibody specificities and, given that autoantibody titres may wax

and wane (Watkins et al. 2014), more frequent sampling in a larger post-transplant cohort may provide greater insight.

ICA was assessed with immunofluorescence, which is time-consuming, non- antigen specific and suffers from operator variability. The use of non-human tissue for testing may also affect antibody binding and therefore results. Serological assessment of further autoantibody subtypes may provide additional detail regarding underlying pathological processes, although caution must be exercised when interpreting the significance of low titre positivity. This analysis was also limited by the size of the cohort suitable for post-transplant analysis. Due to the wide geographical catchment for patient referrals, many stable post-transplant recipients have ongoing clinical care at local transplant centres, where specialised and specific autoantibody testing was not available. It is not known if associations between post-transplant autoantibody positivity and graft outcomes will emerge if analysed in a larger cohort.

It is not known if there is a direct pathogenic role of autoantibodies leading to graft failure through recurrence of diabetic insulitis, or if B lymphocyte activation and antibody production may be sequelae of antigen spreading secondary to underlying inflammatory or rejection processes. Burke et al correlated autoantibodies to markers associated with rejection including creatinine, urinary amylase and biopsies and concluded that failures associated with autoantibodies were due to autoimmune recurrence and not rejection (Burke et al. 2011). Interestingly, the presence of autoantibodies was not associated with DSA in this analysis, and this may suggest either distinct pathological processes or that antibody generation is determined by immunological memory. Tissue was not available from failed grafts for histological examination, and so it is not possible to comment on the

presence or absence of features of T1D recurrence or rejection. Nevertheless, biopsies may not be diagnostic in the case of patchy disease, and studies of pancreas explants from donors with circulating autoantibodies pre-mortem often did not show evidence of insulitis on histological examination (In't Veld et al. 2007). Indeed, recurrence of autoimmune disease post-transplant has been observed in cardiac transplantation for eosinophilic granulomatosis (Groh et al. 2014) , liver transplantation for primary biliary sclerosis (Raczynska et al. 2014) and in renal transplantation for IgA nephropathy, where living related donors , better HLA matching and specific HLA alleles in the recipient have been implicated in conferring greater risk of recurrence (A. Y. Wang et al. 2001), suggesting increased vigilance and perhaps greater immunosuppression is needed for transplantation in the context of autoimmune disease.

Studies in people with recently diagnosed diabetes aimed at halting autoimmune processes have met with limited success, although it is not known how these may translate for pancreas transplant patients with existing immunosuppressive regimes and a different balance of potential risk and benefit. Promising therapies showing early beta-cell preservation, including B cell depletion with rituximab (Pescovitz et al. 2009), CD3-antibody therapy (Herold et al. 2002; Keymeulen et al. 2005) and co-stimulation modulation with abatacept (Orban et al. 2014), showed early preservation of beta-cell function in people with recent onset diabetes. However longer term follow-up has shown a modest delay in progression rather than prevention of decline in the context of diabetes, and experience in the context of pancreas transplantation remains anecdotal. In this analysis, post-transplant autoantibody status was assessed as part of a monitoring protocol and was not used to guide clinical management. It is not known if outcome could have been improved if identification of autoantibody positivity triggered intervention therapies.

5.6 Conclusion

This is the largest reported study examining the impact of diabetes-related autoantibodies on pancreas graft survival after transplantation. Further, this is the only study to distinguish between pre-transplant and post-transplant autoantibody status and between SPK and IP transplantation, and clearly shows that the presence of GADA and ICA pre-transplant is associated with inferior pancreas graft outcome in the IP group.

Chapter 6

Early post-transplant metabolic assessment

6 EARLY POST TRANSPLANT METABOLIC ASSESSMENT

6.1 Introduction

Pancreas transplantation, unlike kidney transplantation, lacks a robust measure of graft function. Early post-transplant, a period of graft dysfunction is well recognised in kidney transplantation as delayed graft function (DGF), and is known to be associated with poorer long-term graft outcome in donors after brainstem death (Yarlagadda et al. 2009). A definition of graft dysfunction in the context of pancreas transplantation has not been established, and the term DGF has also been used to describe early graft dysfunction of the pancreas. This has been defined in some studies as the need for exogenous insulin therapy, however without consistency in either time-point or insulin dose cut-off (Shin et al. 2014; Tan et al. 2004). It is perhaps not surprising, therefore, that these studies have shown conflicting findings regarding the association of DGF with long-term outcome. The temporal pattern of functional decline in pancreas transplantation is not clearly described. However, evidence of the influence of donor factors (Axelrod et al. 2010) and studies in living donors, who have donated part of their pancreas, show that reduction in functional beta-cell mass results in a higher risk of diabetes (Kumar et al. 2008), implying that functional outcome may be to some extent determined at or shortly after transplantation.

In the development of diabetes, evidence of beta cell dysfunction is present long before diagnosis. Increases in glucose levels detected on oral glucose tolerance test (OGTT) can be present two years before the development of T1D and prior to changes in overall c-peptide (Sosenko et al. 2012b). Recently markers derived from OGTTs, such as changes in glucose and c-peptide at one hour, have been shown to further discriminate those most

at risk of developing T1D within three years, in a cohort traditionally defined as high risk (autoantibody positive first degree relatives of people with T1D) (Sosenko et al. 2014). In those at risk of T2D, post-prandial glucose excursions in impaired glucose tolerance (IGT) contribute significant glycaemic load and are predictive of progression to T2D, potentially due to the glucolipotoxic and inflammatory effects of hyperglycaemia on pancreatic beta-cells (Prentki and Nolan 2006). Nevertheless, the OGTT is a single time-point test and continuous glucose monitoring (CGM) is increasingly being used to assess the effect of interventions in people with diabetes (He et al. 2013), particularly to monitor glucose levels in complex patient groups (Ando et al. 2014; Wilinska and Hovorka 2014). Furthermore, hyperglycaemia detected on CGM has been shown to be more sensitive than OGTT in identifying subgroups at the highest risk of progressing to T1D (Steck et al. 2014).

Whilst, a number of studies have used the OGTT to monitor pancreas graft function (Dieterle et al. 2007; A. C. Gruessner et al. 2012), only one assessed the value of OGTT in predicting long term graft function in a small cohort of 41 SPK recipients (Pfeffer et al. 2003) tested at variable time-points. CGM is not routinely used in pancreas transplant monitoring in most transplant centres, and a few studies have examined 24-hour profiles in pancreas transplant recipients with long standing stable function. However, little is known about the detailed 24-hour metabolic profile occurring in the early post-transplant period, or how CGM profiles correlate to OGTT assessment.

6.2 Aims of chapter 6

In our centre, pancreas transplant recipients routinely undergo OGTT post-transplant prior to discharge, and the aims of this chapter were two-fold.

a) Firstly, in a retrospective case controlled study, I aim to test the hypothesis that OGTT performed within 14 days of pancreas transplantation is able to identify recipients at risk of later graft failure.

b) Further, in a second prospectively recruited study, CGM was used to assess the 24 hour metabolic profile of pancreas transplant recipients in the early post-transplant period and to examine how these profiles relate to glucose tolerance.

6.3 Methods

6.3.1 Study A: retrospective assessment of role of OGTT in predicting graft outcome

6.3.1.1 Patient cohort

The database was queried to identify patients with available post-transplant OGTT data. However due to high incidence of missing data at the time of the analysis, a case-controlled methodology was subsequently adopted.

All cases of pancreas graft failure were identified. Patients who underwent pancreatectomy during the transplant admission or failed to achieve insulin-independence were excluded. SPK and IP patients who were discharged with functioning pancreas transplants and not requiring exogenous insulin, but whose grafts subsequently failed were matched to a control group with ongoing graft function on a 1:5 and 1:3 basis respectively. It was not possible to match the IP group on a 1:5 basis due to the smaller number of IP transplants performed and therefore cases available for matching. Cases were matched for year and type of transplant (SPK, PTA, PAK or 2nd graft). Demographic, transplant related and outcome data were collected retrospectively.

6.3.1.2 Oral glucose tolerance test

All pancreas transplant recipients were free from medications for glycaemic control, including insulin, and underwent OGTT prior to discharge at 10-14 days post-transplant. Following an overnight fast, patients consumed a 75g oral glucose drink, and blood samples were taken for serum glucose at 0 and 2 hours. Normal glucose tolerance (NGT) was defined according to WHO criteria (fasting glucose <6.1mmol/L and 2 hour glucose

<7.8mmol/L). OGTTs were performed in the Transplant ward by clinical nursing and medical staff as part of routine protocol. No additional samples were taken. Assays were performed at the Clinical Biochemistry Laboratories at Oxford University Hospital by clinical scientists using enzymatic methods (ADVIA® 2400 Chemistry systems, Bayer® Diagnostics).

6.3.1.3 Statistical analysis

For SPK and IP transplants, variables in the graft failure group were compared to those in the control group (Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables). Cox regression analysis was performed to test for associations between variables and graft outcome. The model was adjusted for type of transplant. Significant variables were added into a multivariate model to identify independent predictive factors. Pancreas graft survival was compared for significant variables using Kaplan-Meier survival analysis.

6.3.2 Study B: prospective assessment of the role of continuous glucose monitoring

6.3.2.1 Patient cohort

To further investigate early metabolic assessment after pancreas transplantation, a second cohort of study participants, either SPK or IP, were prospectively recruited. The only exclusion criterion applied was use of oral hypoglycaemic medications or insulin. Thirty consecutive pancreas transplant recipients were recruited between August 2013 and May 2014. CGM probes (iPro™2, Medtronic Ltd, Watford, UK) were applied to study participants on day 7 post-transplant and remained in situ for 7 days, or until the patient was discharged.

6.3.2.2 Continuous glucose monitoring

The probe was inserted subcutaneously to the anterior abdominal wall and secured, using the iPro™2 device, by myself or the study research nurse. Interstitial glucose readings were taken by the probe. These glucose readings were blinded to patient and clinicians and were not used in clinical management decisions. After removal of the probe, anonymised readings were downloaded using data management software (CareLink *i*Pro Software, Medtronic Diabetes, California).

Patients were supplied with a glucose metre for correlation to finger-prick readings. Patients were asked to eat and drink as normal, and to keep a food diary. All recipients underwent OGTT according to clinical protocol.

Hyperglycaemia was managed according to clinic protocol, with clinical decisions based on finger-prick glucose and OGTT findings. Hyperglycaemia and abnormal glucose tolerance were investigated with radiological imaging to assess for underlying thromboses, which were treated if discovered with anticoagulation. Insulin therapy was not used in any participants in this series.

6.3.2.3 CGM data analysis

For each participant, 7-day data was analysed. CGM readings were analysed for excursions from the normal range (3.9- 7.8mmol/l, as defined by ADA guidelines (Seaquist et al. 2013a). Profiles were compared by type of transplant using the independent samples Mann Whitney U test, and by OGTT result using the independent samples Kruskal-Wallis test with post-hoc analysis of significant values ($p<0.05$). Receiver operating characteristic (ROC) analysis was performed for correlations between CGM profiles and OGTT result. Normal and abnormal OGTT groups were compared for differences in median trough tacrolimus level using the Fisher's Exact test. CGM readings were correlated to finger-prick glucose readings and clinical outcomes.

6.4 Results

6.4.1 Study A: the role of oral glucose tolerance

6.4.1.1 Demographic data

In total 54/ 486 recipients were identified as having suffered pancreas graft failure, of which 12 recipients were excluded based upon early pancreatectomy following graft pancreatitis or surgical complications. The remaining 42 graft failures included 21 SPK transplants and 21 IP transplants, and were matched to 105 and 63 recipients with ongoing pancreas graft function respectively. In total, 210 graft recipients were included in the analysis. Transplants where graft failure occurred were comparable to those with ongoing graft function in terms of donor and recipient characteristics, although the cold ischaemia time (CIT) was longer in the failure group compared to the functioning graft group (table 6.1). Sub-group analyses showed the difference in CIT was significant in the IP group only (780mins v 683mins, p=0.010).

6.4.1.2 Metabolic outcomes

The mean two-hour post-OGTT serum glucose was statistically higher in the failure group compared to the functioning group (p=0.014). When analysed in combination with fasting glucose, the diagnosis of abnormal glucose tolerance (WHO criteria (Alberti and Zimmet 1998)) was significantly more common in the failed graft group (p<0.001), table 6.1. Sub-group analysis showed this finding to be consistent in both the SPK and IP groups, with abnormal OGTT carrying greater significance than 2-hour glucose alone.

Table 6.1 Comparison of baseline characteristics: failed pancreas grafts vs. grafts with ongoing function

	Failed Grafts	Functioning Grafts	p-value
Time of failure (months)	8 (1-53)		
Operation type (%)			
SPK	21 (50.0%)	105 (62.5%)	
PTA	13 (31.0%)	41 (24.4%)	
PAK	6 (14.3%)	15 (8.9%)	
PASPK/ PAPTA	2 (4.8%)	7 (4.2%)	0.320
Recipient Gender (female %)	12 (28.6%)	69 (41.1%)	0.158
Recipient Age (years) ± SD	41.46 ± 7.39	43.64 ± 10.11	0.190
Donor Age (years) ± SD	36.49 ± 12.70	35.83 ± 13.44	0.776
Donor BMI (kg/m^2) ± SD	24.25 ± 3.33	23.67 ± 3.74	0.387
Donor type (DBD %)	32 (76.1%)	141 (83.9%)	0.260
CIT (min) ± SD	753.00 ±158.13	672.21 ± 172.43	0.011
Tacrolimus level ± SD	11.11 ± 5.17	9.79 ± 4.96	0.138
OGTT result 0min ± SD	5.30 ± 0.93	5.61 ± 1.01	0.091
OGTT result 120min ± SD	8.36 ± 3.82	6.80 ± 2.10	0.014
Abnormal OGTT result (%)	21 (50.0%)	39 (23.2%)	0.001
Kidney rejection (%)	7 /21 (33.3%)	8 /105 (7.6%)	0.004
Kidney failure (%)	9 /21 (42,9%)	5 /105 (4.8%)	<0.001

simultaneous pancreas kidney transplant (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK), pancreas after SPK (PASPK), pancreas after PTA (PAPTA). Donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT), oral glucose tolerance test (OGTT), standard deviation (SD)

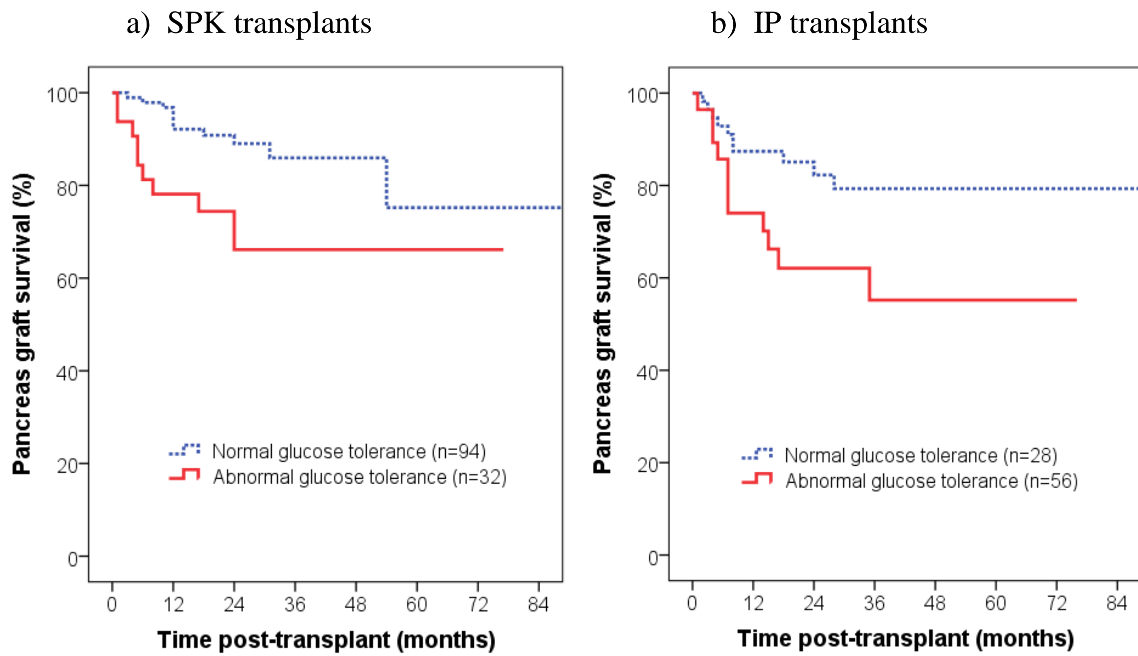
6.4.1.4 Graft survival

Kaplan-Meier analysis showed recipients with early abnormal glucose tolerance had significantly poorer graft survival compared to those with NGT (for SPK: 1-year survival 78% vs 97%, 3-year survival 66% vs 86%, , log rank p=0.008; for IP: 1-year survival 74% vs 87%, 3-year survival 55% vs 79%, log rank p=0.029; figure 6.1).

Of 153 recipients with NGT, 21 (13.7%) suffered pancreas graft failure with a median time to graft loss of 12 months; of 40 recipients with impaired GT (IGT), 14 (35.0%) failed with a median time to graft loss of seven months; of 17 (41.2%) recipients with WHO defined diabetes or DGT (but insulin independent), 7 (38.9%) failed with a median time to graft loss of only four months post-transplant.

A univariate cox regression analysis adjusted for transplant type showed cold ischaemia time, 0- hour serum glucose, 2-hour serum glucose and an abnormal OGTT to be predictive of graft failure. In a multivariate model, although CIT and OGTT result showed an interaction effect, they both emerged as independent predictive factors, with abnormal OGTT showing the highest predictive power (HR 1.66, p=0.001; table 6.2). The early OGTT had low sensitivity (47.6% SPK, 52.4% IP) but high specificity (79.1% SPK, 73.0% IP) for identifying graft failure. Early OGTT has a negative predictive value of 88.3% for SPK and 82.1% for IP.

Figure 6.1 Kaplan-Meier Survival Curve of pancreas graft survival by oral glucose tolerance test result



simultaneous pancreas kidney transplant (SPK), isolated pancreas transplant (IP)

Table 6.2 Cox regression analysis for pancreas graft survival adjusted for transplant type

	HR	95% confidence interval	p-value
Recipient Gender (F)	0.518	0.259, 1.035	0.063
Recipient Age	0.978	0.943, 1.014	0.232
Donor Age	1.008	0.984, 1.032	0.522
Donor BMI	1.045	0.959, 1.140	0.317
Donor type (DBD)	0.730	0.354, 1.505	0.394
CIT (min)	1.002	1.000, 1.004	0.021
Sensitisation	1.217	0.786, 1.883	0.379
Tacrolimus level	1.044	0.989, 1.101	0.119
OGTT result 0min	1.301	1.029, 1.646	0.028
OGTT result 120min	1.207	1.099, 1.325	<0.001
Abnormal OGTT result	1.655	1.221, 2.243	0.001

Donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT), oral glucose tolerance test (OGTT), female (F), hazard ratio (HR)

6.4.1.5 Predictors of abnormal glucose tolerance

Recipients with abnormal glucose tolerance were comparable to those with normal glucose tolerance for demographic characteristics, transplant type and CIT (table 6.3). Recipients with abnormal glucose tolerance had numerically older donors (38.8 years vs 34.8 years, p=0.051) with higher BMI (24.6 vs 23.5kg/m^2, p=0.053), although this did not reach statistical significance and had no predictive value in a logistic regression model (p=0.249 and p=0.302 respectively). Neither kidney graft rejection (10.0% vs 6.0%, p=0.374) nor kidney failure (12.5% vs 10.6%, p=0.752) was statistically greater in recipients who displayed abnormal glucose tolerance compared to those with NGT.

Table 6.3 Comparison of recipients showing normal and abnormal glucose tolerance

	Normal Glucose Tolerance	Abnormal Glucose Tolerance	p-value
Operation type (SPK %)	94 (62.7%)	32 (53.3%)	0.217
Recipient Gender (female %)	60 (40.0%)	21 (35.0%)	0.534
Recipient Age (years)	43.14	43.35	0.892
Donor Age (years)	34.70	38.83	0.051
Donor BMI (kg/m^2)	23.46	24.62	0.053
Donor type (DBD %)	127 (84.7%)	46 (76.6%)	0.228
CIT (min)	677.97	702.89	0.397
Kidney rejection (%)	9 (6.0%)	6 (10.0%)	0.374
Kidney failure (%)	10 (10.6%)	4 (12.5%)	0.752

simultaneous pancreas kidney transplant (SPK), donor after brainstem death (DBD), body mass index (BMI), cold ischemia time (CIT)

6.4.3 Study B: the role of continuous glucose monitoring

Thirty prospective pancreas transplant recipients were included in the analysis. Three iPro probes failed to record any readings, and one probe was inadvertently removed at return to the operating theatre. Data were, therefore, available for 26 recipients, including 22 SPK and 4 PTA. The iPro device was on average inserted on day 7 post-operatively and remained in situ for an average of 6.2 days. An example is displayed as figure 6.2.

6.4.3.1 Comparison by transplant type

The characteristics of the continuous glucose monitoring profile are displayed in Table 6.4, and were comparable for the SPK (n=22) and PTA (n=4) groups in terms of demographics, CGM and OGTT parameters. During the period of monitoring the transplant recipients were within the normoglycaemic range for 77.9% of the time and there were no significant differences between the SPK and PTA groups.

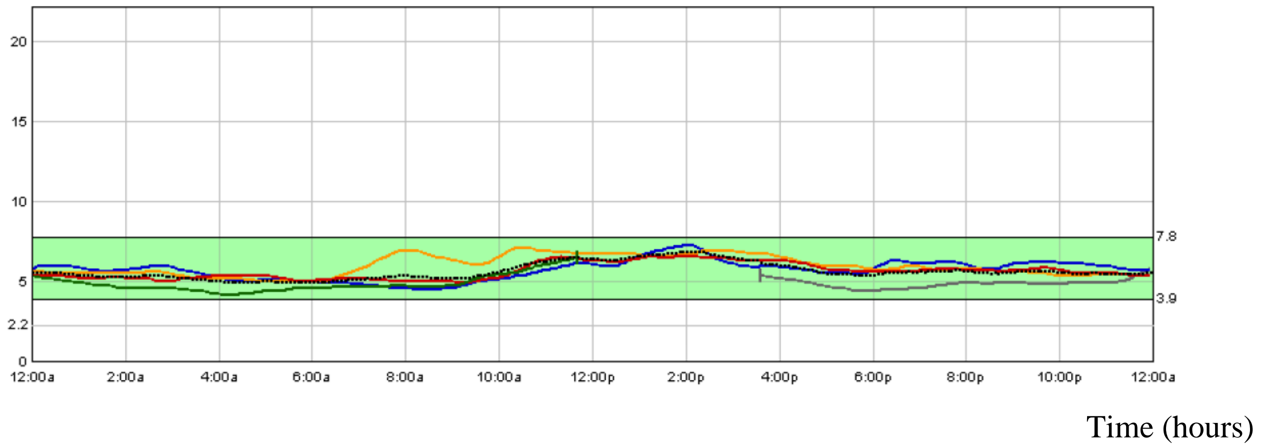
Of note, although mild hypoglycaemic episodes, (blood glucose <3.9 mmol/l (Seaquist et al. 2013a)) occurred in 10/26 (38.5%) pancreas transplant recipients, low excursions were brief (1.38% of study time below 3.9mmol/l) and resolved spontaneously. Four out of ten hypoglycaemic episodes were below 3.1 mmol/l. No symptomatic hypoglycaemic events were observed.

The frequency of hyperglycaemic episodes was however more common (20.69% of study time >7.8mmol/l), with small, non-significant differences between the SPK and PTA groups. CGM data were compared to data from regular finger-prick sugars, as shown in

table 6.5. Finger-prick estimations approximated CGM data, although highest glucose readings were underestimated and hypoglycaemic events were often missed.

Figure 6.2 Example continuous glucose monitoring reading for a patient with (a) normal glucose tolerance and (b) impaired glucose tolerance

a



b

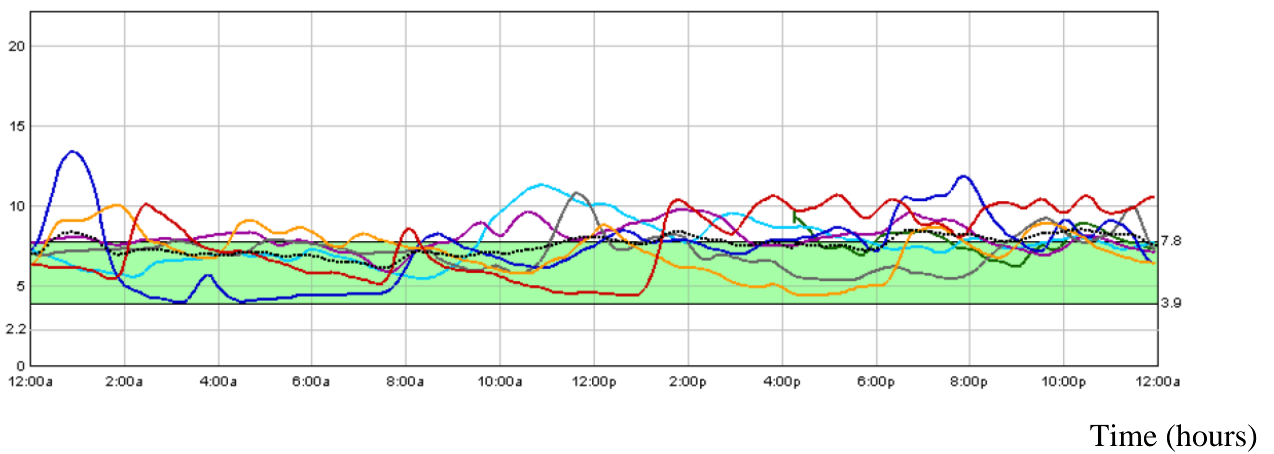


Table 6.4 Demographics and continuous glucose monitoring profile of whole cohort

	Whole cohort N= 26
Recipient age (years)	43.7 ± 10.1
Days post-transplant (days)	7.0 ± 1.0
Highest glucose reading (mmol/l)	10.43 ± 2.43
Lowest glucose reading (mmol/l)	4.07 ± 0.88
Mean glucose value (mmol/l)	6.69 ± 1.20
Total number of high excursions	8.19 ± 7.87
Total number of low excursions	0.69 ± 1.09
AUC glucose above 7.8mmol/l	0.31 ± 0.54
AUC glucose below 3.9mmol/l	0.01 ± 0.02
Time above 7.8mmol/l (%)	20.69 ± 26.94
Time in normal range (%)	77.92 ± 27.01
Time below 3.9mmol/l (%)	1.38 ± 2.59
OGTT – 0 hour - 2 hour	5.68 ± 0.96 8.18 ± 2.73

Area under the curve (AUC), oral glucose tolerance test (OGTT)

Table 6.5 Comparison of finger prick (BM) and continuous glucose monitoring (CGM)

	NGT N= 11		IGT N= 10		DGT N= 2	
	BM	CGM	BM	CGM	BM	CGM
Highest glucose reading (mmol/l)	8.6	9.3	10.8	11.8	12.4	13.6
Lowest glucose reading (mmol/l)	4.7	4.2	5.3	3.9	4.2	4.0
Mean glucose value (mmol/l)	6.4	6.0	7.9	7.3	7.9	8.0
Time above 7.8mmol/l (%)	4.8	4.5	47.8	38.2	37.5	47.5
Time in normal range (%)	95.0	94.1	56.1	59.8	59.0	52.0
Time below 3.9mmol/l (%)	0	1.4	0	2.0	3.6	0.1

Finger prick glucose level (BM), continuous glucose monitoring (CGM), normal glucose tolerance (NGT), impaired glucose tolerance (IGT), diabetic glucose tolerance (DGT)

6.4.3.3 Comparison by glucose tolerance

OGTT data were available for 23 pancreas transplant recipients. 11/23 (47.8%) pancreas transplant recipients had NGT, 10/23 (43.5%) had IGT and 2/23 (8.7%) had DGT. The median trough tacrolimus level was 7.9mg/L (IQR 6.62- 10.41). There was no significant difference in tacrolimus level between those with normal and abnormal glucose tolerance (p=0.684).

The mean continuous glucose profiles were compared by glucose tolerance category (figure 6.3). No significant difference was seen in the frequency or duration of hypoglycaemic episodes between glucose tolerance groups (table 6.6). Hyperglycaemic excursions were more frequent and reached higher levels in recipients with IGT and DGT compared to recipients with NGT. Those with IGT and DGT spent a comparable percentage of the study period above the normal range, which was significantly higher than those with NGT (p=0.012). Those with NGT spent a significantly higher percentage of time within the normal range compared to those with IGT and DGT (94.2% NGT vs 59.8% IGT vs 52.0% DGT, p=0.008; figure 6.4).

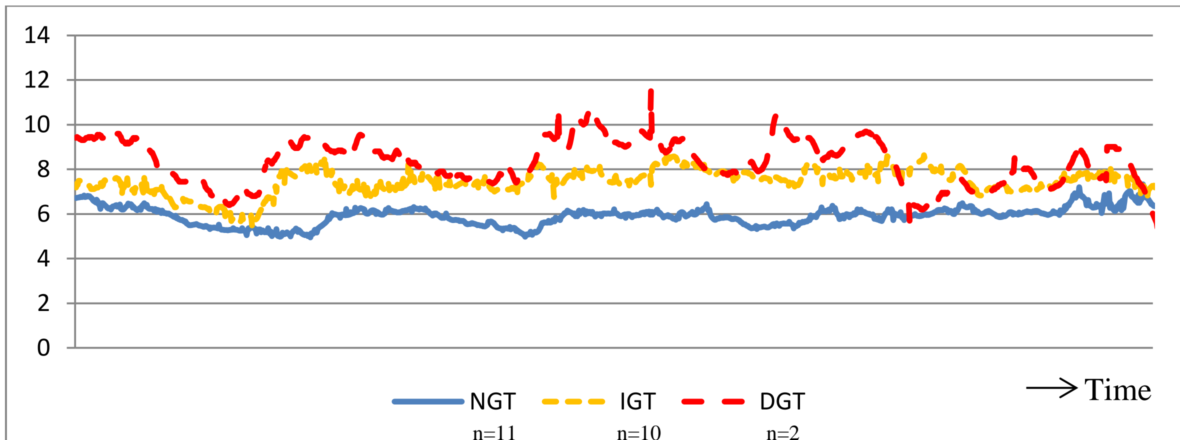
ROC curve analysis showed abnormal glucose tolerance was most associated with % time in hyperglycaemia (area under curve, AUC: 0.86, CI 0.67- 1.00; p=0.004). Abnormal glucose tolerance was predicted with sensitivity of 83.3% and specificity of 100% for time spent in hyperglycaemia of 10.5% (figure 6.4).

Table 6.6 Demographics and continuous glucose monitoring profile of whole cohort and for each transplant type.

	NGT N= 11	IGT N= 10	DGT N= 2
Recipient age (years)	40.1 ± 8.9	47.0 ± 11.1	37.0 ± 7.1
Days post-transplant (days)	7.1 ± 1.4	7.3 ± 0.7	7.0 ± 0.0
Highest glucose reading (mmol/l)	9.25 ± 1.57	11.75 ± 2.39	13.60 ± 1.56
Lowest glucose reading (mmol/l)	4.15 ± 0.75	3.93 ± 1.20	3.95 ± 0.35
Mean glucose value (mmol/l)	5.99 ± 0.57	7.34 ± 1.20	8.05 ± 2.19
Total number of high excursions	4.82 ± 4.64	13.3 ± 9.57	10.0 ± 0.0
Total number of low excursions	0.13 ± 1.35	0.90 ± 0.99	0.50 ± 0.71
AUC glucose above 7.8mmol/l	0.03 ± 0.03	0.54 ± 0.53	1.60 ± 1.41
AUC glucose below 3.9mmol/l	0.01 ± 0.02	0.01 ± 0.02	0.0 ± 0.0
Time above 7.8mmol/l (%)	4.45 ± 4.12	38.2 ± 27.89	47.5 ± 51.62
Time in normal range (%)	94.18 ± 5.79	59.8 ± 27.33	52.0 ± 50.91
Time below 3.9mmol/l (%)	1.36 ± 3.11	2.00 ± 2.58	0.05 ± 0.71

Finger prick glucose level (BM), continuous glucose monitoring (CGM), normal glucose tolerance (NGT), impaired glucose tolerance (IGT), diabetic glucose tolerance (DGT). Area under the curve (AUC)

Figure 6.3 Average continuous glucose profile for pancreas transplant recipients by glucose tolerance

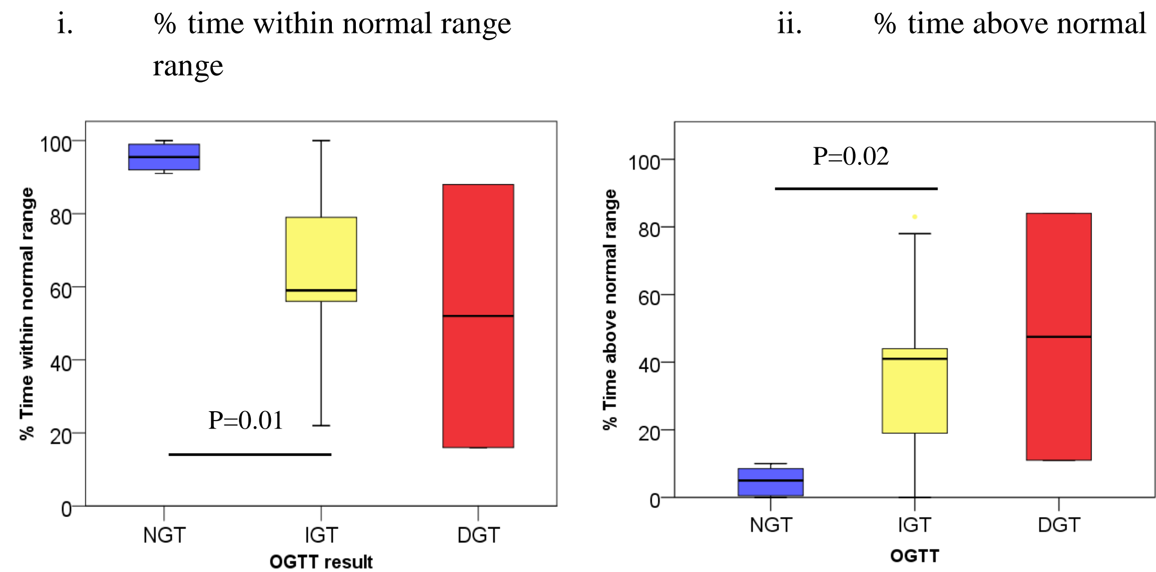


normal glucose tolerance (NGT), impaired glucose tolerance (IGT), diabetic glucose tolerance (DGT)

Figure 6.4 Box-plot depicting percentage of study time spent (i) within normal range (3.9- 7.8mmol) and (ii) above normal range (>7.8mmol/l) by glucose tolerance

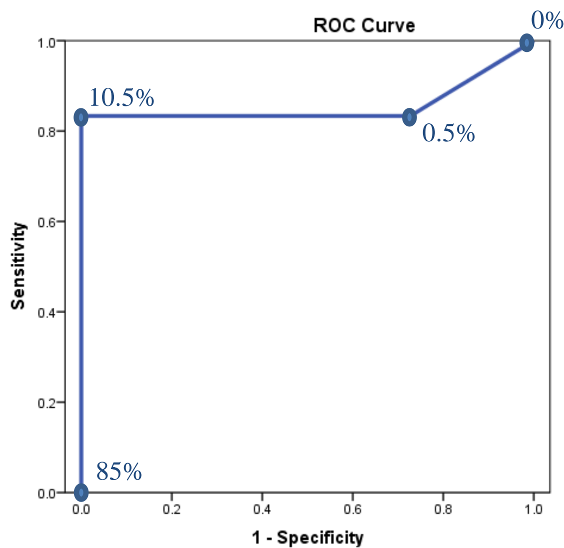
i. % time within normal range

ii. % time above normal range



normal glucose tolerance (NGT), impaired glucose tolerance (IGT), diabetic glucose tolerance (DGT)

Figure 6.5 Receiver operating curve analysis of % time in hyperglycaemia and oral glucose tolerance test result



The ROC curve describes the diagnostic accuracy of a test. In this case, the ability of the time in hyperglycaemia to predict an abnormal OGTT result. The sensitivity describes the true positive rate i.e. probability that time in hyperglycaemia correctly identifies abnormal glucose tolerance. The specificity describes the true negative rate i.e. probability of time in hyperglycaemia correctly identifies a normal glucose tolerance. Therefore, 1-specificity represents the false positive rate. A test that is not diagnositic is represented by a straight 45 degree line. A perfect test would have a sensitivity of 1.0 and specificity of 1.0 (or 1-specifity of 0), and would be represented in the top left corner of the ROC curve, giving a large area under the curve. The ideal threshold for the test is taken as the point with the greatest sensitivity and specificity.

6.4.3.5 Relationship to clinical outcome

5/26 (19.2%) patients had early clinical complications: 3/26 (11.5%) suffered graft pancreatitis, one of which resulted in graft pancreatectomy, 2 patients had radiologically diagnosed partial venous thrombosis relating to the pancreas graft treated with anticoagulation, and 1/26 (3.8%) had delayed kidney graft function. All of the patients with complications, except the patient who underwent pancreatectomy, had episodes of IGT or DGT on oral glucose testing, and increased number of episodes and peak values of hyperglycaemia on CGM. 8/12 (66.6%) patients with abnormal glucose tolerance had an uneventful early clinical course. The remaining 25 grafts were functioning at last follow-up (median 9 months, range 6-15 months), and all except 2 had an HbA1c of less than 5.3% (median 5.2%, range 4.9 – 6.3%). Two patients (8%) had an HbA1c above 6.2% at 1 year post op. Both had shown hyperglycaemia and abnormal glucose tolerance early post-operatively; one patient did suffer graft pancreatitis, and the other had an uneventful clinical course.

6.5 Discussion

This chapter comprises two studies. The first, a retrospective analysis of routine clinical data comprising OGTTs post-transplant (study A), shows for the first time, that abnormal glucose tolerance early after pancreas transplantation is associated with a higher risk of pancreas graft failure, and that recipients showing the greatest degree of early glucose intolerance have the shortest time to graft failure. This association was consistent for both SPK and IP transplants and is independent of demographic factors usually thought to influence graft outcome. Normal glucose tolerance post-transplant is associated with excellent 3-year graft survival of 86% and a normal OGTT has a high negative predictive value. Thus, an early normal result can be considered reassuring of good long-term graft survival. The second study, a prospective analysis in a separate cohort (study B), is the first study to examine CGM profiles in the early post-transplantation period with matched OGTT. CGM profiles were shown to correlate with OGTT result, and identified a marker of glycaemic control previously not utilised in pancreas transplant monitoring.

The OGTT is a simple and accessible test of glycaemic control, which has been used in pancreas transplant recipients, showing abnormal glucose tolerance to be common in recipients with long-standing graft function (Dieterle et al. 2007). A previous study found shorter graft survival to be associated with impaired glucose tolerance in 41 pancreas transplant recipients, however OGTTs were performed at a variable time-point of 1.7 ± 1.7 years post-transplant. This study, conducted in a much larger cohort, with a mean follow up of 30 months has the advantage of utilising an early standardised time-point and thus

has greater potential clinical utility, particularly since the greatest return to exogenous insulin occurs in the first year post-transplant.

Although CIT emerged as a significant factor for predicting graft failure in this study, unlike an abnormal OGTT result, this was not consistent in both SPK and IP groups. It is interesting to note that CIT showed statistical interactions with an abnormal OGTT result, since early glucose intolerance may represent graft damage as a result of ischaemia-reperfusion injury, which is known to be correlated with increased CIT and commonly seen in kidney transplantation as DGF. Early dysfunction may also represent a reduced functional β-cell mass at the time of implantation due donor factors or trauma, which is suggested by the association between abnormal OGTT, and donor age and BMI, although this did not quite reach statistical significance.

Delayed graft function of the pancreas graft, as defined by a need for exogenous insulin, has been associated in other studies with increased donor age although the relationship with graft survival has been less clear (Baitello et al. 2011; Shin et al. 2014; Tan et al. 2004), and this may be due heterogeneity in the definitions used. Troppman et al suggests that DGF may result from a combination of poor functional reserve combined with the increased stress in the early post-transplant period of uraemia, steroid treatment and glucose-rich infusions (Troppmann et al. 1996). The reason why insulin is needed less frequently in the post-transplant period in our cohort compared to the described literature is not known, but may reflect differences in donor selection, immunosuppression protocols and peri-operative management practices.

The findings of study B, in addition to the information provided by the OGTT, show that CGM allowed the identification of minor hypoglycaemia, which was common and present in over a third of patients. In this study, CGM-identified minor hypoglycaemia was generally asymptomatic, infrequent and of short duration, resolving spontaneously such that no clinically significant hypoglycaemic events occurred. This supports findings from other groups that, although frequently missed with finger-prick monitoring alone, hypoglycaemic events often occur, resolve spontaneously and are unlikely to have any clinical consequence (Esmatjes et al. 2003; Rodriguez et al. 2010). CGM data also allowed more detailed comparison of recipients of SPK and PTA transplants, showing they had comparable glucose profiles. This is in contrast to a previous study, which found PTA recipients to have a higher mean glucose concentration when compared to SPK recipients, however in that study the PTA group had a different immunosuppression protocol with higher tacrolimus dosing and steroids, which are likely to have affected glucose control (Lauria et al. 2012).

Importantly, study B showed that CGM correlates to the OGTT result, with recipients displaying excursions above the normal range greater than 10% of a 24-hour period highly likely to be diagnosed with abnormal glucose tolerance. Therefore, CGM could be used to replace OGTT, to identify a group at high risk of graft failure. Transplant recipients with NGT demonstrated near normal 24-hour glucose profiles, which have been shown to be superior to those seen with intensive insulin therapy (Kessler et al. 2002). Whilst it is reassuring to see that those with NGT spend 94% of time within normal range, it is notable that those with IGT or DGT spend significantly higher percentage of time above the normal range, confirming that although the abnormal OGTT is based upon a single

time point blood glucose, subjects with abnormal glucose tolerance spend significant and more prolonged periods of time in the hyperglycaemic range.

A previously published study has shown poorer long-term pancreas graft survival with a mean glucose >7mmol/l on 24 hour glucose profiling performed 1 year post-transplant in 53 SPK recipients (Battezzati et al. 2001). This corresponded in our cohort to a diagnosis of IGT and adds further weight to the hypothesis that early hyperglycaemia precedes and predicts later graft failure. Early hyperglycaemia on CGM was evident in patients who had high HbA1c at 1 year post-transplant. Prolonged periods of hyperglycaemia can induce beta-cell death (Robertson et al. 2003), which may be contributing in itself to poorer long-term graft outcomes and ultimately a higher risk of diabetes-related complications in some of our patients with abnormal glucose tolerance (Stratton et al. 2006).

The CGM analysis involved a relatively small number of subjects in whom complete CGM data were available. Unfortunately 4 probes did not yield data sufficient to be used in the analysis; one was accidently dislodged early after its insertion and three failed to register. In addition OGTT data were not available for three patients. The assessment of OGTT did suffer, as a retrospective analysis, with the inherent bias associated with missing data. Also, IP transplantation was less commonly performed and a smaller cohort was available for analysis compared to the SPK group, so limiting the achievable matching ratio. Nevertheless, the groups showed no significant differences and sub-group analyses confirmed that associations and significances remained in both groups. Importantly, graft failures secondary to surgical complications or early pancreatectomy following sepsis were excluded as glucose tolerance may be altered by these confounding

factors. Whilst the exclusion of these individuals allows for more meaningful interpretation of the data in an important group, our findings may not apply in the context of sepsis and should only be considered relevant to recipients considered to have good graft function 2-4 weeks post-transplant. However, it is also quite possible that CGM, and OGTT, is equally predictive if carried out late in the patient's course, whenever the inflammatory response has settled. Additionally, these associations relate to all-cause graft failure since causes of graft failure were not defined, thus graft loss due to rejection processes cannot be distinguished from those relating to post-transplant diabetes (Neidlinger et al. 2010).

The findings of this study highlight important early post-transplant metabolic markers that can be used to assess the risk of later pancreas graft failure, and a means of providing detailed multiple time-point data with minimal inconvenience to patients. The mechanisms behind early graft dysfunction remain elusive, with studies previously highlighting reduced early phase insulin secretion responses in pancreas transplant recipients with abnormal glucose tolerance (Pfeffer et al. 1996) and Christiansen et al also noting impaired glucose disappearance and reduced glucagon suppression (Christiansen et al. 1997). Longitudinal OGTT and CGM data may better reflect the complex and evolving post-transplant environment, comprising autoimmunity, allo-immunity and the inflammatory manifestations of ischaemia-reperfusion (all of which may contribute to the pathogenesis of beta-cell destruction) and future prospectively planned studies will be necessary to make detailed assessments of defects in insulin secretion and glucose regulation to provide insights into the mechanisms of pancreas graft failure.

6.6 Conclusion

In conclusion, this study shows that an early post-operative OGTT may help define recipients discharged with functioning grafts not requiring exogenous insulin who are high risk for all-cause future graft failure. This early surrogate for graft survival has the potential to direct close surveillance and targeted management aimed at preserving graft function. Further, this study shows that CGM is a feasible, convenient and patient-friendly monitoring tool in the post-transplant setting, and correlates well to OGTT result. CGM has the advantage of frequent time-point data enabling accurate assessment of hyperglycaemia and potentially more sensitive identification of pancreas transplant recipients at the greatest risk of later graft failure, as well as being more appropriate for outpatient assessment and more convenient for patients. This will need further assessment in a larger cohort with long-term follow-up.

Chapter 7

Factors contributing to hyperglycaemia early post-transplant

7 FACTORS CONTRIBUTING TO HYPERGLYCAEMIA EARLY POST-TRANSPLANT

7.1 Introduction

Early graft dysfunction can be seen after whole organ transplantation. The need for dialysis in the first week post-transplant, which defines delayed graft function, is well recognised in kidney transplantation. In this thesis, I have shown that early metabolic dysfunction, as defined by abnormal glucose tolerance, is not uncommon after pancreas transplantation, and may be contributed to by numerous mechanisms. Early dysfunction may represent reduced beta-cell mass at the time of transplantation, the damaging effects of ischaemia-reperfusion or delays in achieving optimal regulation. Early metabolic dysfunction appears to be associated with later pancreas graft failure, and the reasons for this are not fully understood.

The physiology and pathology underlying early pancreas graft dysfunction and how this progresses to graft failure is unknown. Pancreas transplantation represents a unique and abnormal anatomical arrangement, involving implantation of a heterotopically placed, denervated donor pancreas with systemic drainage of insulin and glucagon. The transplant recipient's native pancreas remains in situ and, although there is no remaining beta-cell function, other functions are maintained, including those relating to alpha-cells. After pancreas transplantation, glucose homeostasis is normalised immediately post-transplant in most cases and the mechanisms behind pancreas graft function, and indeed dysfunction, are poorly understood.

In health, insulin is released from the pancreas, principally in response to circulating glucose or other nutrients, into the portal circulation where it initially acts to suppress endogenous glucose production and undergoes first-pass metabolism in the liver. At higher levels, insulin also increases peripheral glucose uptake in adipose and muscle tissue, with the transport of glucose into the intracellular compartment leading to normalisation of hyperglycaemia (Herring et al. 2014). In the context of systemically-drained pancreas transplantation, first-pass metabolism is bypassed resulting in loss of the suppressive effects on the liver and systemic hyperinsulinaemia. Although glucose homeostasis normalises post-transplant, it is not known how these physiologically abnormal changes are compensated for, what exact mechanisms underlie normalisation in glucose homeostasis and what impact the transplanted pancreas is having on the native pancreas.

Diabetes is known to be a bi-hormonal condition, involving the balance of insulin and glucagon. In health, glucagon is released from the pancreas, regulated through responses to glucose and nutrient levels, paracrine regulation from intra-islet insulin and sympathetic innervation. Absolute or relative hyperglucagonaemia is a hallmark of both T1D and T2D, and contributes negatively to hyperglycaemia (Unger 1971). In pancreas transplantation, both native and transplanted alpha-cells are present and it is unknown if normalisation of glucagon regulation occurs. Alternatively, it may be that hyperglucagonaemia persists and contributes to hyperglycaemia or, through demanding equivalent hyperinsulinaemia to achieve euglycaemia, increases pancreatic stress that may impact of graft survival.

The regulation of insulin and glucagon secretion is complex, but includes influence by hormones released form the gastrointestinal tract (GIT), collectively called incretin hormones. Although there are many incretin hormones, GLP-1 (Glucagon-like peptide-1) and GIP (Glucose-dependant insulinotropic peptide) have been considered the most important. These hormones are secreted following a meal, and lead to a three-fold increase in plasma insulin levels in comparison with an equivalent intravenous glucose load, so contributing 80% of mealtime insulin and efficient glucose homeostasis. This phenomenon is called the Incretin Effect (Nauck et al. 1986) and has been seen to be diminished in T2D (Holst et al. 2011).

In normal physiology, GIP is released from K cells situated in the duodenum and jejunum, and promotes glucose-dependent insulin and glucagon secretion during hyperglycaemia and hypoglycaemia respectively (Christensen et al. 2011). GLP-1 from L-cells, principally in the ileum and colon, also has glucose-dependent insulinotropic effects, and instead has an inhibitory effect on glucagon, independent of insulin concentrations (Baggio and Drucker 2007).

In experimental models, both GIP and GLP-1 have been suggested to have anti-apoptotic and even proliferative effects of beta-cells, although the therapeutic value of GIP has been questioned. GLP-1 infusions have also been shown to delay gastric emptying and so reduce food intake and consequently result in weight loss (Yabe and Seino 2011). Experiments examining the effects of GLP-1 and GIP infusions, found that supra-physiological levels of GLP-1, but not GIP, restored the incretin effect in T2D and had insulinotropic effects on vascularised islets in animal studies (Yabe and Seino 2011). The molecular mechanisms underlying this selective response are unknown, and it has been

suggested that GIP effects may be more important at near normoglycaemia, although further investigation is needed.

In turn, data from experimental models with infusion of supraphysiological GLP-1 levels have led to the development of new therapeutic agents, which modulate the incretin axis for use in T2 and more recently T1D (Inzucchi et al. 2012). These agents typically enhance the enteroinsular axis by increasing GLP-1 concentrations. This can be achieved, either through the inhibition of dipeptidyl peptidase-4 (DPP-4), which is usually responsible for the rapid degradation of endogenous GLP-1, thus increasing endogenous GLP-1 concentrations within the normal range; or by providing supra-physiological levels of GLP-1, through the use of GLP-1 mimetics resistant to DPP-4 degradation. These therapies have been of particular interest since, in addition to decreasing post-prandial glucose excursions and reducing insulin doses, they have been postulated to preserve beta cell function in patients with T2D (Bunck et al. 2009) and encourage beta-cell proliferation in animal studies (Hadjiyanni et al. 2008), making their use in pancreas transplantation also appealing. Due to the rapid degradation of incretin hormones (with a half-life of 2-2.5 minutes), many have speculated that the effects of incretin hormones are not mediated via a direct effect on the pancreas, but rather via a neuroendocrine axis, whereby vagal motor neurones respond to GLP-1 and stimulate beta-cell secretion (Holst 2007). In the context of transplantation of a denervated pancreas, it is not known if the incretin effect will be mediated, or if hormone secretion is affected.

7.3 Aims of chapter 7

The aims this chapter are two-fold and will be investigated in two separate studies.

a) The effect of pancreas transplantation on glucagon secretion early post-transplant will be investigated in a prospective study of pre- and post-transplant recipients: Study C.

b) The role of the incretin effect early post-transplant will be examined in a separate prospective study of post-transplant recipients: Study D.

7.4 Methods

7.4.1 Study C: Prospective pre- and post-transplant assessment of glucagon regulation

7.4.1.1 Patient cohort

People on the waiting list for pancreas transplantation were invited to participate in the study. The waiting list was examined by clinical scientists, within the Tissue Typing laboratory, to identify patients considered at the 'top' of the waiting list, and these patients were invited to participate first. This was performed through identifying recipient factors considered as part of the national allocation process, including waiting time and sensitisation status. Patients on the waiting list, who were not sensitised and had longer waiting times were considered to be likely to be allocated a pancreas transplant sooner than those who had been added to the waiting list recently, or were highly sensitised. Potential recipients for SPK and IP transplantation were included, and no specific exclusion criteria were applied.

A control group of healthy volunteers was also included and invited to participate in the study, in order to provide a comparison to the pancreas transplant group. This healthy control group was recruited from University of Oxford staff, who had previously expressed an interest in involvement in research studies. The healthy control group was matched to pancreas transplant recipients for age and BMI.

7.4.1.2 Study procedures

Participants in the pancreas transplant group attended three research visits. The first took place whilst the participant was on the transplant waiting list at a time convenient to them (visit 1). The second visit took place within 14 days post- transplant, prior to discharge from the ward to home (visit 2). The third visit took place 3 months post-transplant (visit 3), often co-ordinated with clinical follow-up appointments (Figure 7.1). Participants in the healthy control group attended for a single research visit. At each research visit, all study procedures were performed.

Clinical data

For each participant, clinical data were collected against a unique study identifier. Data were recorded including date of birth, gender, BMI, comorbidities, weight, height, BP, details of transplant and medications.

Frequently-sampled Oral Glucose Tolerance Test (FSOGTT)

Participants were asked to fast from midnight of the night preceding the research visit, and to avoid strenuous exercise the preceding evening and morning of the research visit.
At the research visit, a peripheral intravenous cannula was placed, through which fasting blood samples were taken, as per sampling protocol (table 7.1).

75g anhydrous glucose was dissolved in 250ml water, which was then consumed steadily over 4-5min by research participants. A timer was started when drinking commenced. Participants were then asked to remain rested in bed while samples were taken at 0, 30, 60, 90 and 120 minutes after the glucose drink was consumed. After 120 minutes, the oral glucose tolerance test was complete and the peripheral cannula was removed. Participants

were provided with something to eat and drink. Participants were observed for at least 30 minutes after the end of the test, and blood glucose tested prior to leaving the research visit.

Sampling protocol

Blood samples were taken at each time-point, having discarded 1 ml of blood at the beginning of each sample and flushing the cannula with 1ml of saline the end of each sample. At all time-points 8ml of blood was taken into a heparinised plasma tube, for testing of glucose, c-peptide and insulin, as well as 2ml of blood into a tube containing EDTA and aprotinin, which inhibits glucagon degradation, for testing for glucagon. All samples were placed on ice and centrifuged at 4°C and 3000rpm 10mins. Plasma was then separated into aliquots. Glucose aliquots were stored at -20°C and all other samples stored at -80°C, pending assaying in batches in order to minimise batch and kit variability. Samples were analysed with commercial radioimmunoassay kits: glucose, c-peptide and human insulin-specific kits (Merck-Millipore®, Missouri, USA), and EURIA-glucagon (Euro Diagnostica, Sweden). Assays were processed by laboratory technicians in the Oxford Centre for Diabetes, Endocrinology and Metabolism research laboratories.

7.4.1.3 Statistical analysis

The pancreas transplant group was compared to the healthy volunteer group for demographic factors. Variables were explored and compared for changes over time (between research visits) in glucose, c-peptide, insulin and glucagon. Comparison was also made between the pancreas transplant group and the healthy control group.

Variables that showed normal distribution and were analysed with parametric tests included glucose. Variables that showed non-normal distribution were analysed with non-parametric tests and included c-peptide, insulin and glucagon.

Figure 7.1 Glucagon study participant timeline

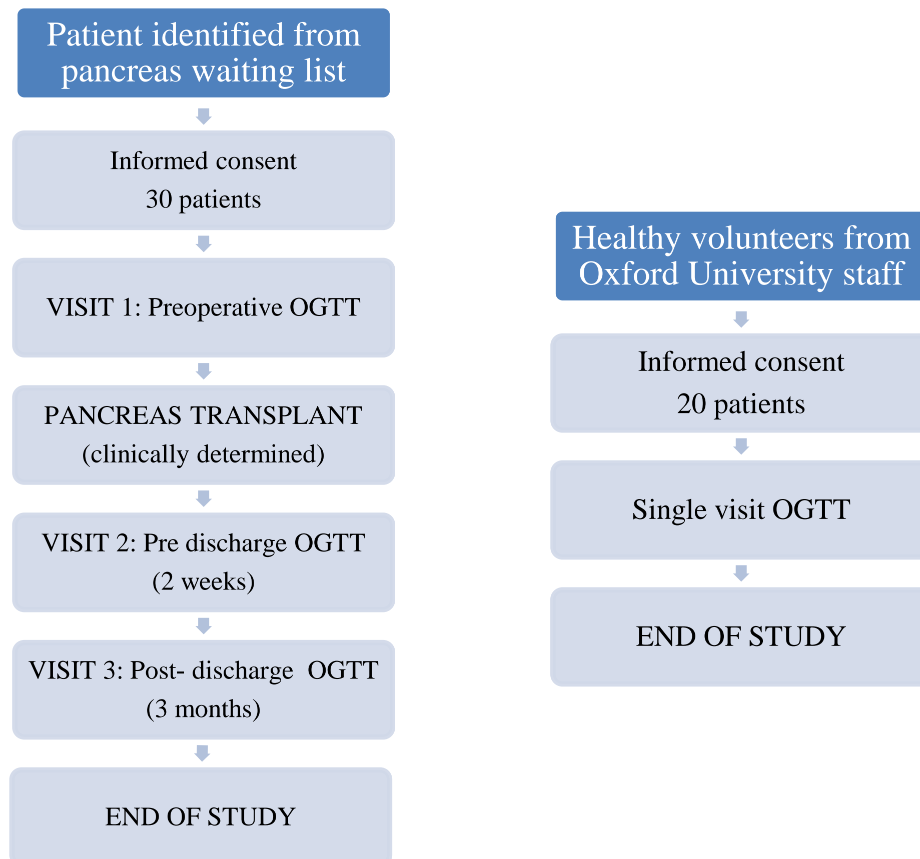


Table 7.1 Glucagon study sampling protocol

<table>
<tr><th>Tube</th><th>Immediate Handling</th><th>Separation and aliquoting</th><th>Storage</th><th></th></tr>
<tr><td>Heparinised plasma (glucose)

10microlitre</td><td>All time points:
Invert tube 3-4 times
Keep cold or store on ice</td><td>Cool spin for 10 minutes</td><td>Store in fridge or freeze – 20 ˚C</td><td rowspan="4">X1 9ml green top heparinised plasma tube – spin at 3000 and separate plasma into 4 0.5ml aliquots with green lid</td></tr>
<tr><td>Heparinised plasma (Insulin)

200mcl</td><td>All time points:
Invert tube 3-4 times
Store on ice</td><td>Cool spin for 10 minutes</td><td>Store at – 80˚C</td></tr>
<tr><td>Heparinised plasma (Pro-insulin)

500mcl</td><td>All time points:
Invert tube 3-4 times
Store on ice</td><td>Cool spin for 10 minutes</td><td>Store at – 80˚C</td></tr>
<tr><td>Heparinised plasma (C-Peptide)

300-500mcl</td><td>All time points:
Invert tube 3-4 times
Store on ice</td><td>Cool spin for 10 minutes</td><td>Store at – 80˚C</td></tr>
<tr><td>Pink tube (Glucagon)</td><td>All time points:
Use glass pipette
Store on ice</td><td>Cool spin for 10 mins</td><td>Store at – 80˚C</td><td>Spin at 3000 and pipette into x1 glass aliquot with black lid</td></tr>
</table>

7.4.2 Study D: Prospective early post-transplant assessment of the role of the incretin effect

7.4.2.1 Patient cohort

Three participant groups were invited to take part in the study. Each group contained 10 participants, with attempts to match for age and BMI.

Group 1: All potential pancreas transplant recipients on the transplant waiting list, who were not already involved in study C, were sent invitations to participate in this study. Those who expressed an interest were approached on the transplant ward after receiving their pancreas transplant, and were recruited. Recipients of SPK, PTA and PAK were all considered suitable for participation.

Group 2: Non-diabetic kidney transplant patients were also invited and recruited in the same manner as group 1. This was to provide a control group to allow for the effects of immunosuppression, surgery and kidney transplantation for renal failure.

Group 3: Healthy volunteers were also recruited to provide a ‘normal control’ comparator group, for comparison to groups 1 and 2.

To evaluate the early post-transplant period, participants in the transplant groups attended for two sets of research visits, making four research visits in total. The first set of two research visits took place 2-3 weeks post-transplant, and the second set (or 3rd and 4th research visit) took place at 3-months post-transplant. Patients that were unwell post-transplant, or had delayed graft function of the kidney transplant were excluded from the study.

Participants in the healthy volunteer group attended for a single time point set of two research visits.

7.4.2.2 Study procedures

Clinical data

For each participant, clinical data were collected against a unique study identifier. Data were recorded including date of birth, gender, BMI, comorbidities, weight, height, BP, details of transplant and medications. For the transplant patients, changes in observations and medications between visits were noted.

Extended frequently sampled OGTT (visit 1 and 3)

For these visits, participants were asked to fast from midnight of the night preceding the research visit, and to avoid strenuous exercise the preceding evening and morning of the research visit. A peripheral intravenous cannula was placed, through which fasting blood samples were taken at -10 min and 0 min before the start of the study procedure, as per sampling protocol (Figure 7.2).

A 50g glucose drink was then consumed steadily over 4-5min by research participants. A timer was started when drinking commenced. Participants were then asked to remain rested in bed while frequent blood samples were taken through the peripheral cannula at 10, 20, 30, 40, 50, 60, 75, 90, 105, 135, 150, 180, 210 and 240 minutes after the glucose drink was consumed for laboratory and HemoCue® analysis (HemoCue, Sweden). After 240 minutes, the extended oral glucose tolerance test was complete and the peripheral cannula was removed. Participants were provided something to eat and drink.

Participants were observed for at least 30 minutes after the end of the test, and blood glucose tested prior to leaving the research visit.

Isoglycaemic intravenous glucose infusions

Visit 2 and 4 took place within 2 weeks of visit 1 and 3 respectively. At these visits, participants underwent isoglycaemic intravenous glucose infusions (IIGI). A peripheral intravenous cannula was inserted into a peripheral vein in each hand; one for blood sampling and the second for 20% glucose infusion. The glucose infusion rate was manually adjusted in increments to reproduce the glucose profile obtained during the preceding extended OGTT. Blood sampling was taken at identical time-points to during the extended OGTT, with occasional additional HemoCue measurements of glucose in order to accurately adjust the glucose infusion.

Sampling protocol

Samples were taken identically during the extended OGTT and IIGI according to Figure 7.2.

Blood samples were taken at each time-point, having discarded 1 ml of blood at the beginning of each sample and flushing the cannula with 1ml of saline the end of each sample. At all time-points 5ml of blood was taken into a heparinised plasma tube, for testing of glucose, c-peptide and insulin, as well as 9ml of blood into a tube containing EDTA, aprotinin and a specific dipeptidyl peptidase IV inhibitor, in order to inhibit glucagon and incretin hormone degradation (gift from Novo Nordisk, Bagsvaerd, Denmark), for testing for glucagon, GLP-1 and GIP. All samples were placed on ice and centrifuged at 4°C and 3000rpm 10mins. Plasma was then separated into aliquots.

Glucose aliquots were stored at -20°C and all other samples stored at -80°C, pending assaying in batches in order to minimise batch and kit variability. Samples for glucose, c-peptide and insulin were analysed by laboratory technicians in the Oxford University Hospital NHS Trust Clinical Biochemistry laboratories: glucose was measured with enzymatic methods (ADVIA® 2400 Chemistry systems, Bayer® Diagnostics), while c-peptide and insulin were measured with radioimmunoassay (ADVIA® Centaur systems, Bayer® Diagnostics). Samples for glucagon, total GLP-1 and total GIP were measured by laboratory technicians at the Department of Translational Metabolism, University of Copenhagen, using in-house assays developed at the University of Copenhagen: glucagon (Bak et al. 2014a), GLP-1 (Bak et al. 2014b) and GIP (Deacon et al. 2000) were measured using radioimmunoassay techniques validated against commercially available assays.

7.4.2.3 Statistical analysis

Groups were compared for demographic characteristics and for differences in glucose, c-peptide, insulin, glucagon, GLP-1 and GIP. Gastrointestinal glucose disposal (GIGD) and incretin effect (IE) were calculated for each group and compared. The difference in the means of the incretin effect was normally distributed and compared using parametric analysis. Other variables were compared using non-parametric analyses.

Calculating GIGD

GIGD reflects the percentage of glucose disposal caused by the oral route of glucose administration, and is mediated by incretin hormones, neuronal reflexes, first-pass hepatic uptake of glucose and other unknown factors. It is calculated as below:

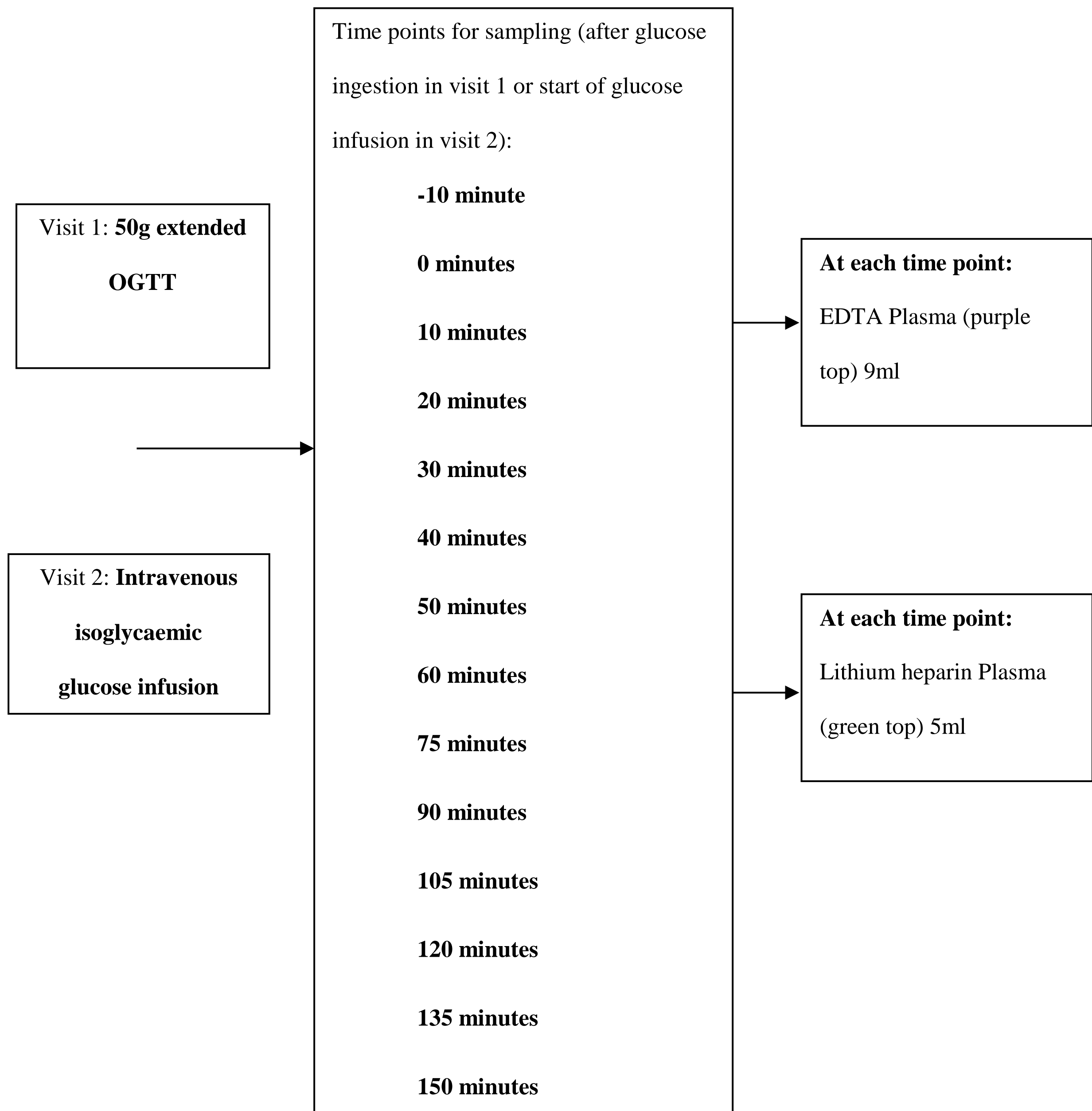
$$100\% \times \frac{(\text{glucose}_{OGTT} - \text{glucose}_{IIGI})}{\text{glucose}_{OGTT})}$$

Calculating IE

The incretin effect describes the phenomenon whereby there is increased secretion of insulin for a given glucose in response to an oral glucose load compared to an intravenous glucose load, and is mediated by gut-derived incretin hormones. It is calculated as below:

$$100\% \times \frac{(\text{insulin}_{\text{OGTT}} - \text{insulin}_{\text{IIGI}})}{\text{insulin}_{\text{OGTT}}}$$

Figure 7.2 Sampling protocol for study D



7.5 Results

7.5.1 Study C: Pre- and post-transplant assessment of glucagon

7.5.1.1 Demographics

Thirty potential pancreas transplant recipients were recruited to the study. 24 participants were transplanted within the study period: one participant was removed from the pancreas transplant waiting list in favour of a kidney only transplant and five participants had periods of suspension from the waiting list and are awaiting transplantation. Following transplantation, 2 participants had early pancreatectomy and were excluded from the study. Three further participants attended for post-transplant visits but were unable to tolerate oral glucose tolerance testing due to vomiting, and one participant dropped out of the study. In total, 6 participants were excluded after pancreas transplantation. Eighteen participants were included in the final analysis.

The mean age of the pancreas group participants at the time of transplant was 40.5 years (±SD 12.1 years). There was an approximately equal gender division (8 female, 10 male). The mean BMI was 25.3kg/m^2 (±SD 7.4 kg/m^2) pre-transplant and was stable over the study period. The healthy control group were comparable for demographics, with a mean age of 39.2 year (±SD 9.8 years), gender division of 10 female, 9 male and BMI of 24.1 kg/m^2 (±SD 3.0 kg/m^2).

In the pancreas transplant group, all participants included in the analysis had pancreas transplantation: 17 SPK, 1 PTA. The mean duration of diabetes prior to transplantation was 27.2 years (±SD 8.6 years). 13/18 participants (72.2%) were established on dialysis prior to transplantation. The mean duration between visit 1 (on the waiting list) and visit

(2 weeks post-transplant) was 148 days (±SD 109 days). All participants received standard immunosuppression, and were insulin- and oral hypoglycaemic agent-independent at completion of the study.

7.5.1.2 Comparison of glucose levels

Seven participants (38.9%) had raised fasting serum glucose at visit 1 (>7mmol/l), and were given a bolus of short-acting insulin greater than 2 hours prior to the start of the OGTT, in order to standardise starting glucose through all procedures.

In the pancreas transplant group, all participants were diabetic pre-transplant. At 2 weeks post-transplant, glucose tolerance had improved and 11/18 (61.1%) had NGT. Glucose tolerance improved further by 3 months post-transplant, at which time 16/18 (88.8%) had NGT (table 7.2).

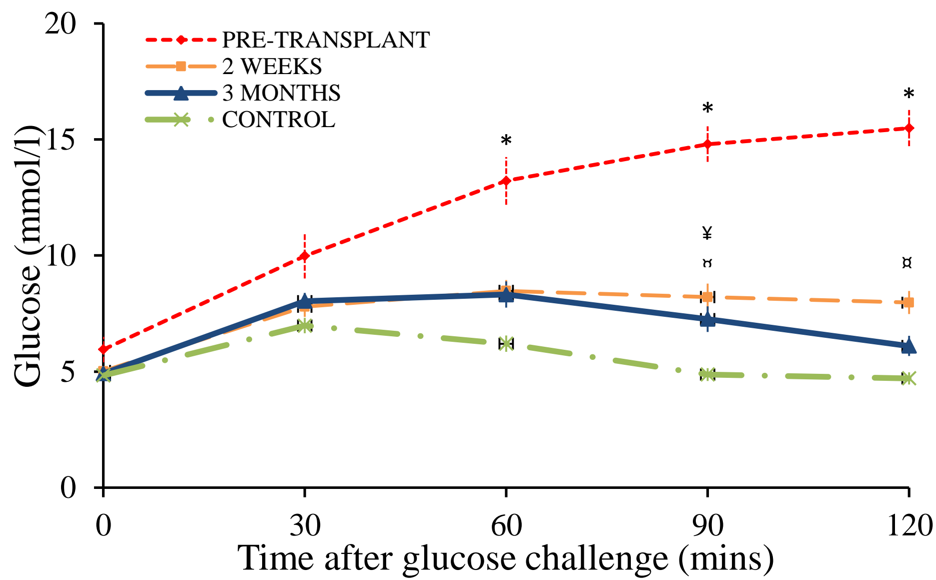
There was no significant difference in fasting glucose in the pancreas group over time, nor when compared to the healthy control group. However, beyond 60-mins pre-transplant subjects had significantly higher blood glucose compared to the post-operative and control groups ($p<0.001$, Figure 7.3). Glucose profiles improved from 2 weeks to 3 months post-transplant, with 120-min glucose significantly higher than controls at 2 weeks ($p<0.001$) but comparable at 3 months ($p=0.364$).

Table 7.2 Oral glucose tolerance test result at each research visit in the pancreas transplant group

	DGT	IGT	NGT
Visit 2: 2 weeks post-transplant	2/18 (11.1%)	5/18 (27.8%)	11/18 (61.1%)
Visit 3: 3 months post-transplant	1/18 (5.6%)	1/18 (5.6%)	16/18 (88.8%)

Diabetic glucose tolerance (DGT), impaired glucose tolerance (IGT), normal glucose tolerance (NGT)

Figure 7.3 Glucose profiles after OGTT



a) Pre-transplant (red spotted line); b) 2 weeks post-transplant (orange dashed line); c) 3 months post-transplant (blue solid line); d) healthy control (green dot-dash line)

* significant difference between pre-transplant and post-transplant and control groups, $p<0.001$;
¤ significant difference between 2 weeks post-transplant and controls, $p<0.001$;
¥ significant difference between 3 months post-transplant and controls, $p<0.05$;

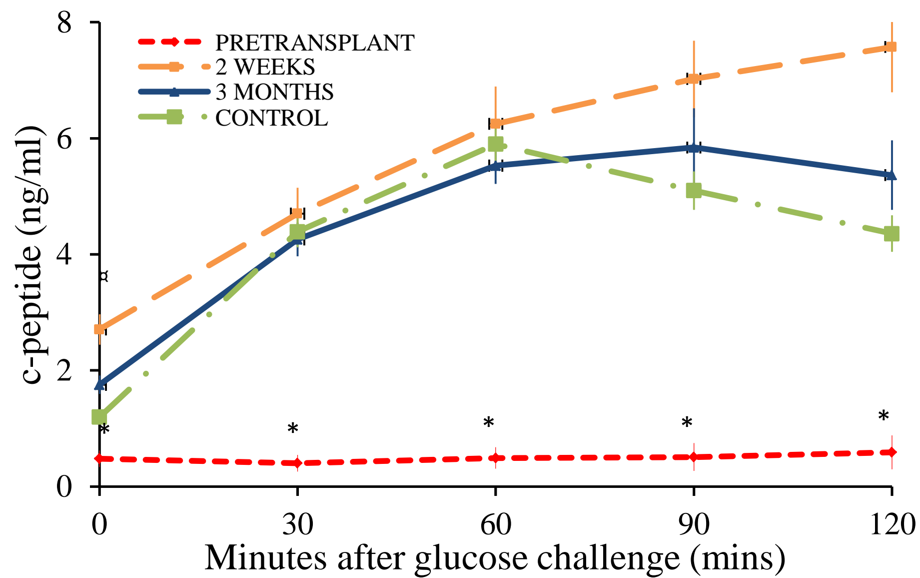
7.5.1.3 Comparison of c-peptide and insulin levels

The pre-transplant pancreas group showed no c-peptide response, and c-peptide levels were significantly different to other groups at all time-points, figure 7.4. At 2 weeks post-transplant, higher fasting c-peptide levels were present compared to the control group (2.71 ng/ml vs 1.19 ng/ml; $p<0.001$), however by 3 months post-transplant fasting levels were comparable to controls (1.75 ng/ml vs 1.19 ng/l; $p=0.385$). At 2 weeks post-transplant, c-peptide levels continued to rise throughout the 120 minute OGTT, compared to at 3 months when a secretory response similar to that of the healthy control group was seen. However, these differences in secretory pattern did not reach statistical significance (c-peptide at 120 min: 2 weeks 7.57 ng/ml vs 3 months 5.37 ng/ml vs controls 4.53 ng/ml; $p=0.084$).

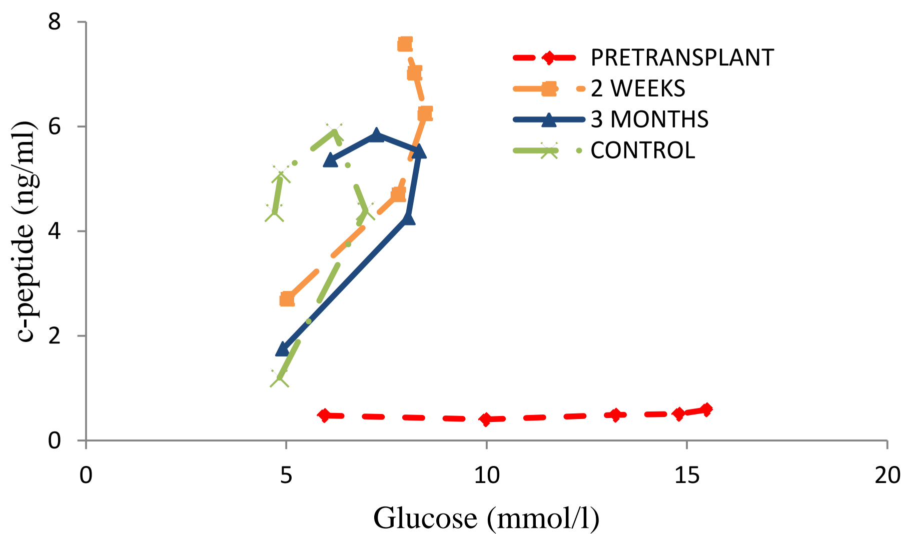
The pre-transplant pancreas group showed no evidence of insulin secretion in response to an oral glucose load, although exogenous insulin was detected, figure 7.5. The post-transplant pancreas groups showed higher fasting insulin compared to the healthy control group (2 weeks 129 pmol/l and 3 months 114 pmol/l vs. control 57 pmol/l; $p<0.001$). However, following a glucose challenge, insulin levels at 30, 60 and 90 minutes were comparable. At 120 minutes after glucose load, insulin levels were significantly higher at 2 weeks after transplantation compared to controls (440 pmol/l vs 201 pmol/l; $p=0.007$), however by 3 months post-transplant, insulin levels were comparable (359 pmol/l vs 201 pmol/l; $p=0.105$).

Figure 7.4 C-peptide profiles after OGTT

a) Over time



b) Against serum glucose

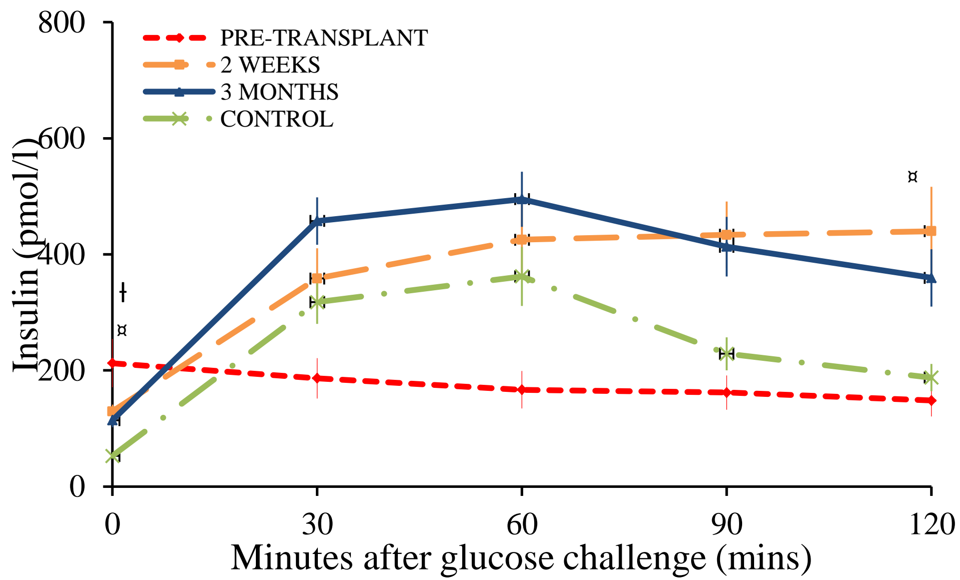


Pre-transplant (red spotted line); 2 weeks post-transplant (orange dashed line); 3 months post-transplant (blue solid line); healthy control (green dot-dash line)

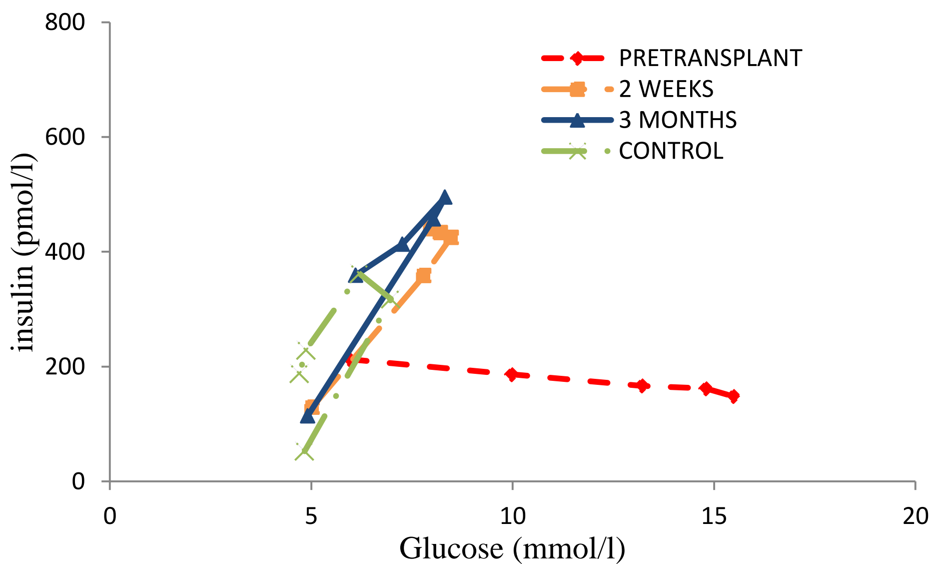
* significant difference between pre-transplant and post-transplant and control groups, $p<0.001$;
¤ significant difference between 3 months post-transplant and controls, $p<0.001$;

Figure 7.5 Insulin profiles after OGTT

a) Over time



b) Against serum glucose



Pre-transplant (red spotted line); 2 weeks post-transplant (orange dashed line); 3 months post-transplant (blue solid line); healthy control (green dot-dash line)

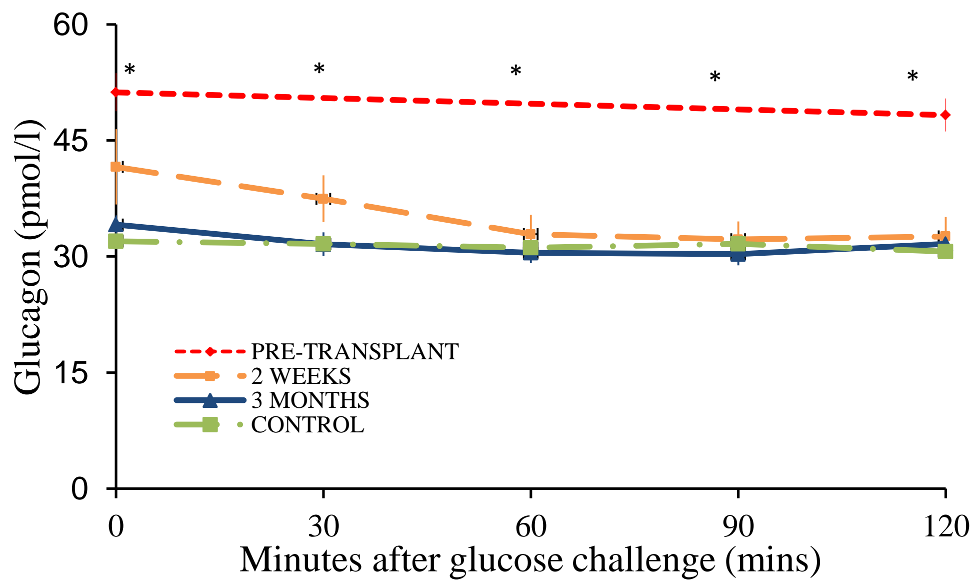
¤ significant difference between 2 weeks post-transplant and controls, $p<0.005$;
ǂ significant difference between 3 months post-transplant and controls, $p<0.001$;

7.5.1.4 Comparison of glucagon levels

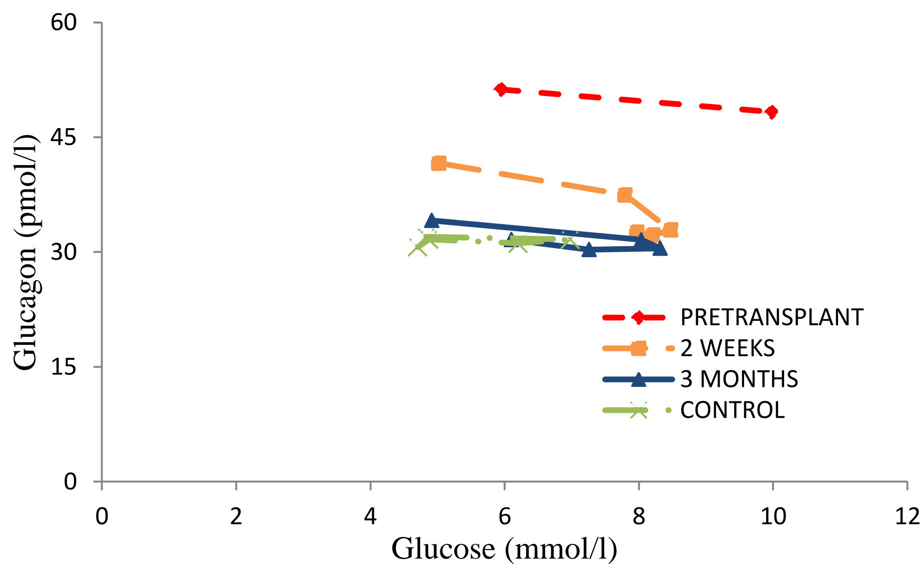
Pre-transplant participants showed hyperglucagonaemia at all time-points, with significantly higher glucagon levels compared to all other groups ($p<0.001$, figure 7.6). Fasting glucagon was numerically higher at 2 weeks post-transplant compared to 3 months post-transplant but this was not statistically significant (41.6 pmol/l vs 34.1 pmol/l; $p=1.00$). Glucagon levels at 2 weeks were otherwise comparable to those at 3 months and to healthy controls. However, when compared considering serum glucose values, glucagon values at 2 weeks post-transplant did appear elevated compared to healthy controls, and were completely normalised by 3 months post-transplant.

Figure 7.6 Glucagon profiles after OGTT

a) Over time



b) Against serum glucose



Pre-transplant (red spotted line); 2 weeks post-transplant (orange dashed line); 3 months post-transplant (blue solid line); healthy control (green dot-dash line)

* significant difference between pre-transplant and post-transplant and control groups, $p<0.001$;

7.5.2 Study D: Post-transplant assessment of the incretin effect

7.5.2.1 Demographics

The healthy control, kidney transplant control and pancreas transplant groups were comparable for demographics factors (Table 7.3). The pancreas control group contained 9 SPK and 1 PTA, all of whom received alemtuzumab induction immunosuppression and tacrolimus and mycophenolate maintenance. The kidney control group contained 5 recipients of deceased donor kidneys and 5 recipients of living donor kidney transplants. Immunosuppression regimes in this group followed clinical protocol, as determined by risk group or involvement in other research studies. 4/10 kidney recipients had alemtuzumab induction with tacrolimus and mycophenolate maintenance, 4/10 had basiliximab induction with tacrolimus and azathioprine maintenance, and 2/10 had basiliximab induction with tacrolimus and mycophenolate maintenance.

There was no change in BMI over time in either the kidney transplant (2 weeks 25.8 kg/m^2, 3 month 25.8 kg/m^2) or pancreas transplant group (2 weeks 26.4 kg/m^2, 3 month 26.8 kg/m^2). There was also no statistically significant change in serum creatinine in either the kidney transplant (2 weeks 148 µmol/l, 3 month 155 µmol/l) or pancreas transplant group (2 weeks 118 µmol/l, 3 month 111 µmol/l), and there were no recipients with delayed kidney graft function in either group included in the study.

Table 7.3 Demographics for participants of study D

	Healthy control n=10	Kidney transplant n=10	Pancreas transplant n=10	p-value
Age (years)	46.2	43.6	45.6	No sig diffs
Gender (%female)	6 (60%)	2 (20%)	3 (30%)	No sig diffs
BMI (kg/m^2)	25.9	25.8	26.4	No sig diffs
Creatinine (µmol/l)		147	118	No sig diffs

Body mass index (BMI)

7.5.2.2 Comparison isoglycaemic clamps

Extended OGTTs and IIGIs were performed on 10 participants in each group successfully, and isoglycaemia was achieved in each case, figure 7.8.

7.5.2.3 Gastrointestinal glucose disposal

In the healthy control group, 23.8 ± 9.4g of glucose was infused to match the 50g OGTT, giving a GIGD of 52.5 ± 18.8%. In the kidney group, at 2 weeks post-transplant GIGD was comparable to healthy controls (p=1.00). By three months post-transplant, the glucose infusion required had reduced and GIGD had increased to 47.4 ± 12.3%, although this remained comparable to controls (p=1.00) (Table 7.4 and Figure 7.7). However, in the pancreas group at 2 weeks, higher glucose infusions were required resulting in a GIGD of 16.0 ± 62.8%. This had improved by 3 months, by which time a GIGD of 50.4 ± 21.5% was calculated. These changes in GIGD over time did not reach statistical significance in either the kidney transplant group (p=0.181) or pancreas transplant group alone (p=0.098).

7.5.2.4 Incretin Effect

Insulin secretion profiles in response to an oral glucose load and intravenous glucose load are displayed in Figure 7.8. For each participant group, the incretin effect was calculated for each visit. The healthy control showed an incretin effect of 48.0 ± 12.4%, which was comparable to the kidney transplant group at 2 weeks (vs 38.0 ± 23.9%, p=1.00) and 3 months post-transplant (vs 64.4 ± 19.1%, p=1.00). However, at 2 weeks post-transplant, the pancreas transplant group showed a reduced incretin effect of -1.5 ± 47.6%, which was significantly lower than the healthy control group (p=0.001) and the kidney transplant group (p=0.018). By 3 months post- pancreas transplant, the incretin effect had increased

to 29.1 ± 14.2%, which was comparable to healthy controls ($p=1.00$) and kidney transplant recipients ($p=1.00$); table 7.4 and figure 7.7. The change in incretin effect from 2 weeks to 3 months was not statistically significant in either the kidney ($p=0.052$) nor pancreas group alone ($p=0.096$). There was no significant correlation in GIGD and incretin effect, except in the pancreas transplant group at 2 weeks post-transplant, where significant correlation was observed ($R^2=0.84$, $p<0.001$).

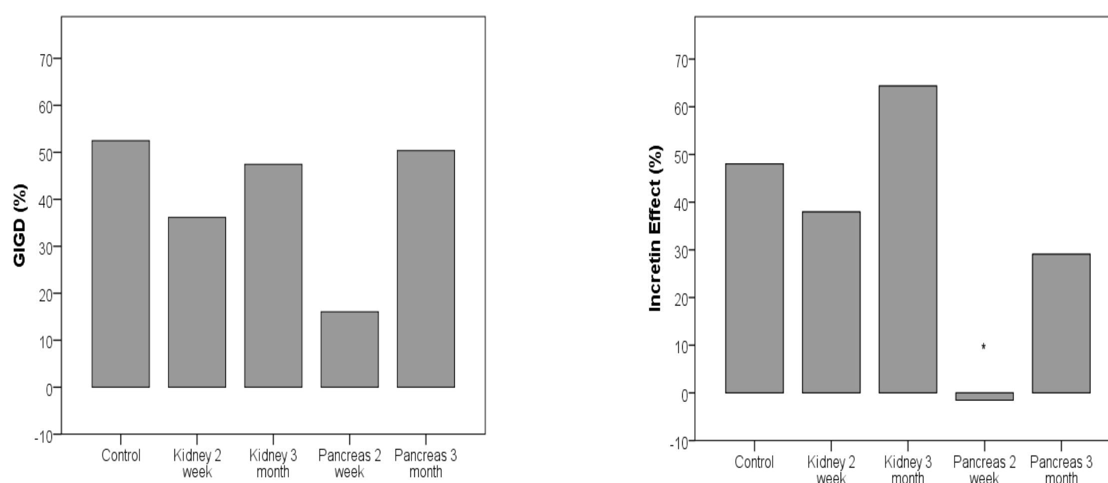
When the incretin effect is calculated using c-peptide secretion (table 7.4), the incretin effect is comparable between groups, and does not change statistically in the pancreas transplant groups over time. However, there is significant correlation between insulin incretin effect and c-peptide incretin effect in all groups over time.

Table 7.4 Gastrointestinal glucose disposal and incretin effect for study D

	Healthy control	Kidney transplant 2 weeks	Kidney transplant 3 months	Pancreas transplant 2 weeks	Pancreas transplant 3 months
Glucose infused (g)	23.8 ± 9.4	31.9 ± 9.0	26.3 ± 6.2	42.0 ± 31.4	24.8 ± 10.8
GIGD (%)	52.5 ± 18.8	36.2 ± 18.0	47.4 ± 12.3	16.0 ± 62.8	50.4 ± 21.5
IE insulin (%)	48.0 ± 12.4	38.0 ± 23.9	64.4 ± 19.1	-1.5 ± 47.6	29.1 ± 14.2
IE c-peptide (%)	28.9 ± 16.8	30.8 ± 21.9	21.2 ± 19.7	17.5 ± 25.5	20.3 ± 11.8

Gastrointestinal glucose disposal (GIGD), incretin effect (IE)

Figure 7.7 Bar chart of a) GI glucose disposal and b) Incretin Effect



* significant difference, p<0.001, compared to control and kidney transplant groups;

Figure 7.8 Hormone profiles after OGTT and IIGI (OGTT, oral glucose tolerance test: dark blue, solid line; IIGI, intravenous glucose infusion: light blue dotted line)

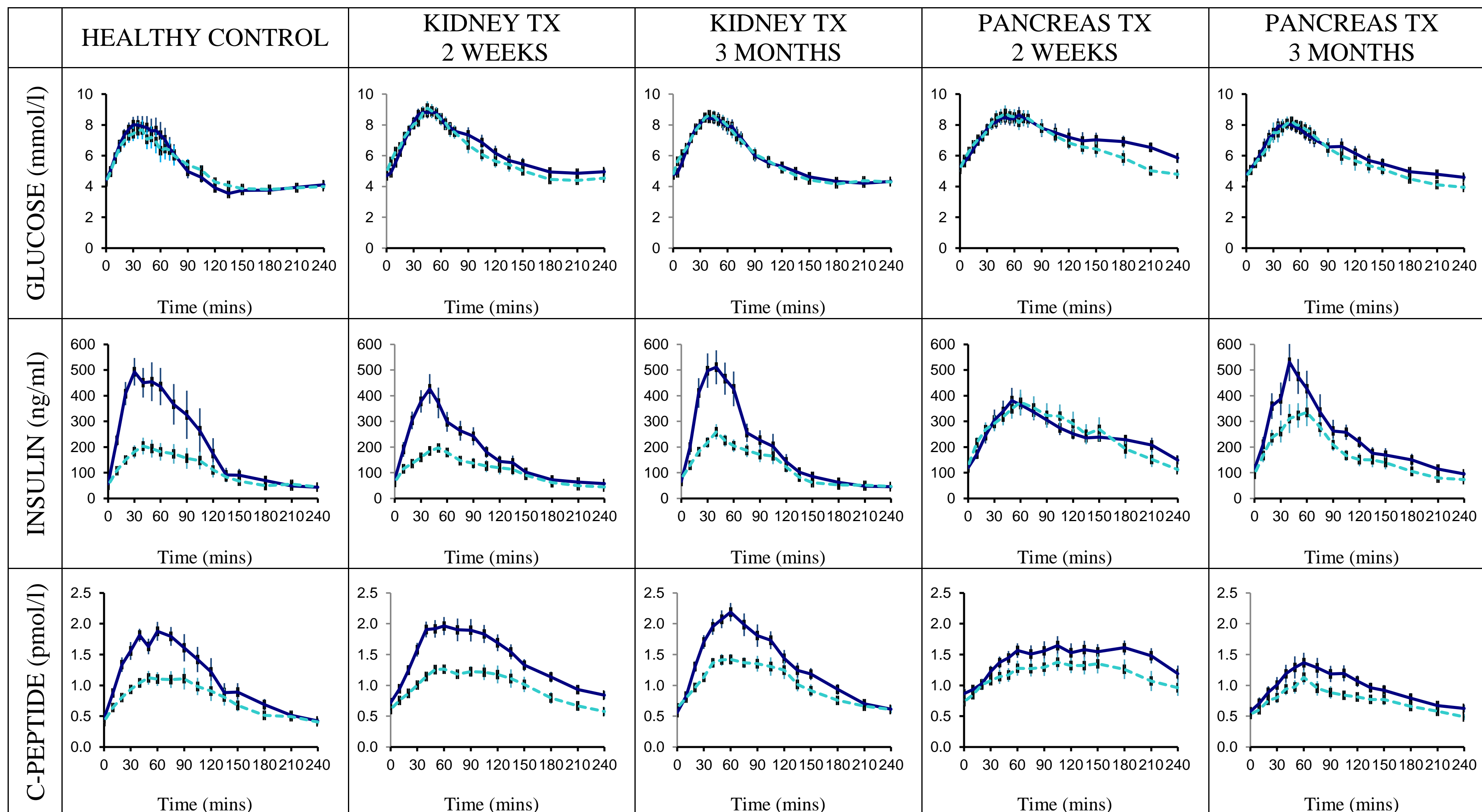
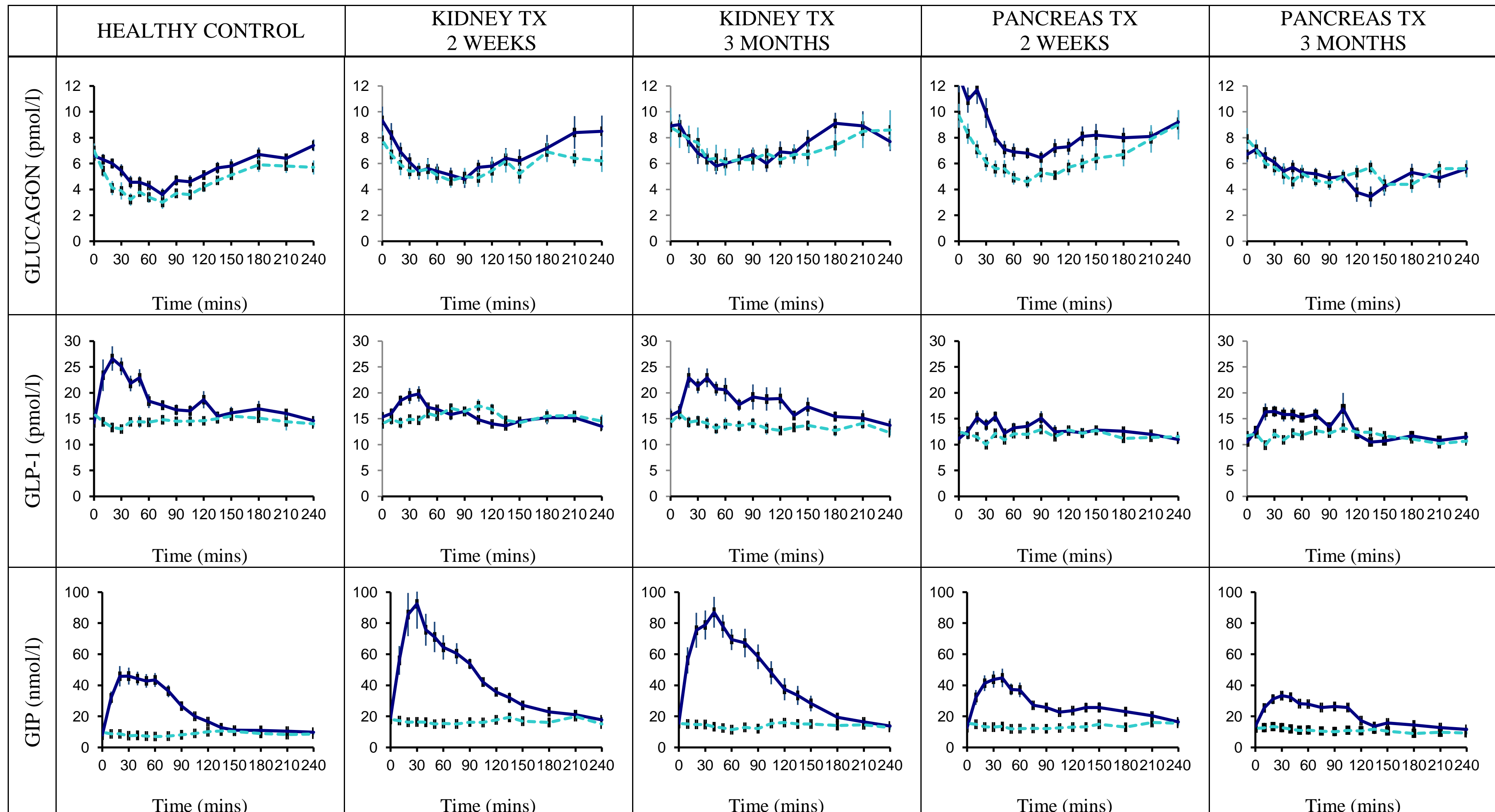


Figure 7.9 Hormone profiles after OGTT and IIGI (OGTT, oral glucose tolerance test: dark blue, solid line; IIGI, intravenous glucose infusion: light blue dotted line)



7.5.2.5 Comparison of incretin hormone levels

Glucagon

All groups showed suppression of glucagon from baseline in response to oral and intravenous glucose. Suppression was greater in the pancreas transplant group at 2 weeks compared to healthy controls due to higher fasting glucagon in the pancreas group ($p=0.019$), and comparable in all other groups, figure 7.9. There was no significant difference in glucagon response to oral or intravenous glucose, except in the pancreas group where there was higher fasting glucagon and greater suppression in response to an oral load compared to an intravenous load at 2 weeks post-transplant ($p=0.022$). Glucagon responses in study D differed to those in study C due to the greater sensitivity of the assay used in study D.

GLP-1

GLP-1 responses to oral glucose appeared blunted at 2 weeks after both kidney and pancreas transplant, with some improvement by 3 months transplant, however these differences did not reach statistical significance when groups were compared (figure 7.9).

GIP

All groups showed a GIP secretory response to oral glucose and no response to intravenous load. The kidney transplant group appear to show an exaggerated GIP response to oral glucose, however, this is statistically comparable to controls.

Table 7.5 Total incretin hormone secretion to OGTT and IIGI

	Healthy control	Kidney transplant 2 weeks	Kidney transplant 3 months	Pancreas transplant 2 weeks	Pancreas transplant 3 months
AUC GLP-1 (pmol/l x 4 h)					
Oral	834.8 ± 670.6	412.3 ± 376.6	651.0 ± 887.4	395.3 ± 461.4	504.0 ± 571.8
Iv	-435.5 ± 545.6	298.0 ± 801.8	-260.8 ± 614.2	-120.8 ± 635.1	-225. ± 381.3
Difference (oral- iv)	1270.3 ± 835.5	114.5 ± 831.8^	911.8 ± 592.0	516.0 ± 483.0	729.0 ± 437.5
AUC GIP (nmol/l x 4 h)					
Oral	3.40 ± 1.39	6.02 ± 2.83≠	6.49 ± 4.38≠	3.38 ± 1.78	1.60 ± 2.05
Iv	-0.33 ± 0.45	-0.23 ± 1.28	-0.20 ± 0.81	-0.36 ± 1.99	-0.35 ± 0.49
Difference (oral- iv)	3.72 ± 1.30	6.24 ± 3.08≠	6.69 ± 4.48≠	3.74 ± 2.75	-1.96 ± 1.78
AUC glucagon (pmol/l x 4 h)					
Oral	-287.3 ± 273.4	-585.3 ± 332.9	-363.8 ± 301.8	-1300.3 ± 1032.2*	-456.3 ± 217.0
Iv	-637.8 ± 339.6	-478.0 ± 239.0	-403.3 ± 461.15	-716.8 ± 404.7	-633.3 ± 364.0
Difference (oral- iv)	350.5 ± 347.5	-107.3 ± 470.5	38.5 ± 305.1	-583.5 ± 1109*	117.0 ± 493.9

Oral glucose tolerance test (OGTT), intravenous glucose infusion (IIGI), area under the curve (AUC)
Data expressed as mean ± standard deviation

* significant difference to controls, $p<0.05$
^ significant difference to controls, $p<0.01$
≠ significant difference to pancreas transplant at 3 months, $p<0.01$

7.6 Discussion

These studies, which are the first to investigate factors contributing to early glucose homeostasis after pancreas transplantation, show that several changes are occurring in the early post-transplant period.

First, both studies show that glucose homeostasis is near normalised post-transplant when compared to pre-transplant, and that although impaired glucose tolerance is common at 2 weeks post-transplant in study C, there is improvement by 3 months. This lends support to the concept of early impaired glucose tolerance as a marker of delayed graft function, however, it is also important to note that in a large longitudinal study of children at risk of T1D, it was found that metabolic deterioration occurred over time with transient improvements and declines progressing in a ratcheting fashion (Sosenko et al. 2012b). Therefore, to fully understand the patterns of metabolic function after pancreas transplantation, further serial assessments will be necessary in larger cohort studies.

Study C clearly demonstrates normalisation of glucagon levels by 3 months after pancreas transplantation, with some improvement in glucagon levels as early as 2 weeks post-transplant, so confirming that hyperglucagonaemia does not play a significant role in driving hyperglycaemia or hyperinsulinaemia. In study D, higher fasting glucagon was observed in the oral glucose test compared to the intravenous infusion test at 2 weeks post-transplant, and this difference may be due to rapid normalisation occurring during the time period between tests, or may relate to inadequate paracrine regulation due early impaired insulin secretion. The apparent differences between the glucagon responses observed in study C and study D are likely to relate to the superiority of the assay used in study D, and

this highlights a weakness of study C. Although several *in vivo* studies have examined the effect of insulin-induced hypoglycaemia on glucagon regulation, the only study examining glucagon in the context of hyperglycaemia was from Christiansen et al, who showed fasting hyperglucagonaemia in 9 segmental pancreas transplant recipients and 6 non-diabetic kidney transplant recipients when compared to controls, with comparable patterns of suppression in response to a 75g OGTT (Christiansen et al. 1997). However, in that study, transplanted groups shared an immunosuppression protocol comprising prednisolone, cyclosporin and azathioprine, which could account for the significantly lower insulin sensitivity observed compared to controls. The only evidence for the effect of whole pancreas transplantation comes from animal studies. Minossi et al demonstrated persistent hyperglucagonaemia 96 hours after systemically drained pancreas transplantation in 17 rats, and concluded that although there was normoglycaemia, dysregulation of glucagon occurred due to denervation and systemic drainage, however recognised that the effect of alloxan used to induce diabetes could not be quantified (Minossi et al. 1998). Kissler et al, also experimenting in rats, found that hyperglucagonaemia was associated with systemic, but not portally-drained transplants (Kissler et al. 2001). However, unlike insulin, glucagon is principally extracted renally and not through the liver, making associations between these surgical techniques and differences in glucagon concentrations most likely due to corresponding changes in insulin concentrations. The findings of this, first in man, whole pancreas transplant study do not reflect what is seen in rodents, highlighting the differences between man and rodents, and the need for human studies.

Study C appears to show that systemic drainage and denervation of the transplant pancreas do not prevent early normalisation of glucagon homeostasis. It has been observed that in

longstanding T1D alpha cell expansion may occur (Li et al. 2000), and it had been thought that hyperglucagonaemia resulted in part from the absence of intra-islet feedback by insulin. It is therefore intriguing that, in our study, glucagon concentrations are normalised in the absence of paracrine regulation within the native pancreas. This may allude to the greater importance of normoglycaemia and/or circulating insulin in glucagon control over paracrine regulation.

Higher fasting insulin and c-peptide levels were observed in pancreas transplant recipients compared to controls, and this may relate to systemic venous drainage of the pancreas graft. Evidence of hyperinsulinaemia has been previously observed following pancreas transplantation, and reduced or delayed insulin secretion has been associated with impaired glucose tolerance (Christiansen et al. 1997). In the presence of normal glucagon levels, it appears that dysglycaemia in our cohort is due to early insulin secretory defects, which recover to normal patterns by 3 months, resulting in fasted and stimulated hyperinsulinaemia. Reduced insulin secretion in response to an oral glucose load at 2 weeks may secondary to beta-cell defects, which may represent reversible ischaemic injury, insensitivity to incretin hormones due to long standing hyperglycaemia (Creutzfeldt 2001) or may be secondary to reduced GLP-1 secretion (discussed below).

In study C, c-peptide concentrations showed considerable variation at each time point, and depicted slower responses to those seen when examining the insulin concentrations in the same participants. In study D, incretin effects calculated using c-peptide levels correlated statistically to incretin effects calculated using insulin levels, however the patterns of change observed were visually and numerically different. It is not clear why patterns should differ when considering c-peptide in place of insulin, however, it is likely that the

utility of c-peptide is limited by its long half-life (30-40mins) and renal excretion, making interpretation of dynamic changes in a group with a variable degree of renal dysfunction challenging or impossible.

C-peptide is secreted in equimolar concentrations to insulin and is often used to calculate pre-portal insulin secretion (Polonsky et al. 1986), since direct measurement is not feasible *in vivo*. Mathematical models have been developed and validated based on the complex dynamics between c-peptide and insulin secretion at various glucose concentrations (Hovorka et al. 1998), and while it can be assumed that c-peptide and insulin continue to be equimolar in the case of pancreas transplantation, the use of published models to derive insulin secretion rates may not be viable. These deconvolution models, all developed for use in portally-drained situations, do not consider the abnormal physiological state and altered dynamics involved in transplantation, including differences in the hepatic effects of insulin and glucagon or differences in the incretin effect. The unique dynamics arising from the loss of first-pass metabolism will need to be further studied, and a bespoke model developed more suitable for estimating beta-cell function in this context.

The principle finding of study D is that there is absence of the incretin effect 2 weeks after pancreas transplantation, with restoration of the effect at 3 months post-transplant. Nauck et al conducted a similar investigation in 9 systemically drained SPK and 7 non diabetic kidney transplant recipients at 10 ± 9 months and 14 ± 11 months post-transplant respectively, and observed a preserved incretin effect in both groups (Nauck et al. 1993b). Although this may appear to conflict with our study, the findings may be explained by the later time-point at which the participants were investigated, since our results show that the incretin effect had re-emerged by 3-months post-transplant. Previous to this, Clark et al

had examined five paratopically placed portally-drained SPK and had also found a normal incretin effect and normal GIP hormone secretion (Clark et al. 1989), however this again was not early post-transplant. There have been no human *in vivo* studies to examine the incretin effect in the early post-transplant period. However, Jakob et al did find a reduction in insulin secretion to oral but not intravenous glucose loads in allotransplanted dogs 10 days post-transplant compared to controls (Jakob et al. 1970). Early post-surgical studies have been performed in the context of bariatric surgery, however these have shown elevation in incretin hormone secretion early post-operatively, thought principally due to surgical alteration in gastric and enteric anatomy (Sala et al. 2014).

The reason for an absent incretin effect early post-transplant is unknown. One hypothesis is that the changes over the first 3 months correspond to re-establishment of the neural network necessary to mediate the incretin effect. Absence of neuronal connections after pancreas transplantation has been demonstrated by Secchi et al. They investigated cephalic phase insulin secretion, thought to be mediated via the enteroinsular axis, and found a loss of cephalic phase insulin secretion in SPK recipients at 2-3 years post-transplant (Secchi et al. 1995), likely due to denervation. Further, Ginier et al, investigated a case 9 years after auto-transplantation of the pancreas to the left iliac fossa, and found absence of pancreatic polypeptide secretion and loss of the incretin effect (Ginier et al. 1988). Although these studies support our hypothesis, reinstatement of the incretin effect in these cases had not occurred over a prolonged time period unlike in our study. The reasons for this are unclear but may be related to differences in the anatomical placement of the pancreas graft. Certainly, there is good evidence that reinnervation of transplanted tissue does occur and has been demonstrated clinically (Pomahac et al. 2014) and histologically (Biedermann et al. 2014) in skin grafts, and through measures of heart

rate variability in cardiac transplantation (Imamura et al. 2014). However, the timescale for nerve growth appears to be variable. In the context of pancreas transplantation, it has been seen that repair of diabetic neuropathy occurs post-transplant (Tavakoli et al. 2013), however, direct innervation of the pancreas graft may be difficult to quantify.

Nevertheless, there is also data to suggest that the incretin effect can be mediated in the absence of innervation. There have been several animal studies showing the presence of a normal incretin effect post-transplant (Kissler et al. 1999) or after surgical denervation (Kohler et al. 1992), that is comparable to incretin effects demonstrated in control and sham laparotomy experiments. Alterations in the incretin effect have been observed in other patient groups with intact innervation, and may be linked to changes in incretin hormone secretion and beta-cell secretory capacity. The incretin effect is diminished in T2D, and in those with impaired glucose tolerance (Jensen et al. 2012). Interestingly, Henchoz et al also noted a blunted incretin effect in 9 liver and 8 heart transplant recipients 1-3 years post-operatively (Henchoz et al. 2003), suggesting factors common to these groups may prevent full recovery of the incretin effect in pancreas transplant recipients well beyond 3 months post-transplant, and this is most likely related to immunosuppressive medications.

It was surprising to note there was diminished GLP-1 secretion and preserved GIP secretion in this study, and that this was present in both transplant groups, although the differences to the control group did not reach statistical significance. Similarly to our study, Nauck et al observed in their investigation of transplant recipients that there was lower GLP-1 and GIP secretion after pancreas transplantation compared to kidney transplantation (Nauck et al. 1993b). However, this was of borderline statistical

significance and was not compared to healthy controls. Henchoz et al observed GIP and GLP-1 secretion to be statistically comparable to controls in their study, and suggested that immunosuppression may inhibit the action of incretin hormones. It is thought that the incretin effect may in part be mediated by receptors in the pancreas (Orskov and Poulsen 1991), which may be down-regulated as a result of chronic hyperglycaemia (Xu et al. 2007), and upregulated with restoration of normoglycaemia (Drucker 2013). The effect of immunosuppression on these receptors is unknown. GLP-1 infusions have been shown to mediate improvements in insulin secretion even in the presence of a severely diminished incretin effect (Nauck et al. 1993a) and Rickels et al showed that SPK recipients were able to show increased glucose-dependent insulin secretion with GLP-1 infusion (Rickels et al. 2009), implying that any incretin insensitivity can be overcome at supraphysiological levels.

GIP is thought to be suppressed through negative feedback and in the presence of hyperinsulinaemia (Morgan et al. 1988), and increased in insulin resistance (Irwin et al. 2011). This may contribute to the numerical but non-significant difference in GIP levels between the pancreas and kidney transplant groups. GLP-1 is thought to be reduced in slow gastric or gallbladder emptying, insulin resistance and long duration diabetes (Holst et al. 2011), although levels have been seen to be normal in T1D (Greenbaum et al. 2002), suggesting insulin resistance may have a role in the hormone patterns seen. In experiments inducing insulin resistance in healthy individuals and those at risk of T2D, a reduction in the incretin effect was observed, however this was due to an increase in the response to intravenous without a corresponding response to oral glucose (Hansen et al. 2010; Jensen et al. 2012). This pattern was not observed in the kidney transplant group, and it is not clear if the increased insulin response to intravenous glucose in the pancreas

transplant group represents the effects of insulin resistance or is the consequence of systemic venous drainage. In both Study C and Study D, there was fasting hyperglucagonaemia at 2 weeks post-pancreas transplant, particularly in response to the OGTT. At this time point, high GIP concentrations relative to GLP-1 were observed, and may contribute, alongside poor early neuronal connections, to impairment in early regulation. Certainly the factors mediating the incretin effect are complex, and a normal incretin effect appears to require adequate GLP-1 secretion and an intact neuronal network, both of which are impaired after pancreas transplant.

These studies do suffer limitations. Both studies are comprised of a small number of participants, which limits interpretation of patterns observed in variables other than those for which they were powered. Therefore, care must be taken not to over-interpret these preliminary data and, although recruitment to similar studies may pose challenges, larger studies will be needed before robust associations and conclusions can be drawn. Study C clearly shows glucagon concentrations after pancreas transplantation to equal those in healthy controls. Nevertheless, an additional control group of kidney transplant recipients would have enabled assessment of the impact on glucagon of the correction of renal failure. In our centre, diabetic patients with end-stage nephropathy are offered SPK transplantation, and therefore identification of a matched group would not have been feasible. A collaboration with another centre without a pancreas transplant programme was established, however, delays in recruitment prevented inclusion in this thesis.

In study D, the nature of the methodology means it is not possible to rule out an order effect when comparing OGTT and IIGI responses. However, since the tests were repeated at a second time-point in the transplant groups, it is unlikely that this has had an impact on

the findings. Second, it would have been interesting to investigate changes in exocrine function in parallel to changes in endocrine function, as would have been possible with bladder-drainage of ductal secretions. However, enteric ductal drainage was employed as per clinical protocol. Also in study D, there was heterogeneity in the matching of participant groups for immunosuppressive regimens. Unfortunately, this was unavoidable since it would not be possible or ethical to interfere with clinical protocol. It would be interesting to examine the effect of immunosuppressive medications in a separate appropriately powered and matched study.

Nevertheless, these studies have clearly shown that after pancreas transplantation, glucose homeostasis is quickly normalised, and this is also associated with normalisation of glucagon suppression in response to an oral glucose load. These studies demonstrate for the first time that the incretin effect is diminished early post pancreas transplant and improved by 3 months. Further, this is associated with reduced GLP-1 responses, which also improve by 3 months, while GIP appears unaffected. The difference in the incretin effect by 3 months post-transplant may represent reestablishment of neuronal connections in the donor pancreas, and likely also contributed to by reduced GLP-1 secretion. Interestingly, reduction in GLP-1 occurred in both transplant groups, thus may result from the effects of surgery or immunosuppression and one could speculate that this may have a role in new-onset diabetes after transplantation.

Further, these studies highlight pancreas transplantation as a unique model that can be used to elucidate the mechanisms of glucose homeostasis with direct relevance to physiology in health and in diabetes. While further study into the impact of immunosuppression, with direct measurement of hepatic glucose metabolism and

measures of insulin insensitivity will be informative, these studies have made significant progress in understanding the metabolic changes occurring in the post-transplant period. Furthermore, this work provides a basis for onward investigation of the factors leading to metabolic dysfunction post-transplant and gives insight into potential targets for therapeutic interventions aimed at maintaining glucose homeostasis and avoiding return to insulin-dependence.

7.7 Conclusion

In conclusion, this series of investigations has shown that glucagon is normalised after pancreas transplantation, despite the presence of a native pancreas and systemic drainage. Insulin secretory defects that are evident early post-transplant may be contributed to by an absence of the incretin effect, which becomes established by 3 months post-transplant. The emergence of the incretin effect may represent reinnervation of the pancreas graft, and may be contributed to by the diminished GLP-1 response observed after both pancreas and kidney transplantation. The mechanisms underlying this diminished response are not known and may relate to increased insulin resistance and immunosuppression or surgery. GIP secretion, however, has been seen to be normal and may contribute to maintaining normoglycaemia in the post-operative period. Further investigation will be necessary to fully understand the significance of these results.

Chapter 8

Discussion

8 DISCUSSION

8.1 Summary of findings

Pancreas transplantation is a successful treatment option for selected people with diabetes and, as well as offering insulin-independence, is likely to confer considerable benefit in halting the progression of diabetic complications. However, pancreas graft failures still occur too commonly and are associated with risk of mortality, with graft survival rates after IP transplantation particularly poor. If the potential benefits of transplantation are to be fully realised, then improvements in graft outcomes are needed, and this requires the development of effective post-transplant monitoring strategies and early identification of graft dysfunction.

The first aim of this thesis was to investigate the utility of donor factors in predicting graft survival in a UK cohort. In chapter 3, I have demonstrated that donor demographic variables, which currently form much of the basis of risk stratification in pancreas transplantation, are not a particularly sensitive means of discriminating organs at risk of graft failure. Although the Pancreas Donor Risk Index does offer some discriminatory ability in predicting one-year graft survival after SPK, the difference in overall survival between the highest and lowest risk group was too small to be able to recommend any change in clinical practice based on a PDRI value, as acceptable graft outcomes can be expected through the range. Further, the PDRI had no role in predicting graft survival in IP transplantation, which is exactly where a predictive tool would have had the greatest impact and value. The UK cohort is too small to generate and validate a new PDRI, and the development of a risk model to be used in IP transplantation would require the use of international databases. It is highly plausible, however, that the impact of differences in

regional practices and the lack of depth of registry data will prove to be a major limitation to developing a model that can be robustly applied in a given population to accurately predict graft outcome. Given the existing narrow practice in pancreas donor selection, it is unlikely that further retrospective analyses of demographic risk indices will greatly affect organ acceptance practice. Also, while donor criteria is being expanded in transplantation of other solid organs, to focus on further limiting the donor pool in pancreas transplantation seems counter-intuitive. Therefore, it is arguable that research into preserving and improving the quality and function of donor organs is likely to be more worthwhile. This may involve research into less damaging organ preservation and storage techniques, and intervention studies aimed at optimising metabolic function in the peri-transplant period.

Monitoring of HLA antibodies and diabetes autoantibodies is routinely performed after pancreas transplantation at the Oxford Transplant Centre. I aimed to investigate if these markers were associated with pancreas graft failure in chapter 4 and 5 respectively. I demonstrated that development of de novo donor-specific antibodies conferred significant risk of graft failure in SPK and IP transplantation, and that pre-transplant autoantibodies were also associated with graft failure in the IP group. It is evident that immunological processes are paramount in determining graft outcomes after pancreas transplantation. In the transplant recipient, the immunosuppressive effects of renal failure, inflammatory processes in the donor, consequences of ischaemia-reperfusion and the stress of surgery all interact with the effects of immunosuppressive medication. However, in the pancreas transplant recipient, additional consideration is needed of the proinflammatory state of diabetes and the role of autoimmunity. Antibodies, against either donor- or self-antigens, appear to denote poorer graft survival, particularly in IP transplantation- a group who

already have the worst outcomes. Whether these antibodies directly lead to graft failure, due to rejection or recurrence of diabetes respectively, or if the emergence of antibodies is secondary to underlying immune processes (perhaps subclinical rejection episodes) is not known. Examination of the histological and cellular characteristics of pancreas biopsy and pancreatectomy specimens may be useful in understanding the processes that lead to graft loss: whether rejection, recurrence of diabetes, or beta cell exhaustion and depletion. Sub-group analysis by cause of failure may strengthen associations and further inform potential intervention strategies.

From the current studies, causality cannot be attributed between antibody development and pancreas graft failure. Thus, it cannot be assumed that direct treatment or removal of antibodies will be useful in improving graft outcomes. Trials in renal transplantation aimed at ameliorating the risk associated with DSA have not yet demonstrated benefit. Immunotherapy studies in young people at high risk of developing T1D have also failed to demonstrate significant preservation of beta-cell function. Currently, proposed interventions are expensive and invasive with significant risk profiles, and as such may not be justifiable. The lack of efficacy demonstrated could be because the antibodies themselves may not be pathogenic. As opposed to inducing damage, emerging allo- and auto-antibodies may represent markers of the underlying immune environment, which may be inherently different in the SPK and IP recipient. Certainly, the IP recipient has phenotypically different diabetes, at an earlier stage and without the presence of diabetic nephropathy and subsequent effects of renal failure (which may be associated with a genetic predisposition).

It is possible that strategies aimed at preventing antigen presentation will be efficacious in preventing de novo antibody formation, and may result in improved graft outcomes. Studies to identify novel immunological signatures present in these high risk individuals pre- and post-transplant, may provide information regarding underlying mechanisms and processes, and could inform the nature and timing of immunosuppressive interventions. The work presented in this thesis does not aim to further knowledge on appropriate interventions, or the likelihood of preventing graft failure. However, it does highlight the very important role of immunological processes (both allo- and auto-immune) in pancreas graft failure, and identifies high risk groups in need of close monitoring and further investigation.

In chapter 6, I aimed to investigate the role of early function measures, namely oral glucose tolerance testing and continuous glucose monitoring, in predicting graft survival in two separate studies. I have demonstrated in a retrospective analysis that early metabolic dysfunction is important in predicting graft failure, and that this can be measured with an OGTT. Further, in a prospective study I confirmed that abnormal glucose tolerance could be detected more easily with CGM. These easily measured, well-established and accepted tests can be translated quickly into routine clinical care, and identify a high-risk group with evidence of functional disturbance in need of further investigation and close monitoring. It follows, that since there is by definition already evidence of graft dysfunction, recipients with abnormal glucose tolerance are ideal candidates for interventional trials aimed at improving graft function.

The underlying cause for early impaired glucose tolerance is not known. Early dysfunction may represent an inadequate absolute beta-cell mass, as determined in the

donor, which will continue to decline over time. Alternatively, it may represent temporary or permanent injury taking place during retrieval and preservation. It is likely that early graft dysfunction is predominantly related to ischaemia-reperfusion injury and that this may trigger subsequent alloimmune processes. It is possible, though not proven, that this progression is responsible for the poor prognosis of pancreas grafts that do not function well from the outset. How early abnormal OGTT relates to functional assessment at 1 year and beyond in functioning grafts remains to be established. Clearly, identifying functional markers associated with graft survival that can be measured sequentially or at later time-points will be important in pancreas transplant monitoring, and may form surrogate end-points in clinical trials. This needs to be investigated further in large longitudinal studies involving oral and intravenous tolerance tests, CGM and other functional measures, so that changes over time can be correlated to graft outcomes.

In terms of clinical monitoring, in kidney transplantation a rise in serum creatinine prompts repeat assessment and escalation to ultrasound imaging and biopsy. There has been no comparable biochemical marker in pancreas transplants that can trigger further investigation. Also, although practiced in some units, histological assessment of donor or transplanted pancreases is far from routine practice for reasons of technical difficulty and (mostly perceived) risk. Therefore, the foundation of pancreas transplant monitoring should ideally be based on a direct measure of graft function. This thesis has demonstrated that functional monitoring of pancreas transplant recipients can be simply, feasibly and usefully implemented using OGTT or CGM.

The final aim of this thesis was to investigate the role of glucagon and incretin hormones in glucose homeostasis early after pancreas transplantation, and this was investigated in

two separate prospective studies, detailed in chapter 7. I have demonstrated that glucose homeostasis is normalised early after pancreas transplantation, and that this is also associated with complete normalisation of glucagon concentrations. Further, it was observed that insulin secretion is impaired early after pancreas transplantation, and this was associated with absence of the incretin effect, which was reinstated by 3 months post-transplant. Interestingly, abnormalities in GLP-1 secretion were also observed early after both pancreas and kidney transplantation, with improvement at 3 months.

These studies highlight the unique physiology involved in glucose homeostasis after whole organ pancreas transplantation. In general terms, the work in this thesis highlights pancreas transplantation as an important research model, which provides a unique opportunity to investigate the mechanisms underlying the regulation of glucose. Complete normalisation of pre-transplant hyperglucagonaemia, despite the presence of a native pancreas with functioning alpha-cells, may refute previous thinking regarding the need for intra-islet regulation of glucagon by insulin. The absent incretin effect early after pancreas transplant may allude to the need for pancreatic innervation for the effect to be mediated, confirming an enteroinsular mechanism of action. These hypotheses related to the physiology of glucose homeostasis will need testing in further studies.

Of particular importance for transplantation, these studies show that hyperglucagonaemia is not contributing to hyperglycaemia after pancreas transplant, and that early dysfunction relates to insulin secretory defects due to a diminished incretin effect. The significant reduction in GLP-1 secretion after both pancreas and kidney transplantation also suggests unknown factors may be inhibiting secretion of this important hormone, potentially on a cellular level, and related to immunosuppression. This highlights an important potential

target for therapeutic intervention aimed at improving pancreas graft function. Furthermore, it is possible to speculate that abnormalities in GLP-1 secretion may also be implicated in the pathogenesis of new-onset diabetes after transplantation (NODAT), which occurs in 15-30% of non-diabetic kidney transplant recipients and most frequently presents 3-6 months post-transplant. The aetiology and pathogenesis of NODAT is poorly understood. Associations with raised BMI, calcineurin inhibitors and corticosteroid therapy have been observed, and defects in insulin sensitivity and insulin secretion have been implicated (Chakkera et al. 2013), however, the role of incretin hormones has not been previously investigated. This study suggests that reduced GLP-1 responses early post-transplant maybe contributing to the development of NODAT and are worthy of further research aimed at identifying underlying mechanisms and novel therapeutic interventions.

It is evident that many questions remain unanswered beyond the scope of this thesis, and it is clear that further investigation is needed to identify and trial appropriate interventions before improvements in graft survival can be realised. There will be value in large longitudinal studies examining changes in immunological and metabolic measures over time, with correlation to graft and patient-reported outcomes. These are likely to inform the design of interventional studies in the high risk groups identified herein

8.3 Conclusion

This thesis has made a substantial contribution to knowledge presented in the existing literature, enabling identification of patients at high risk of graft failure and discovering metabolic regulatory defects contributing to early graft dysfunction.

As a result several conclusions can be made:

1. *Predictive value of donor factors:* pre-transplant demographic risk factors have limited value in identifying organs at high risk of graft failure in pancreas transplantation, and changes in clinical practice cannot be recommended based on the Pancreas Donor Risk Index.

2. *Recommendations for monitoring:* there is clear benefit in routine pre- and post-transplant HLA antibody assessment and autoantibody monitoring to identify groups at high risk of graft failure. An early OGTT and/or CGM could also be performed in patients prior to discharge, with an abnormal result triggering further investigation and close monitoring with a low threshold for intervention.

3. *Informing the design of intervention trials:* the incretin effect is diminished early post-transplant and GLP-1 secretion is reduced, highlighting a potential target for therapeutic intervention that may be important both in pancreas and kidney transplantation.

9 APPENDIX 1: References

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10 APPENDIX 2: Publications resulting from work presented in this thesis

Mittal S, Lee FJ, Bradbury L, Collet D, Reddy S, Sinha S, Sharples E, Ploeg RJ, Friend PJ, Vaidya A. Validation of the Pancreas Donor Risk Index for use in a UK population. Transplant International. Published online March 2015

Mittal S, Page S, Friend PJ, Sharples E, Fuggle S. De novo donor-specific HLA antibodies: biomarkers of pancreas transplant failure. Am J Transplant. 2014 Jul;14(7):1664-71

Mittal S, Nagendran M, Franklin R, Sharples EJ, Friend PJ*, Gough SCL*. Post-operative Impaired Glucose Tolerance is an Early Predictor of Pancreas Graft Failure. Diabetologia. 2014 Oct;57(10):2076-80

Mittal S, Franklin R, Sharples E, Friend P, Gough S. The use of early post-operative continuous glucose monitoring in pancreas transplant recipients. Transplant International.. Published online February 2015

Mittal S, Gough S. Pancreas transplantation: a treatment option for people with diabetes. Diabetic Medicine 2014 Apr. 31(5):512-521

Mittal S, Johnson P, Friend PJF. Pancreas Transplantation: Solid Organ and Islet. Cold Spring Harbour Perspectives in Medicine 2014. 4(4):a015610

11 APPENDIX 3: Prizes and commendations resulting from work presented in this thesis

IPITA Young Investigator Award
- Outcomes after Pancreas Transplant: a single centre experience"
Oral presentation at International Pancreas and Islet Transplantation Association Conference 2013

BTS Poster Prize
- "A United Kingdom Pancreas Donor Risk Index for predicting Outcome after Deceased Donor Pancreas Transplantation"
Poster presentation at British Transplant Society Conference 2013

Best Abstract Challenge Award
- "Donor-specific HLA Antibodies predict graft failure "
Oral presentation at European Society of Organ Transplantation Conference 2013

NIHR Research Training Poster Prize
- "Metabolic profiles after pancreas transplantation"
Poster presentation at NIHR Research Training Camp 2013

Elsevier Junior Researcher Award (finalist)
- "Impaired glucose tolerance predicts early failure in pancreas transplantation"
Oral presentation at OCDEM, University of Oxford 2013

Nick Hales Young Investigator Award (1st runner up)
- "The role of the incretin effect after pancreas transplantation"
Oral presentation in prize session at Diabetes UK Conference 2015

12 APPENDIX 4: Construction of a UK Pancreas Donor Risk Index

Recipient Model

A recipient model was derived and found to include organ transplanted, transplant centre and cold ischaemia time ($p<0.001$).

Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)
Organ			12.661	2	.002	
Organ(1)	.756	.268	7.970	1	.005	2.129
Organ(2)	.861	.317	7.357	1	.007	2.365
Centre			12.953	8	.113	
Centre(1)	-1.510	.626	5.811	1	.016	.221
Centre(2)	-2.360	.769	9.420	1	.002	.094
Centre(3)	-1.700	.706	5.799	1	.016	.183
Centre(4)	-1.437	.678	4.485	1	.034	.238
Centre(5)	-2.065	.692	8.911	1	.003	.127
Centre(6)	-1.638	.768	4.550	1	.033	.194
Centre(7)	-1.744	.612	8.111	1	.004	.175
Centre(8)	-1.311	.681	3.712	1	.054	.270
CIT	.000	.001	.394	1	.530	1.000

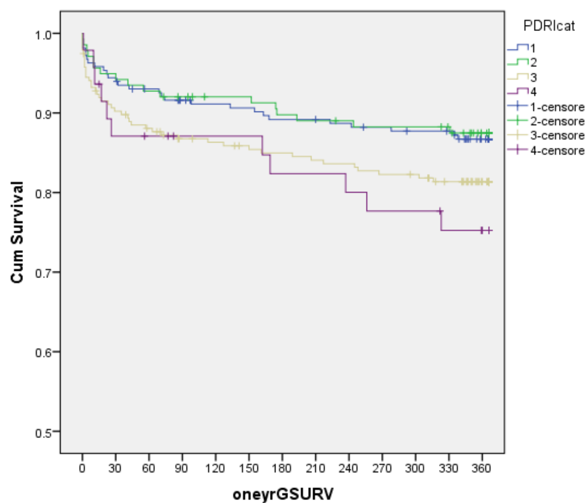
Donor Model

Adjusting for the recipient model, significant donor factors predicting poorer pancreas graft outcome were found to include donor age, donor type (DBD vs DCD), donor serum sodium, evidence of pancreas inflammation, cerebrovascular cause of death and donor ALT. In a multivariate model, pancreas inflammation emerged as the most predictive of one-year graft failure (HR 3.081, p=0.001) with donor sodium 146-150mmol/L(HR 1.985, p=0.04), donor ALT (HR 1.195, P=0.044) and cerebrovascular cause of donor death also highly predictive (HR 1.892, p=0.04). Other donor factors including donor gender, ethnicity, BMI, biochemistry, serology and past medical history were not significant predictors of graft outcome.

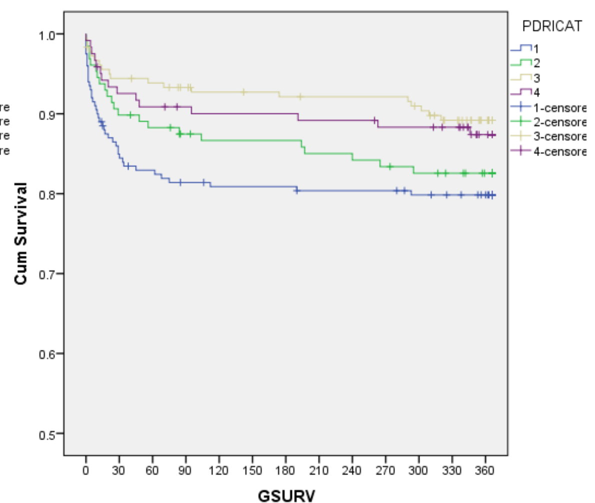
Variables in the Equation

	B	SE	Wald	df	Sig.	Exp(B)
Organ			11.790	2	.003	
Organ(1)	1.141	.360	10.050	1	.002	3.129
Organ(2)	.896	.460	3.800	1	.051	2.449
Centre			10.831	8	.211	
Centre(1)	-1.691	.826	4.192	1	.041	.184
Centre(2)	-2.775	.957	8.409	1	.004	.062
Centre(3)	-2.154	.924	5.431	1	.020	.116
Centre(4)	-1.659	.873	3.609	1	.057	.190
Centre(5)	-2.309	.916	6.356	1	.012	.099
Centre(6)	-1.573	.931	2.855	1	.091	.207
Centre(7)	-1.946	.815	5.704	1	.017	.143
Centre(8)	-1.596	.891	3.210	1	.073	.203
CIT	.000	.001	.379	1	.538	1.000
DAgeCat			1.536	3	.674	
DAgeCat(1)	-.439	.361	1.474	1	.225	.645
DAgeCat(2)	-.256	.410	.388	1	.533	.774
DAgeCat(3)	-.139	.504	.076	1	.783	.870
DType	.677	.416	2.650	1	.104	1.967
DNaCat			4.751	3	.191	
DNaCat(1)	.238	.384	.384	1	.536	1.268
DNaCat(2)	.686	.334	4.216	1	.040	1.985
DNaCat(3)	.063	.772	.007	1	.935	1.065
PancInflam	1.125	.349	10.374	1	.001	3.081
DALTCat	.650	.323	4.041	1	.044	1.915
DcodCat	.638	.310	4.218	1	.040	1.892

Model dataset



Validation dataset



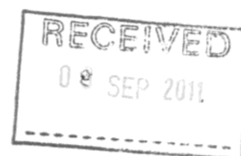
13 APPENDIX 5: Research Ethics Approval

National Research Ethics Service

NRES Committee South Central - Oxford A
South West Research Ethics Committee Centre
Whitefriars
Level 3 Block B
Lewins Mead
Bristol
BS1 2NT

Telephone: 01173421331
Facsimile: 01173420445

31 August 2011



Professor Peter Friend
Professor of Transplant Surgery
Oxford Radcliffe Hospitals NHS Trust and University of Oxford
Oxford Transplant Centre
Old Road
Headington
OX3 7LJ

Dear Professor Friend

Study title: **Metabolic Function and Patient Outcomes following Pancreas Transplantation: A Cohort Observational Study**
REC reference: **11/SC/0308**

Thank you for your letter of 18 August 2011, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

This Research Ethics Committee is an advisory committee to the South Central Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		
GP/Consultant Information Sheets	1	02 June 2011
Investigator CV		
Investigator CV		
Investigator CV		
Letter from Sponsor		07 July 2011
Letter from Statistician		01 July 2011
Letter of invitation to participant	2	11 August 2011
Other: Funder Letter		15 September 2010
Other: Clinical Research Unit SOP 010	2	21 March 2010
Participant Consent Form: Participant consent Form	2	11 August 2011
Participant Information Sheet: Patient Information Sheet	2	11 August 2011
Protocol	1.7	01 July 2011
Questionnaire: SF-36 Health Survey		
Questionnaire: Medical Questionnaire / Visit data	1	11 August 2011
Questionnaire: Diabetes Quality of Life Questionnaire		
REC application		08 July 2011
Referees or other scientific critique report		21 October 2010
Response to Request for Further Information		18 August 2011

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

11/SC/0308	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Miss Sara Owen
Vice-Chair

Email: scsha.oxfordreca@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to:

Heather House, Research and Development
Heather.house@admin.ox.ac.uk

NRES Committee South Central - Oxford C
Room 002
TEDCO Business Centre
Rolling Mill Road
Jarrow
NE32 3DT

Telephone: 0117 342 1333
Facsimile: 0117 342 0445

04 July 2012

Miss Shruti Mittal
Research Fellow
Oxford University NHS Trust
Oxford Transplant Centre
Churchill Hospital
Oxford
OX3 7LE

Dear Miss Mittal

Study title: **Prospective investigation into the effect of pancreas transplantation on glucagon secretion**
REC reference: **12/SC/0341**

The Research Ethics Committee reviewed the above application at the meeting held on 29 June 2012. Thank you for attending to discuss the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Miss Shruti Mittal (Chief Investigator) and Professor Peter Friend (Co-Investigator) attended to discuss the application.

Ethical issues raised by the Committee in private discussion, together with responses given by the researcher when invited into the meeting.

1. The Committee requested under the „What are the benefits of taking part?" section of the PIS (Page 4), the wording is changed to „Although you will not benefit from taking part….."

2. The Committee suggest the REC reference details are added to all participant documentation; 12/SC/0341 South Central- Oxford C REC.

3. The Committee request the CI"s details are added to the Participant documents.

The Researchers agreed to do this.

4. The Committee felt the PIS would benefit from proof reading for grammatical errors.

The Researchers agreed to do this.

5. The Committee asked for the following changes to the PIS:

- In the third paragraph of the „What is the purpose of the study?" section of the PIS (page 1) is replaced by „An oral glucose tolerance test will be performed to measure how well your body processes glucose and to measure glucagon levels".

- The measurement of blood should be described as "15 tea-spoonsful".

- Under the „What are the potential risks and disadvantages of taking part?" (Page 4), the final sentence should read, „ Should the research team discover there be any abnormalities during your participations you will be directly referred to____ within the hospital".

- The REC reference details are added: South Central- Oxford C REC.

Decision

The Committee gave a favourable opinion of the application (with additional conditions)

Additional Conditions:

a) Under the „What are the benefits of taking part?" section of the PIS (Page 4), the wording is changed to „Although you will not benefit from taking part….."

b) The REC reference details to be added to all participant documentation; 12/SC/0341 South Central- Oxford C REC.

c) The CI"s details to be added to the Participant documents.

d) Changes to the PIS:

- In the third paragraph of the „What is the purpose of the study?" section of the PIS (page 1) is replaced by „An oral glucose tolerance test will be performed to measure how well your body processes glucose and to measure glucagon levels".

- The measurement of blood should be described as "15 teaspoonsful".

- Under the „What are the potential risks and disadvantages of taking part?" (Page 4), the final sentence should read, „Should the research team discover there be any abnormalities during your participations you will be directly referred to_____________ within the hospital".

- The REC reference details are added: South Central- Oxford C REC.

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering Letter	Miss Shruti Mittal	
Investigator CV	Miss Shruti Mittal	
Investigator CV	Peter J Friend	23 May 2012
Investigator CV	Edward J Sharples	20 June 2011
Letter from Sponsor	Heather House	25 May 2012
Letter of invitation to participant	1	17 November 2011
Other: Letter from Funder	Professor Stephen Gough	26 April 2012
Participant Consent Form	1	24 April 2012
Participant Information Sheet	1	24 April 2012
Protocol	1.0	24 April 2012
REC application	IRAS Version 3.4, 95347/327038/1/932	23 May 2012
Referees or other scientific critique report	Parth Narendran, University of Oxford	29 April 2012

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/SC/0341 **Please quote this number on all correspondence**

With the Committee"s best wishes for the success of this project

Yours sincerely

Professor Nigel Wellman
Chair

Email: scsha.oxfordRECC@nhs.net

NRES Committee South Central - Oxford C
Room 002
TEDCO Business Centre
Rolling Mill Road
Jarrow
NE32 3DT

Telephone: 0117 342 1333
Facsimile: 0117 342 0445

04 July 2012

Miss Shruti Mittal
Research Fellow
Oxford University NHS Trust
Oxford Transplant Centre
Churchill Hospital
Oxford
OX3 7LE

Dear Miss Mittal

Study title:	**Investigation of the Incretin Pathway after Pancreas Transplantation in Type 1 Diabetes**
REC reference:	**12/SC/0335**
Protocol number:	**N/A**

The Research Ethics Committee reviewed the above application at the meeting held on 29 June 2012. Thank you for attending to discuss the study.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Miss Shruti Mittal (Chief Investigator) and Professor Peter Friend (Co-Investigator) attended to discuss the application.

Ethical issues raised by the Committee in private discussion, together with responses given by the researcher when invited into the meeting.

1. The Committee queried the mention of an advert for healthy volunteers in the application documentation.

The Researchers advised this was an error as they will be using data collected from a previous study for the healthy volunteers. Therefore the PIS for healthy volunteers is no longer needed.

2. The Committee suggest the REC reference details are added to all participant documentation; 12/SC/0335 South Central- Oxford C REC.

3. The PIS should clearly state this is a doctoral study.

The Researchers agreed to do this.

4. The Committee request points three, five and six are removed from the Consent Forms. The

Researchers agreed to revise the PIS.

5. The Researchers were asked to revise the order of the „Do I have to take part?" and „Why have I been invited?" sections of the PIS. The

Researchers agreed to do this.

Decision

The Committee gave a favourable opinion of the application (with additional conditions)

Additional Conditions:

a) The second line of the first paragraph of the PIS should read: "This research is being undertaken as part of a phD study".

b) The REC reference details should be added to all participant documentation; 12/SC/0335 South Central- Oxford C REC.

c) Confirmation the Healthy Volunteer PIS is no longer needed. d)

Remove points three, five and six from the Consent Forms.

e) Under the section „Do I need to take part?" of the PIS, please move the 6th sentence to below the „You will be given 24 hours…." sentence.

f) Under the section „Why have I been invited", please state that „the study will take place after the surgery" and that Group 1-15 will include „people who will have a pancreas transplant…"

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Covering Letter	Miss Shruti Mittal (University of Oxford)	
Investigator CV	Miss Shruti Mittal	
Investigator CV	Peter J Friend	21 May 2012
Letter from Sponsor	Ms H House (University of Oxford)	22 May 2012
Letter of invitation to participant	Version 1	02 April 2012
Other: Letter from Funder	Professor Stephen Gough (OCDEM)	26 April 2012
Participant Consent Form	Version 1.1	10 April 2012
Participant Information Sheet: Group 1	Version 1.1	10 April 2012
Participant Information Sheet: Group 2	Version 1.1	11 April 2012
Protocol	Version 1.1	16 April 2012
REC application	IRAS Version 3.4 104854/325715/1/715	18 May 2012
Referees or other scientific critique report	Parth Narendran	29 April 2012

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/SC/0335 Please quote this number on all correspondence

With the Committee"s best wishes for the success of this project

Yours sincerely

Professor Nigel Wellman
Chair

Email: scsha.oxfordRECB@nhs.net

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
"After ethical review – guidance for researchers" [Emailed]

Copy to: Ms Heather House
heather.house@ouh.nhs.uk

NRES Committee South Central - Oxford C

Attendance at Committee meeting on 29 June 2012

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Leonard Brookes	Consultant to the Pharmaceutical Industry	Yes	
Dr Avinash Gupta	Clinical Research Fellow	No	
Mrs Sue Hallett	Paediatric Research Nurse	Yes	
Miss Kate Hicks	Clinical Trials Coordinator	Yes	
Ms Laura Kirkbride	Committee Coordinator	No	
Mrs Susan Lousada	Lay Member	Yes	
Mrs Lynch Mason	Occupational Therapist	No	
Mr Barry Muir	Lay Member	Yes	
Mrs Rachael Quinn	Nurse Member	No	
Dr David Scott	Pharmacist	Yes	
Dr Sabeena Sharma	Consultant Anaesthetist	Yes	
Mr Roy Staley	Retired Officer of the Civil Aviation Authority	Yes	
Dr Laurence Villard	Senior Lecturer/Epidemiologist	Yes	
Professor Nigel Wellman	Professor of Health and Human Sciences	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Siobhan McDonagh	Committee Coordinator