Peri-Operative Assessment and Optimisation in Simultaneous Pancreas
and Kidney Transplantation

A thesis submitted to The University of Manchester for the degree of

Doctor of Philosophy

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Hussein Ahmed Khambalia

School of Medicine
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Thesis Abstract

University The University of Manchester
Name Hussein Ahmed Khambalia
Degree Doctor of Philosophy, 2015
Thesis Title Peri-Operative Assessment and Optimisation in Simultaneous Pancreas and Kidney Transplantation

Pancreas transplantation (PT) is considered a gold-standard cure for brittle insulin dependent diabetes mellitus. In over 90% of cases, this is conducted simultaneously with a kidney transplant, providing concurrent treatment for end-stage renal failure (Simultaneous pancreas and kidney transplantation, SPKT). However, since its inception in the 1960’s, SPKT has been associated with considerable morbidity and mortality. Despite significant recent improvements in graft and patient survival, the multi-factorial nature of the procedure has resulted in persistently high peri-operative morbidity.

This thesis has identified four areas to study in the peri-operative assessment and management of these patients, potentially resulting in improved clinical outcomes.

1. Pre-operative risk-prediction scoring systems aide in the consent process and the peri-operative planning of care following major surgery. In PT, multi-system risk-prediction tools are deficient. We therefore assessed the utility of commonly used general surgical risk prediction models in PT recipients. Our finding suggested that The Waterlow Score, a multi-system tool originally developed for predicting the development of decubitus skin ulcers, identified high-risk individuals and has value in predicting outcome following SPKT.

2. Peri-operative physiological optimisation (Goal-directed therapy, GDT) is well-recognised to improve outcomes following major general surgery in high-risk individuals. A randomised controlled trial was therefore performed to investigate the benefits of GDT in the peri-operative period following SPKT. The findings demonstrated improved short-term outcomes following GDT in our cohort.

3. The temporal evolution of biomarkers following major physiological stresses allow for application in the diagnosis, management surveillance and treatment of diseases. In our cohort the acute evolution of inflammatory and diabetes biomarkers were delineated and correlated to clinical outcome. We identified that cold ischaemic time is significantly negatively related to early pancreatic function and CRP provides an easily measurable predictor of recipient morbidity.

4. The final study aimed to evaluate the feasibility and assess the benefits of contrast enhanced ultrasound (CEUS) in the immediate post-operative period following PT. We found CEUS to be a clinically useful adjunct in the post-operative assessment of allograft morphology and perfusion, although further validation and correlation with outcomes is required.
DECLARATION

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.
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This thesis incorporates my work from conception in September 2011 to its completion in September 2014, at the Department of Renal and Pancreas Transplantation, Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust and The Cardiovascular Research Unit, University of Manchester.

All studies and their findings reported in this thesis represent my own original work.
ACKNOWLEDGEMENTS

This work has resulted from the experiences of patients suffering with insulin dependent diabetes mellitus and end-stage renal failure and their difficult journey through the transplantation process. I thank all the patients and their families and friends for allowing me to conduct this work through a difficult period in their lives.

I also thank all the professionals involved with the Transplant Unit, Critical Care Unit, Anaesthetic and Radiology teams for their assistance in carrying out and completing this work.

I have been afforded a number of opportunities during my PhD and have gained invaluable clinical and research expertise that I owe to my supervisors, Mr. Titus Augustine, Prof. Yvonne Alexander and Prof. Mahesh Nirmalan. I am also very grateful to Mr. David van Dellen and Dr. Angela Summers for their valuable support and advice throughout the course of this research.

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS WORK

Publications


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Ischaemia in Pancreas Transplantation. Transplantation 2013; 96 (Suppl. 6): S53 (Abstract)


Presentations

1. **Invited Lecture**- *Cardiac Output Monitoring in Transplantation*. Association of Vascular Anaesthetists, Manchester (September 2013)

2. **Invited Lecture**- *Cardiac Output Monitoring in Transplantation*. Association of Indian Anaesthetists, Manchester (September 2013)

3. **Invited Lecture**- *Cardiac Output Monitoring in Transplantation*. Association of Vascular Anaesthetists, Portsmouth (September 2012)


7. **Oral Presentation**- *Contrast Enhance Ultrasonography in Simultaneous Pancreas and Kidney Transplantation*. European Society of Organ Transplantation, Brussels (September 2015)

9. **Oral Presentation**- *Does Cardiac Risk Quantification Have a Role in Assessment and Stratification for Pancreas Transplantation?* Association of Surgeons of Great Britain and Ireland (April 2015)


17. **Oral Presentation**- A Prospective Cohort Study of Risk Prediction in Simultaneous Pancreas and Kidney Transplantation, Association of Surgeons of Great Britain and Ireland, Harrogate (March 2014)


19. **Poster Presentation**- Outcome of Pancreas Allograft Salvage Following Segmental Ischaemia: A Single Centre Experience. British Transplant Congress, Glasgow (February 2014)


21. **Oral Presentation**- Short Cold Ischaemic Times Improve Cytokine, Diabetic and Clinical Markers in Pancreas Transplantation. International Pancreas and Islet Transplant Association, Monterey, CA, USA (September 2013)


25. **Oral Presentation**- Management of Pancreas Allograft Head or Duodenal Ischaemia in Pancreas Transplantation. International Pancreas and Islet Transplant Association, Monterey, CA, USA (September 2013)
26. **Oral Presentation**- *Risk Assessment in Pancreas Transplantation: Utilisation of the Waterlow Score.* European Society of Organ Transplantation, Vienna (September 2013)

27. **Oral Presentation**- *Waterlow Scoring System: An Independent Predictor for Delayed Graft Function in Renal Transplantation.* European Society of Organ Transplantation, Vienna (September 2013)


30. **Poster Presentation**- *The Waterlow Scoring System: A Robust Method to Predict Length of Hospital Stay in Pancreas Transplantation.* British Transplant Society, Bournemouth (March 2013)
ABBREVIATIONS

ANOVA, Analysis of Variance

APP, Acute phase proteins

ASA Score, American Society of Anaesthesiologists Score

AT, Anaerobic Threshold

BMI, Body mass index

C-peptide, Connecting protein

CCU, Critical Care Unit

CD, Cluster of differentiation

CEUS, Contrast enhanced ultrasound

CI, Confidence Interval

CIT, Cold ischaemic time

CoD, Cause of Death

CPET, Cardiopulmonary Exercise Test

CRP, C-reactive protein

CT, Computed tomography

DBD, Donation after brainstem death

DCD, Donation after cardiac death
DGF, Delayed graft function

DO_{I,}, Indexed oxygen delivery

ELISA, Enzyme-linked immunosorbent assay

EMA, European Medicines Agency

ESRD, End-stage renal disease

ESRF, End-stage renal failure

EVAR, Endo-vascular Aneurysm Repair

FAST, Focussed Assessment with Sonography in Trauma

FGF, Fibroblast growth factor

FiCM, Faculty of Intensive Care Medicine

G-CSF, Granulocyte colony stimulating factor

GDT, Goal-directed therapy

GM-CSF, Granulocyte macrophage colony stimulating factor

HES, Hydroxyethyl starch

HTK Solution, Histidine-tryptophan-ketoglutarate solution

IBMIR, Instant, blood-mediated inflammatory reaction

ICU, Intensive care unit

IDDM, Insulin Dependent Diabetes Mellitus

IFN-γ, Interferon gamma
IL, Interleukin

IM, Inflammatory marker

IP-10, Interferon-γ induced protein 10

IPTR, International Pancreas Transplant Registry

IQR, Inter-quartile range

IRI, Ischaemia-reperfusion-injury

K CIT, Kidney cold ischaemic time

L, Litre

MAG-3, Mercaptoacetyltriglycine

MCP-1, *Monocyte chemoattractant protein-1*

MELD, Model for End-Stage Liver Disease

MHRA, The Medicines and Healthcare products Regulatory Agency

MIA syndrome, Malnutrition Inflammation and Atherosclerotic syndrome

Min, Minute

MIP, Macrophage inflammatory protein

ml, Millilitre

mmHg, Millimetres of mercury

MODS, Multiple Organ Dysfunction Score

MR imaging, Magnetic resonance imaging
MWU-test, Mann-Whitney U-test

NA, Nor-adrenaline

NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells

NHS BT, National Health Service, Blood and Transplant

NHS, National Health Service

NICE, National Institute for Health and Clinical Excellence

ODM, Oesophageal doppler monitoring

PAI, Plasminogen activator inhibitor

PAK, Pancreas after kidney [transplant]

P CIT, Pancreas cold ischaemic time

PDGF, Platelet derived growth factor

P-DRI, Pancreas Donor Risk Index

pg, Picogram

PIS, Patient Information Sheet

POMS, Post-Operative Morbidity Survey

P-PASS, Pre-Procurement Pancreas Suitability Score

P-POSSUM, Portsmouth Physiological and Operative Severity Score for the Enumeration of Mortality and Morbidity

PT, Pancreas transplantation
PTA, Pancreas transplant alone

QoL, Quality of Life

RA, receptor against

RANTES, Regulated on activation, normal T cell expressed and secreted

RCT, Randomised clinical trial

RCRI, Revised Cardiac Risk Index

ROI, Region of interest

S, seconds

ScVO₂, Central venous oxygen saturations

SD, Standard deviation

SF₆, Sulphur hexafluoride

SIRS, Systemic Inflammatory Response Syndrome

SPKT, Simultaneous pancreas and kidney transplantation

SSTs, Serum separating tubes

ST, Standard therapy

Th1, T-helper 1 cell

Th2, T-helper 2 cell

TNF-β, Tumour Necrosis Factor- β

UK, United Kingdom
US, Ultrasound

USA, United States of America

UTI, Urinary tract infection

UW solution, University of Wisconsin Solution

VEG-F, Vascular endothelial growth factor

WCC, White cell count

WS, Waterlow Score
Dedicated to Insiya, Umme-Hani & Abbas
If you don’t invest very much, then defeat doesn’t hurt very much and winning is not very exciting; Dick Vermeil

Success is the prize for those who stand true to their ideas; Josh S Hinds

The tragedy of life is often not in our failure, but rather in our complacency; not in our doing too much, but rather in our doing too little; not in our living above our ability, but rather in our living below our capacities; Benjamin E. Mays

Only those who will risk going too far can possibly find out how far one can go; T S Eliot

The only way to find true happiness is to risk being cut open; Chuck Palahniuk

Risk is like trying to control something you are powerless over; Eric Clapton

To write something, you have to risk making a fool of yourself; Anne Rice

Difficulties are opportunities; Joseph E Murray

Our knowledge of an individual’s risks involved remains at best uncertain, and at worst ignorant; H A Khamalia

Decisions to proceed with transplantation are associated with uncertainty at every step; H A Khamalia
**Peri-Operative Assessment and Optimisation in Simultaneous Pancreas and Kidney Transplantation**

**PhD Thesis, Hussein A Khambalia**

**A Prospective Cohort Study of Risk Prediction in Simultaneous Pancreas and Kidney Transplantation**

A multi-system co-morbidity index identifies high-risk individuals undergoing SPKT

**Peri-Operative Goal-Directed Haemodynamic Optimisation Improves Short-Term Outcomes Following Simultaneous Pancreas and Kidney Transplantation: A Randomised Clinical Trial (NCT01619904)**

Peri-operative, supra-physiological optimisation improves short-term outcomes post-SPKT

**A Proof of Principle Study Investigating the Temporal Evolution of Inflammatory and Diabetes Markers Following Simultaneous Pancreas and Kidney Transplantation**

The temporal evolution of inflammatory and diabetes markers have been delineated

**The Temporal Evolution of Inflammatory and Diabetes Biomarkers Following Simultaneous Pancreas and Kidney Transplantation**

CRP is an early predictor of morbidity post SPKT

**Contrast Enhanced Ultrasonography after Simultaneous Pancreas and Kidney Transplantation: Development of a Novel Imaging Technique (NCT02104024)**

CEUS is feasible in SPKT recipients

Quantification data requires correlation to clinical outcome

There are potentially several advantages of CEUS identified

Provides evidence for the use of targeted anti-inflammatory therapies

CIT is significantly related to pancreatic endocrine
Introduction

H A Kambalia
Chapter 1, Introduction

Diabetes Mellitus (DM) affects over 3 million people in the United Kingdom (Diabetes UK, April 2014). The direct costs [diagnosis and treatment] of managing DM and its associated multisystem morbidity currently accounts for approximately 10% of the annual National Health Service spend, £9.8 billion per annum (Hex, Bartlett et al. 2012). When the indirect costs [salary loss and life-years lost] are included in this estimation the current annual estimated cost of DM rises to £23.7 billion and is projected to increase to £39.8 billion per annum in 2036 (Hex, Bartlett et al. 2012). DM has evolved to become the largest single cause of end-stage renal failure (ESRF) in the western world. 20- 25% of people admitted to hospital suffer with DM, whilst insulin dependent diabetes mellitus (IDDM) accounts for approximately 15% of all cases. DM and its complications are associated with low socio-economic status and social deprivation, with 2.5 times higher incidence of the disease in this cohort across all age ranges. Furthermore, the World Health Organization estimate that 3.4 million people died as a result of the effects of diabetes in 2010, and 80% of these people are from low- and middle-income countries (UN WHO, October 2014).

These costs are partly due to on-going treatment and management (£561.4 million per year), but primarily reflect the challenges of managing diabetic complications. Diabetes UK estimate that the presence of diabetic complications cause a five-fold increase in both the risk of a person requiring hospital admission and the cost of a patient’s care (Diabetes UK, April 2014). Despite advances in the medical management of DM, the rising cost of healthcare delivery and the impact and cost of diabetic complications continues to increase.
While major refinements have been made in insulin therapy including pump therapy, combined with out-patient continuous glucose monitoring, beta-cell replacement in the form of pancreas transplantation (PT) is considered a gold-standard therapy for patients with end-organ failure secondary to IDDM. It replaces the defective β-cells in the islets of Langerhans of the recipient, with those from an implanted whole-organ donor pancreas. The aim of treatment is to reinstate normal physiological control of blood glucose levels via endogenous insulin production and re-establish the defective native closed loop control system, utilising insulin and glucagon produced by the transplanted pancreas as feedback markers. In diabetic patients, this provides instant normalisation of blood glucose levels, thereby preventing the hypo-/hyper-glycaemic attacks associated with poorly controlled DM and the worsening of vascular complications. This results in improvements in the co-morbidities associated with IDDM, leading to significantly increased life-expectancy and improvement in quality of life (QoL) (Katz, Homan et al. 1991, Piehlmeier, Bullinger et al. 1991, Landgraf 1996, Piehlmeier, Bullinger et al. 1996, Gross, Limwattananon et al. 1998, Ryan, Bigam et al. 2006), results currently unachievable with best medical therapy or islet cell transplantation.

The History of Pancreas Transplantation

The 1923 Nobel Prize for Medicine was awarded to Frederick Banting, a surgeon from Toronto, and Prof John Macleod, a world leader at the time in the field of diabetes research at The University of Toronto, for their work in diabetes research and the discovery of insulin. Banting shared his award and the seminal paper on the first successful use of pancreatic tissue to treat Type 1 DM with Dr Charles Best, a medical student at the time of the discovery (Banting, Best et al. 1922). Their work laid the foundation for advances in the
Chapter 1, Introduction

treatment of diabetes. Subsequent to these findings, it was envisaged that DM would evolve into an easily treatable disease and these hopes were reflected with a dramatic decrease in diabetic acidosis mortalities in the subsequent 50 years (Lillehei, Weil et al. 1970). However, this break-through led to improved longevity of diabetic sufferers at the cost of considerable macro- and micro-vascular morbidity, previously an unrecognised phenomenon in diabetic patients.

The first ever whole organ PT was performed in Minnesota in 1966 and the recipient achieved a two month graft survival (Kelly, Lillehei et al. 1967). The first ten pancreas transplants performed achieved less than one-year graft survival in all cases with maximum patient survival of 11 months post-transplant (Lillehei, Simmons et al. 1970). These dismal outcomes were related to the intrinsic complexity of the pancreas as an allograftable organ, unregulated immunosuppression, un-controlled re-perfusion injuries and subsequent pancreatitis, ultimately resulting in complications due to the exocrine secretions of the pancreatic allograft.

Despite poor initial results, refinements in techniques of exocrine drainage and immunosuppression have led to significant improvements in recipient and graft survival. The advances in immunosuppression with the advent of cyclosporine (a calcineurin inhibitor) which, in combination with azathioprine and prednisolone resulted in vastly improved outcomes in comparison to the “pre-cyclosporin era”, despite the recognised nephrotoxic effects of a calcineurin inhibitor (Squifflet, Van Ophem et al. 2004).

In addition, the continued evolution of surgical techniques to drain the exocrine secretions of the pancreas had led to improved graft and patient survival and reduced post-operative
morbidity. In the first reports of SPKT, Kelly trialed two techniques of enteric anastomosis (Kelly, Lillehei et al. 1967). In the first case, the pancreas was implanted without the duodenum and the cut pancreatic duct was tied, leading to a gross pancreatitis requiring allograft explant after two months and patient death after three months. In the second case, the pancreas was implanted with the entire duodenum, the distal end of which was exteriorized as a duodenostomy. In this case, the patient suffered with two episodes of rejection, leading to insulin recommencement two months post-implantation. Encouragingly, insulin production was detected from both allograft pancreata with normalisation of blood sugars in the recipients, prior to failure. The authors concluded by stating their intent to trial a third technique, performing an anastomosis between the allograft duodenum and a Roux-en-Y loop. In a report of the following ten patients to undergo SPKT by the same group (Lillehei, Simmons et al. 1970), the first four underwent duodenostomy to drain the pancreatic exocrine secretions, while the following six underwent duodenal anastomosis to a Roux-en-Y. Unfortunately, the longest graft survival was eleven months (at date of publication), with graft losses due to; death with functioning graft (5), primary graft non-function (1) and graft infarction (2), whilst all bar two patients had died at one year post-SPKT due to; sepsis (7) and hyper-kalaemic arrest (1). Of those patients with a Roux-en-Y, two were alive at time of publication, whilst the four which had died, three were related to sepsis secondary to complications with the anastomosis.

Since then, a number of pancreatic duct management methods have been described. Little and Dubernard described injecting the pancreatic duct to occlude the secretion of pancreatic exocrine enzymes, but this also resulted in graft loss secondary to pancreatitis, and fibrosis (Little, Lauer et al. 1977, Dubernard, Traeger et al. 1978). Kyriakides described leaving the duct open to the peritoneum, a technique which had been successfully used in
swine models (Kyriakides, Nuttall et al. 1979), but resulted in chemical peritonitis and subsequent pancreatectomy in humans (Sutherland, Goetz et al. 1981).

A breakthrough in duct management came in 1983 when Cook (Cook, Sollinger et al. 1983) retrieved the pancreas with duodenum and used this as a conduit to perform a duodenocystotomy, therefore excreting the enteric contents of the pancreas directly into the bladder. This had the additional advantage of being able to protect the anastomoses with a bladder catheter and resulted in one-year graft survivals approaching 80%. The technique’s reproducibility and improved safety profile, when compared to the other methods of duct management being used at the time, resulted in the wide-spread use of this method. In addition, at a time when rejection rates were high, this technique offered the advantage of being able to monitor urinary amylase as a marker of graft rejection, as a rise in urinary amylase (signifying rejection of the exocrine function of the pancreas) precedes rejection of the endocrine function of the pancreas by up to one week (Gruessner and Gruessner 2013). Finally, it was suggested that if anastomotic leaks did occur with bladder drained pancreata they were less likely to be patient or graft threatening (Pirsch, Odorico et al. 1998).

However, bladder drainage was dogged by multiple metabolic, urinary and infective complications, in the most serious cases it resulted in severe metabolic acidosis due to excess urinary loss of extracellular sodium bicarbonate (Schang, Timmermann et al. 1991). Less serious, but more common were urinary symptoms (dysuria, recurrent cystitis/ urinary tract infections, haematuria, neurogenic bladder, urethral strictures and prostatitis), reflux pancreatitis, duodenitis and poorer quality of life suffered by up to 80% of recipients when compared to enteric drained pancreata (Sollinger, Ploeg et al. 1993, Sollinger, Odorico et al. 2009). Despite the non-life- or graft-threatening nature of these side-effects they continue

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to result in greater than 50% enteric conversion rates at 15 years post-PT, with higher complication rates the longer after the primary procedure the conversion takes place (Sollinger, Odorico et al. 2009). This large single-centre data (over 1000 PTs) is consistent with The International Pancreas Transplant Registry (IPTR)/ United Network for Organ Sharing (UNOS) data (A. Gruessner, personal communication (email) 12/11/2014).

For these reasons, attempts at improving the technique of enterically draining the pancreatic secretions continued. The re-evolution of the duodeno-enteric anastomosis came in the 1990s when a Swedish group modified and simplified the duodeno-enteric drainage of pancreatic exocrine secretions initially described by Dejode et al (Dejode and Howard 1962), and anastomosed the donor duodenum to recipient jejunum with no Roux-en-Y loop. This technique resulted in 0% anastomosis leak rates, compared to 5% and 10% when using a Roux-en-Y loop, either with (n= 20) or without a pancreatic duct catheter (n= 20) respectively (Tyden, Tibell et al. 1996). Although their entire series was small (n= 61), the results were promising and led to one-year patient and pancreas graft survival rates following SPKT of 100% and 87% respectively with no graft losses due to anastomotic complications, whilst eliminating the longer-term metabolic and urinary complications observed in bladder-drained pancreata. More recently, in an effort to become “more physiological”, a Brazilian group is now performing duodeno-duodenostomy (Peresa, Noujaim et al. 2014) with comparable surgical complication rates to duoden-jejunostomy, but the potential advantages of this specific enteric drainage procedure are yet to be elucidated.

The continued complication of concern with enteric drainage of pancreatic secretions compared to bladder drainage is anastomotic leak, leading to; peritonitis, re-laparotomy
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(24%- 35%), graft loss, stoma formation, recurrent intra-abdominal sepsis and occasionally death (Reddy, Stratta et al. 1999, Troppmann 2010, Ziaja, Krol et al. 2011). However, with familiarity of the surgical technique, there is now no significant difference in patient and graft survival between enteric- and bladder drainage following PTs (Sollinger, Odorico et al. 2009, Gruessner and Gruessner 2013) and given the disadvantages of bladder-drained pancreata discussed above, enteric drainage is now the most widely chosen technique for duct management (A. Gruessner, personal communication (email) 12/11/2014) In 2012 90% and 78% of SPKT and PAK transplants respectively were enterically drained. (Gruessner and Gruessner 2013).

These improvements in surgical technique, immunosuppression and donor and recipient selection have led to vast improvements in outcome, since the International Pancreas Transplant Registry (IPTR) began data collection. In 1980 the IPTR reported 1-year graft and patient survival rates of 21% and 67% respectively (Sollinger, Odorico et al. 1998). In 1988 2-year graft and patient survival rates were 44% and 76% respectively (Sollinger, Stratta et al. 1988) and in 2013 these had improved further to 1-year graft and patient survival rates of 89% and 98% respectively (A. Gruessner, personal communication (email) 12/11/2014).

World-wide, 41,854 PTs had been reported to the IPTR by the end of 2013 (A. Gruessner, personal communication (email) 12/11/2014). In the UK, up to July 2015, 2,579 PTs (2,137 SPKT and 442 pancreas only) have been performed (NHS BT, personal communications (email) 26/08/2015). Internationally, approximately 2,300 are carried out annually, of which 800 are performed in Europe and 200 in the UK (Gruessner 2011, NHS BT 2014). PT has evolved to become the most effective management strategy for patients with IDDM and severe end-organ failure. The specific indications for PT are discussed below.
Indications for Pancreas Transplantation

PT is currently performed in distinct clinical contexts, each of which has well defined indications and criteria.

1. Simultaneous Pancreas and Kidney Transplantation (SPKT). This is the largest group and accounts for 86% of all PTs performed in the UK in 2013 and 83% of all PTs performed over the course of the UK program (NHS BT 2014). The recipients are diabetic patients with chronic renal failure either already on renal replacement therapy or within 6 months of a predicted date of commencing dialysis (PAG 2014). Commonly, both pancreas and kidney are transplanted from the same deceased donor as a combined procedure. Variations of this include living donor SPKT (Gruessner and Sutherland 1996, Sutherland, Radosevich et al. 2012) or a living donor kidney transplant carried out synchronously with a deceased donor pancreas (Farney, Cho et al. 2000), but neither are common practice.

2. Pancreas Transplant Alone (PTA). This accounts for a minority of all PTs performed. Patients considered eligible for transplantation should have preserved renal function (Isotopic Glomerular Filtration Rate >80ml/ min/1.73m²) to account for a potential subsequent deterioration due to the nephrotoxic effects of post-operative immunosuppression. PTA should be considered in patients with significant diabetic or life threatening complications; namely frequent and severe episodes of hypoglycaemia with particular emphasis on unawareness, normally culminating in referral by a diabetologist (PAG 2014).
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In the patients considered for PTA, stable renal function is a critical component of the assessment process. A major disadvantage of PTA is deterioration in renal function both due to the deleterious effects of major surgery but also due to the effects of subsequent nephrotoxic immunosuppression (Gruessner, Sutherland et al. 1997). Patients with an isotopic glomerular filtration rate of less than 80ml/min/1.73m² are likely to need a renal transplant at some point after the PTA and 30% of patients given a PTA will need a kidney transplant at 9-10 years post PTA (Sutherland, Gruessner et al. 2001).

3. **Pancreas after Kidney Transplant (PAK).** In this situation the patient will have received a kidney transplant as a modality of renal replacement therapy either from a living (related or un-related) donor or from a cadaveric donor prior to receiving a PT. Pancreas after living donor kidney transplant offers improved renal graft survival combined with the renoprotective effects of physiologic glycaemic control associated with PT. It also provides the advantage of treating the renal failure prior to the PT, thereby slowing the rate of deterioration of renal function (estimated glomerular filtration rate) after the PT, when compared to PTA. (Odorico, Voss et al. 2008). Therefore PAK should be considered in patients with end-organ failure secondary to IDDM, with stable renal function from a previous renal transplant (PAG 2014).

**Benefits of Pancreas Transplantation**

The immediate clinical benefit of successful whole organ PT is an improvement in quality of life associated with euglycemia, albeit at the expense of immunosuppression (Zehrer and Gross 1991, Gross and Zehrer 1992, Gross, Limwattananon et al. 1998). In over 80% of
patients, who undergo simultaneous kidney transplant, the procedure also confers the benefit of dialysis independence.

Sustained euglycemia translates into benefits across all organ systems with an increased life expectancy being the most evident (Boggi, Vistoli et al. 2012). Patients remaining on the waiting list have a relative risk of mortality of 2.67, compared to those post-SPKT (van Dellen, Worthington et al. 2013). SPKT also increases life-expectancy when compared to IDDM patients who received kidney transplant alone, by up to 17% at 8 years post-transplant (Tyden, Bolinder et al. 1999, Ojo, Meier-Kriesche et al. 2001, Reddy, Stablein et al. 2003), and diabetic patients on renal replacement therapy (Smets, Westendorp et al. 1999).

The improvement in life expectancy could be related to the reduction in cardiovascular mortality. Blood pressure and left ventricular functional improvements have been described (Fiorina, La Rocca et al. 2000) as has the slowing in progression of coronary artery disease post-SPKT (Jukema, Smets et al. 2002).

In individuals who have had successful isolated PT, preexisting renal diabetic lesions have been shown to regress after 5-10 years of euglycaemia (Fioretto, Mauer et al. 1993, Fioretto, Steffes et al. 1998). At 5 years following PT the mesangial fractional volume had increased, but at 10 years, the glomerular and basement membrane was thinner in the PT group when compared to baseline values.
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At six months following transplantation small fibre nerve repair within the cornea can be demonstrated (Mehra, Tavakoli et al. 2007). Within one year of successful transplantation a significant improvement in visual acuity is established (Koznarova, Saudek et al. 2000) and at 17 month follow-up, PT recipients not only have a stabilisation of diabetic retinopathy, but 41.7% suffering from non-proliferative diabetic retinopathy have an improvement of diabetic lesions (Giannarelli, Coppelli et al. 2005).

PT improves peripheral motor and sensory nerve conduction in studies within one year of transplant and this improvement continues long-term (Navarro, Sutherland et al. 1997). Improvements to autonomic neuropathy can occur post-transplantation, but these are not significant, can take up to two years to become apparent and are dependent upon severity of symptoms prior to transplant (Kennedy, Navarro et al. 1990).

Finally, SPKT recipients have a lower rate of major limb amputations compared with diabetic patients who have not undergone PT (Woeste, Wullstein et al. 2003) and the earlier the transplant is conducted, the less likely patients will require a major amputation.

In terms of chargeable cost analysis, in the USA SPKT is the most cost-effective treatment for patients with end-stage renal failure (ESRF) secondary to diabetes, when compared to living or cadaveric donor kidney transplant either followed by PAK or life-long insulin therapy (Douzdjian, Escobar et al. 1999).
Surgical Complications of Pancreas Transplantation

Ever since its introduction, complications have tarnished the reputation of the procedure, due to their prevalence and potentially serious nature. Even in an era where outcomes are continually improving (Sollinger, Stratta et al. 1988, Sollinger, Odorico et al. 1998, Gruessner 2011, Gruessner and Gruessner 2013), serious life- and graft- threatening complications persist. This reflects the inherent fragility of the pancreas as a transplantable organ, on a background of diabetes, renal failure, immunosuppression and a systemic inflammatory response syndrome (SIRS) associated with transplant pancreatitis.

The procedure is associated with 35% re-laparotomy rates (Troppmann 2010). Vascular complications account for 70% of technical graft failures. Portal vein thrombosis rates vary between 3- 10% (Troppmann 2010) inevitably leading to hyperglycaemia, re-exploration and graft loss, but rarely are a risk to patient life. Surgical thrombectomy (Nghiem 1995) and thrombolysis (Stockland, Willingham et al. 2009) have been described as potential methods of pancreas graft salvage in this scenario, but graft monitoring techniques are reliant upon testing blood glucose levels and once hyperglycaemia occurs the chance of salvage is very low. Therefore, success of salvage procedures is exceptionally rare (Gruessner and Gruessner 2013). In an effort to identify vascular complications at an early stage, and instigate pre-emptive treatment and/or increase rates of salvage, a clinical trial was instigated to study the feasibility of using a novel imaging technique in the peri-operative period following SPKT and investigate the potential utility and advantages of peri-operative elective imaging post-SPKT (Chapter 6).

In the medium- and long- term following PT pseudoaneurysms at the arterial anastomosis of the graft could lead to massive haemorrhage and death, and can occur months after the
initial procedure. Due to the rarity of these complications, only case reports and small case series have been published. Pseudoaneurysms tend to be mycotic in nature, but may also represent technical implantation errors or result from iatrogenic injuries during the retrieval process or biopsy surveillance of the graft. Occasionally they may be found incidentally allowing for salvage/reconstructive surgery (Verni, Leone et al. 2001, Akhtar, Jones et al. 2011). More commonly they present with rupture (Benedetti, Gruessner et al. 1996), putting at risk the patient and graft. Aorto-enteric fistulae may also occur following PT, but again are exceptionally are. They may present secondarily to intra-abdominal sepsis, leading to major gastro-intestinal bleeding, once again putting at risk the graft and the patient.

Bowel complications may present following SPKT in patients who have had both enteric and bladder drained grafts. In one case series, 19% of patients had a bowel complication ranging from self-limiting adhesive small bowel obstruction and internal hernias to anastomotic leak and intra-abdominal abscess formation following entero-enteric anastomosis. Rarely (<1%) entero-cutaneous fistula, major gastrointestinal haemorrhage and colitis may also occur. However minor the complication may appear, all these complications potentially lead to repeated surgery, potential graft loss and occasionally mortality (Lall, Sandrasegaran et al. 2006).

Up to 75% of PT recipients could have an infective complication (Bassetti, Salvalaggio et al. 2004) and although the majority are minor, this has implications on long-term patient survival (Khuri, Henderson et al. 2005). Graft pancreatitis is difficult to diagnose as hyperamalaysaemia can occur in up to 35% of PT recipients in the peri-operative period (Troppmann 2010) and due to the lack of a severity scoring system, it is an enigma to
manage. It potentially leads to intra-abdominal collections, pancreatic pseudocysts and a decrease in graft survival rates (Grewal, Garland et al. 1993).

As a result of these significant potential complications, clinician reticence persists in referral and listing for PT. In an effort to reduce the risk associated with the procedure careful donor and recipient selection is mandated.

Donor Selection

Donor selection and assessment of the pancreas at retrieval is critical to improving outcomes. The factors that make the ideal pancreas donor are summarised in Table 1.1.

The best graft outcomes are achieved from donors who have succumbed to trauma, rather than a cardiac or intracranial event (Gruessner, Sutherland et al. 1997). A time of less than 48 hours from hospital admission to brain death also leads to improved outcomes (Douzdjian, Gugliuzza et al. 1995). There is no agreement between studies regarding age, but donor age greater than 40-45 (Douzdjian, Gugliuzza et al. 1995, Gruessner, Sutherland et al. 1997) seem to lead to poorer graft survival and a higher rate of re-laparotomy rates in the recipient, due to increased risk of graft thrombosis and intra-abdominal infections (Humar, Kandaswamy et al. 2000).

Increased donor BMI leads to a higher risk of intra-abdominal infections, graft loss and recipient mortality (Ziaja, Krol et al. 2011). However, donor hyperamylasaemia and acute hyperglycaemia do not affect outcomes (Gruessner, Gruessner et al. 1993, Douzdjian, Gugliuzza et al. 1995) as might be expected, because these are most likely due to endocrine
disturbances associated with brain stem death. Donors with a history of pancreatitis are treated with caution, as are intra-venous drug users, due to the potential of disease transmission in these cases.

In an attempt to standardise donor selection, The Eurotransplant Pancreas Advisory Committee have developed a Pre-procurement Pancreas Suitability Score (P-PASS), aimed at aiding the clinician in accepting viable organs for transplantation (Summarised in Table 1.2). Originally, a review of acceptance factors was conducted, finding that a P-PASS of $>17$ or $=17$ was three times more likely to be declined for transplantation, compared to a P-PASS $<17$ (Vinkers, Rahmel et al. 2008). Furthermore, a P-PASS $>17$ or $=17$ has been shown to lead to higher rates of venous thrombosis post-implantation, higher re-laparotomy rates and longer hospital lengths of stay, but in this study the P-PASS was not related to long-term outcomes (1, 5 or 10 year graft or patient survival) (Schenker, Vonend et al. 2010). In another study, a P-PASS $>17$ was associated with poorer graft and patient survival, but this may also be linked to individual factors (increased age and BMI), both of which were also present in the higher P-PASS group and are independently related to reduced graft and patient survival (Ziaja, Krol et al. 2011). Finally, two studies have found no link between P-PASS and any major peri-operative morbidity, length of hospital stay, graft and/or patient survival (Woeste, Moench et al. 2010, Foltys, Kath et al. 2011). Aside from the initial study by Vinkers, all studies investigating P-PASS are small, single-centre analysis of data and the results must be interpreted with caution.

The Pancreas Donor Risk Index (P-DRI, summarised in Table 1.3) (Axelrod, Sung et al. 2010) has also been developed using data from the Scientific Registry of Transplant Recipients [international data] to help in the quantification of pancreas graft survival using donor
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factors. A formula was created to estimate graft survival using ten donor factors, but this has not been validated specifically in the UK population (Mittal, Sharples et al. 2013).

The single most important modifiable parameter in the transplantation process is cold preservation time (cold ischaemic time, CIT), which has a significant effect on outcomes (Humar, Kandaswamy et al. 2000). This group suggested that a CIT less than 20 hours leads to significant reductions in early graft loss (9% compared to 20.5% when CIT is greater than 20 hours). Furthermore, in this cohort, grafts with longer CIT suffered with higher rates of thrombosis and anastomotic leaks. More recently, IPTR data suggest that CIT less than 12 hours is associated with a reduced relative risk of pancreas graft failure, compared to a CIT greater than 12 hours (Gruessner 2011). Therefore, in the UK, the Pancreas Advisory Group guidance aims for a pancreas CIT less than 12 hours (PAG 2014).

Recipient Selection

The benefits of PT must counter the considerable morbidity and potential mortality of the procedure, especially in view of the requirement for subsequent life-long immunosuppression (Gruessner, Sutherland et al. 1997). This therefore mandates careful selection and thorough evaluation of the recipient prior to activation on the waiting list.

Recipient selection is based upon analysis of large registry data and studies of retrospective case series and their correlation to outcome. At present a cumulative risk assessment score for PT recipients does not exist. However, recipients with a BMI greater than 30 are generally not listed for PT as they are at increased risk of post- transplant complications. These include a double risk of pancreas graft thrombosis and a 38% increased risk of overall
transplant related complications when compared to recipients with a BMI between 20 and 25. Obese patients (BMI > 30kg/m²) are also at higher risk of mortality and pancreas and kidney graft loss at three years following SPKT (Humar, Ramcharan et al. 2004, Sampaio, Reddy et al. 2010). Therefore, in the UK, NHS BT account for recipient BMI when adjusting for outcomes and publishing results. Recipients aged over 50 have up to a 40% increased risk of lower respiratory tract infections when compared to recipients aged less than 50 (Ablorsu, Ghazanfar et al. 2008). However, recipient age demonstrates no significant difference in one-year graft or patient survival compared to their younger counterparts (Humar, Ramcharan et al. 2004, Ablorsu, Ghazanfar et al. 2008). Therefore, in clinical practice recipient age is used as a soft marker to guide fitness for transplantation and taken into consideration with a holistic assessment of the patient.

Although specific recipient factors have been linked to post-transplant outcomes, as yet a multi-system recipient risk prediction score to help guide risk stratification following PT does not exist. Ideally, a transplant specific scoring system would require the consideration of recipient, donor and organ factors and aim to predict organ survival and/ or patient morbidity/ mortality post-operatively. Given the large number of potential confounders and the low volume nature of the procedure, development of such a score is challenging.

In liver transplantation the various scoring systems (Model for End-Stage Liver Disease, Child-Pugh) are used to score the severity of disease in the recipient and correlate to post-transplant mortality and length of stay (Kamath, Wiesner et al. 2001, Washburn, Meo et al. 2009). In PT there are few multi-factorial scoring systems (e.g. P-PASS and P-DRI) and these do not take into consideration recipient factors. In addition, they have limited predictive
value, either for organ survival or recipient morbidity/ mortality. This is likely due to; the high rate of unpredictable technical graft failures post-PT, the multi-system nature of diabetes, the difficulty in objectively differentiating the severity of the co-morbidities between recipients and the subjective nature of organ assessment prior to transplantation.

**Chapter 2** therefore compares a number of different scoring systems, already in use in general surgery, with varying post-operative predictive reliability, to assess their utility in predicting morbidity following PT.

**Outcomes of Pancreas Transplantation**

SPKT demonstrates both long-term improved patient and graft survival in comparison to PAK and PTA. UK one- and five- year patient survival following SPKT are 96% and 90% respectively, compared to 95% and 85% for pancreas only transplant (PTA and PAK). One- and five- year graft survival rates for SPKT are 85% and 77% respectively, compared to 65% and 44% for pancreas only transplants (PTA and PAK) (NHS BT, 2014). Internationally one- and five- year pancreas graft survival following SPKT are currently 86% and 73% respectively, compared to 79% and 58% respectively for PAK and 78% and 54% respectively for PTA (A. Gruessner, personal communication (email) 12/11/2014). Current one- year patient survival following SPKT is 97%, compared to 98% for PAK and 96% for PTA (A. Gruessner, personal communication (email) 12/11/2014).

International immunologic graft loss within the first year is significantly lower in SPKT recipients (2.1%) when compared to PTA (6.6%) and PAK (5.5%) (Gruessner, Sutherland et al. 2010). In a review of 1000 PTs at a single-centre in the USA (University of Wisconsin, Madison), 5, 10 and 20 year pancreas graft survival following SPKT are 76%, 63% and 36% respectively with patient survival quoted at 89%, 80% and 58% respectively (Sollinger,
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Odorico et al. (2009). This compares with a predicted 10 year survival of less than 5% for patients on the waiting list.

Current patient and graft survival in the UK compares favourably with equivalent IPTR data (Gruessner and Gruessner 2013). The long-term (> 10 years) UK data is only now emerging and must be seen in context of a developing programme. They represent a period when the procedure in the UK was still in its infancy. There is now international recognition that; developments in surgical technique and immunosuppression, greater centre experience of recipient and donor selection and improvements in peri-operative management lead to improvements in re-laparotomy rates, thrombosis rates and rates of intra-abdominal collections (Humar, Kandaswamy et al. 2000) which suggest that we would expect these improvements to be reflected in long-term UK outcomes when analysed over the following 10-years.
Peri-Operative Optimisation

Improvements in the technical approach to surgery, immunosuppression and careful donor and recipient selection have led to significant improvements in graft and patient survival following PT, which have now begun to plateau at an acceptable level (Gruessner and Gruessner 2013). However, high complication rates persist and remain unacceptable. They are accounted for by the multi-system morbidity caused by IDDM in our patients and the surgical, physiological and immunological insult inflicted upon them at the time of transplantation. These risks and co-morbidities are unique to this cohort of patients and comparisons are difficult to make with other critically ill patients. However, in other types of major acute physiological insults (major surgery, trauma and sepsis) affecting high-risk patients, supra-physiological peri-operative optimisation (Goal Directed Therapy, GDT) has been shown to improve outcomes in numerous clinical trials (Shoemaker, Appel et al. 1988, Boyd, Grounds et al. 1993, Bishop, Shoemaker et al. 1995, Wilson, Woods et al. 1999, Lobo, Salgado et al. 2000, Rivers, Nguyen et al. 2001, Gan, Soppitt et al. 2002, Pearse, Dawson et al. 2005, Wakeling, McFall et al. 2005) and systemic reviews/metanalysis (Gurgel and do Nascimento 2011, Hamilton, Cecconi et al. 2011, Pearse, Harrison et al. 2014).

GDT is the physiologic haemodynamic optimisation of patients with the aim of improving clinical outcomes. The ultimate physiological end-point of all GDT protocols is to improve tissue oxygenation, whether this is measured directly or as a surrogate. This is achieved with the use of a cardiac output monitor guiding inotrope treatment and fluid optimisation per protocol, with the aim of achieving supra-normal predefined physiological goals. The concept came to the fore in the 1980’s with the advent of the Swan-Ganz pulmonary artery (PA) catheter (Swan, Ganz et al. 1970, Ganz, Donoso et al. 1971) which gave clinicians the
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ability to dynamically monitor cardiac output in the critically ill patient. Shoemaker et al. (Shoemaker, Montgomery et al. 1973) used the PA catheter to identify physiologic differences between shocked patients and correlated these parameters to clinical outcomes. The group initially described “lower cardiac output, higher pulmonary vascular resistance, reduced oxygen transport, acidosis, higher arterial carbon dioxide tension, greater reductions in haematocrit reading and blood volume” as all being factors in predisposing to peri-operative mortality. Following on from this seminal paper, the group narrowed this list down to “blood flow, oxygen transport and most intravascular pressures” as being the key physiological factors which required optimising to supra-normal values to ensure improved clinical outcomes (Bland, Shoemaker et al. 1978), and if this was not achieved spontaneously by the patient an “oxygen debt” developed, resulting in increased morbidity and mortality. Given these observations and theories, they instituted the first randomised clinical trial (RCT) investigating the benefits of supra-normal physiological, protocol driven optimisation in high-risk surgical patients. They admitted high-risk patients (defined in Table 1.4a) pre-operatively to the intensive care unit (ICU), inserted PA catheters and optimised them, using a combination of fluid resuscitation, inotropes, vasodilators and vasopressors per protocol, to achieve supra-normal values of cardiac index (CI >4.5l/min/m²), oxygen delivery (DO₂I >600ml/min/m²) and oxygen consumption (VO₂I >170ml/min/m²) compared with “normal” values (CI 2.8- 3.5 l/min/m², DO₂I 400-550ml/min/m², VO₂I 120- 140ml/min/m²). This therapy resulted in reduction in mortality rates from 33% to 4%, as well as reduced post-operative morbidity and shorter length of critical care unit and hospital stays (Shoemaker, Appel et al. 1988).
Advantages of Peri-Operative Haemodynamic Optimisation

Since this seminal trial the benefits of GDT have been well published. Multiple trials in general surgery (Boyd, Grounds et al. 1993, Wilson, Woods et al. 1999, Lobo, Salgado et al. 2000, Pearse, Dawson et al. 2005, Wakeling, McFall et al. 2005), trauma (Bishop, Shoemaker et al. 1995) and sepsis (Rivers, Nguyen et al. 2001) have found reductions in peri-operative mortality (28-days) by up to 75% (Boyd, Grounds et al. 1993), reduction in lengths of hospital stay by up to 28% (Donati, Loggi et al. 2007), reductions in intensive care unit stays by up to 30% (Shoemaker, Appel et al. 1988) and improvements in post-operative morbidity by up to 55% (Pearse, Dawson et al. 2005), findings further supported by a number of meta-analysis (Kern and Shoemaker 2002, Gurgel and do Nascimento 2011, Hamilton, Cecconi et al. 2011). In general surgical cases, multiple studies have shown an overall reduction in post-operative complications including respiratory failure, renal failure, cardiac complications and sepsis, whether the intervention is initiated pre- (Shoemaker, Appel et al. 1988, Boyd, Grounds et al. 1993, Wilson, Woods et al. 1999), intra- (Gan, Soppitt et al. 2002, Wakeling, McFall et al. 2005, Donati, Loggi et al. 2007, Mayer, Boldt et al. 2010) or post- (Pearse, Dawson et al. 2005) operatively. When incorporated in a recent systematic review, this translates to a 25% reduction in overall complication rates with GDT (Pearse, Harrison et al. 2014).

Furthermore, GDT increases splanchnic perfusion, thereby reducing gastrointestinal complications post-operatively (Mythen and Webb 1995, Wilson, Woods et al. 1999, Gan, Soppitt et al. 2002, Wakeling, McFall et al. 2005). Again meta-analysis and reviews have supported these findings (Bundgaard-Nielsen, Holte et al. 2007, Giglio, Marucci et al. 2009), but no study has investigated the effect of GDT on gastrointestinal complications as a primary outcome.
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One paper has retrospectively investigated the benefits of peri-operative GDT on 15-year outcomes following surgery, finding a survival advantage of GDT, compared to ST by over 1000 days (Rhodes, Cecconi et al. 2010). They postulate that the avoidance of peri-operative complications (infections, renal and cardiac dysfunction) as a result of GDT led to an improved long-term survival advantage.

These improvements in clinical outcomes correlate to considerable cost savings. Shoemaker noted savings of over $20,000 per patient in the intervention arm (Shoemaker, Appel et al. 1988), and numerous authors have subsequently stated an expected cost reduction due to the improved post-operative morbidity and lengths of stay associated with GDT, despite the inherent start-up costs. In sepsis, Rivers suggests that hospital-related costs decrease by 20% with a reduction in hospital length of stay by 5.02 days. Therefore, a hospital treating 250 such patients annually would benefit with 1,250 bed days per year resulting in an estimated cost saving of greater than $11.98 million (Rivers, Jaehne et al. 2010).

The Debate over Peri-Operative Haemodynamic Optimisation

Despite the evidence available, questions were quickly raised about the efficacy of GDT, the monitoring technique, the ideal intervention group, the ingredients of the therapy and its timing. GDT was potentially regarded as a homogenous therapy with benefits over a heterogeneous group of patients. This is not the case and this lack of clarity has resulted in an underutilisation of the technique (Rivers 2010).
Very quickly and unintentionally, the fate of GDT became intertwined with that of the PA catheter. In the late 1980s and early 1990s numerous studies brought into question the benefits obtained by the use of the PA catheter. Initially, observational studies noted increased rates of morbidity and mortality specifically related to the insertion of the PA catheter, with a possible three-fold increased risk in cardiac events associated with its use (Gore, Goldberg et al. 1987, Connors, Speroff et al. 1996, Polanczyk, Rohde et al. 2001). Additionally, in a large RCT (n= 1994, (Sandham, Hull et al. 2003)) investigating the effect of GDT using the PA catheter as a mode of cardiac output monitoring, significantly increased rates of PE were noted in the intervention arm compared to the standard therapy arm (0.8% vs. 0%) with no clinical benefit associated with the intervention, a finding corroborated by numerous other authors (Tuman, McCarthy et al. 1989, Hayes, Timmins et al. 1994, Gattinoni, Brazzi et al. 1995, Harvey, Harrison et al. 2005). Despite this, other investigators persisted and did note improvements in morbidity, mortality and lengths of stay following use of the PA catheter to guide optimisation strategies following trauma (Bishop, Shoemaker et al. 1995), major vascular and general surgery (Wilson, Woods et al. 1999, Lobo, Salgado et al. 2000, Polonen, Ruokonen et al. 2000). However, the reported morbidity associated with the use of the PA catheter was considered too high in non-cardiac surgery and as a result, under-utilization of the PA catheter outside of this specialty resulted in a growing lack of knowledge associated with its use and the interpretation of the results among these anaesthetists and intensivists (Iberti, Fischer et al. 1990, Iberti, Daily et al. 1994, Soni 1996, Gnaegi, Feihl et al. 1997). Given that GDT almost exclusively requires cardiac output monitoring, and with no other viable alternative, GDT also became unused.
However, with the advent of minimally invasive cardiac output monitors the risks of PA catheter insertion have been nullified. The breakthrough initially came with the Oesophageal Doppler Monitor (ODM) (Cathignol, Lavandier et al. 1985). This allowed clinicians to use the ODM and successfully reduce peri-operative complications and critical care unit and hospital lengths of stay by between 20-30%, following major abdominal, general, vascular and cardiothoracic surgery (Gan, Soppitt et al. 2002, McKendry, McGlione et al. 2004, Wakeling, McFall et al. 2005). However, limitations of the ODM include; its use is confined to unconscious patients, it is not calibrated and therefore unable to give absolute values with which to guide protocols and the quality of readings obtained by the ODM are user dependent.

In 1993 the Lithium Dilution Cardiac Output monitor (LiDCOplus) (Linton, Band et al. 1993) was developed as one of the first minimally invasive cardiac output monitors available. It served as a calibrated means of measuring cardiac output and oxygen delivery based on pulse pressure and waveform analysis of the in-situ arterial catheter. In 2005 Pearse used LiDCOplus post-surgery to reduce post-operative complications by 35% and hospital stay by 27% after high-risk general surgery (Pearse, Dawson et al. 2005).

In addition to the controversy about which cardiac output monitor to use, investigators brought into question the targets that should be aimed for. Hayes claimed that attempting to achieve unrealistic targets with aggressive resuscitation might be detrimental (Hayes, Timmins et al. 1994). They aimed for the same targets as those set by Shoemaker (Shoemaker, Appel et al. 1988), but 70% of their intervention arm did not achieve all the targets set. Gattinoni (Gattinoni, Brazzi et al. 1995) also found no benefit of GDT, having
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aimed for a supra-normal cardiac index, but again, 55% of their intervention arm did not achieve the goals set. In reality investigators have varied their goals depending on the mode of cardiac output monitoring used and success of attaining the targets in trials which have observed improvements in outcome has ranged from 60% (McKendry, McGloin et al. 2004)- 100% (Gan, Soppitt et al. 2002). Commonly, those choosing to use the PA catheter have used supra-normal DO₂I and/ or CI as an ultimate goal. Occasionally, groups have also used lactate (Polonen, Ruokonen et al. 2000) or mixed venous oxygen saturations (ScVO₂) greater than 70% (Rivers, Nguyen et al. 2001) as end-goals for their protocol, all noting improvements in outcomes. In studies using ODM as a monitor, the end-goal used is stroke volume optimisation (Gan, Soppitt et al. 2002, Wakeling, McFall et al. 2005).

Furthermore, there has been much debate about the timing of the intervention. In modern clinical practice, pre-operative admission to ICU for physiological optimisation, as in the original Shoemaker protocol (Shoemaker, Appel et al. 1988), is logistically prohibitive, despite potential longer-term advantages. In a systematic review, Heyland et al claim that an intervention is more likely to be successful if initiated pre-operatively (Heyland, Cook et al. 1996). Kern and Shoemaker in their review, included septic patients, where it is obviously not possible to start the optimisation protocol prior to the physiological insult (Kern and Shoemaker 2002). They found a significant reduction in mortality, but rather than it being directly related to the physiological insult, it is related to the timing of the intervention relative to onset of organ failure. Therefore, optimisation strategies instituted prior to organ failure were more likely to succeed than those initiated after the onset of organ failure. This is consistent with an early observation by the Shoemaker group stating that rather than the pre-operative nature of the intervention, optimisation needs to occur prior to the buildup of an irreversible oxygen debt and prior to the commencement of
organ failure (Bland, Shoemaker et al. 1978). Their findings suggest a window of opportunity between 4-12 hours following the initial physiological insult, when optimisation can be instituted and lead to improved outcomes. The findings in trauma patients add further support to this theory (Bishop, Shoemaker et al. 1995). Studies have now consistently showed improvements in post-operative outcomes following initiation of either an intra-operative (Mythen and Webb 1995, Lobo, Salgado et al. 2000, Gan, Soppitt et al. 2002, Wakeling, McFall et al. 2005, Donati, Loggi et al. 2007, Lopes, Oliveira et al. 2007, Mayer, Boldt et al. 2010, Cecconi, Fasano et al. 2011, Salzwedel, Puig et al. 2013) or post-operative (Polonen, Ruokonen et al. 2000, McKendry, McGloin et al. 2004, Pearse, Dawson et al. 2005, Jhanji, Vivian-Smith et al. 2010) optimisation protocol in heterogeneous groups of critically ill surgical, trauma and septic patients. The benefit of these later studies is that they suggest that pre-operative admission to ICU is not warranted. One can deduce that whilst optimisation to achieve improvements in clinical outcome is possible at later stages following the physiological insult, it is likely that the earlier it is instituted, the higher the chances of improvements in outcome and the greater the effect will be.

Moreover, just as the therapy in previous studies has not been homogenous, the target cohort has not been homogenous either, further muddying the indications and applications of GDT. High-risk surgery in the UK accounts for 12.5% of procedures carried out, but for 80% of the deaths (Pearse, Harrison et al. 2006). Furthermore, at the outset of GDT, Shoemaker recognised that the highest risk patients were likely to benefit most from GDT (Shoemaker, Appel et al. 1988). Subsequently, systematic reviews and meta-analysis have found that patients with greater than 20% predicted mortality are likely to benefit most from optimisation strategies (Kern and Shoemaker 2002, Gurgel and do Nascimento 2011). However, this group of patients is small and is difficult to predict prospectively. Studies
have classified risk according to patient and/or operative characteristics, in a similar way to Shoemaker (Shoemaker, Appel et al. 1988) (Table 1.4 a and b) and although these criteria are well accepted, subsequent studies have created their own criteria, likely dependent upon their individual patient cohorts. Despite this disparity between studies, the selection of patients based on the terms “high-risk surgery” or a “high-risk patient” follow a common theme.

High-risk surgery is often categorised according to the extent or invasiveness of surgery, length of surgery and National Confidential Enquiry into Patient Outcome and Death (NCEPOD) category. The high-risk patient is often categorised according to age, pathology, the number of co-morbidities and the number of systems involved. Both of these lend themselves to a subjective, rather than objective assessment of risk. Even use of recognised scoring systems such as American Society of Anaesthetists (ASA) scoring are primarily subjective (Mayer, Boldt et al. 2010). Multiple articles used Physiologic and Operative Severity Score for the enUmeration of Mortality and Morbidity (POSSUM) scoring as a pre-operative risk stratification tool, but none used POSSUM as inclusion/exclusion criteria for the studies. To date, only one group have attempted to differentiate patient risk according to objective physiological measures. Challand defined high-risk patients according to anaerobic threshold (AT) based upon cardiopulmonary exercise test (CPET) results (Challand, Struthers et al. 2012). In this study, all patients were undergoing major colorectal surgery and were recruited independent of their aerobic fitness levels. High-risk was defined by an AT of less than 11ml/kg/min. They noted no benefit with GDT in either high or low risk group, but claim a higher rate of blood loss in the aerobically fit GDT group when compared to the standard therapy arm. However, the aerobically fit patients perhaps should not have been considered as high-risk and therefore not recruited to the study and
the intervention only used fluids to optimise the patients, not the full armory of medical interventions available, therefore casting some doubt over the validity of the intervention and the study.

This final point highlights the final enigma in studying GDT as a single entity. The timing of the therapy, the monitoring used, the end-goals and the target group are not homogenous in assessing the benefits of GDT. However, neither are the ingredients which provide the therapy. Again, at the outset, Shoemaker used a combination of fluid replacement, blood products, inotropes and vasopressors to optimise these patients. In an effort to simplify protocols and make the intervention more acceptable to clinicians, subsequent investigators have used varying combinations of these ingredients to test for similar clinical outcome benefits. With the use of the ODM, due to the nature of the information it provided, investigators who chose this monitoring technique were almost entirely dependent upon using fluid replacement in their optimisation strategies (Gan, Soppitt et al. 2002, McKendry, McGloin et al. 2004, Wakeling, McFall et al. 2005). With the advent of minimally invasive calibrated cardiac output monitors, the option was available to consider a more aggressive approach to optimisation. Since Pearse (Pearse, Dawson et al. 2005) a number of investigators have once again used the full armamentarium of interventions available to improve outcomes following GDT, whilst using calibrated cardiac output monitors to guide their interventions and achieving improvements in clinical outcome (as discussed above). In the most recent attempt to simplify a GDT protocol whilst using an uncalibrated cardiac output monitor (LiDCOrapide) with fluid optimisation and a fixed rate inotrope infusion (Pearse, Harrison et al. 2014), Pearse et al. failed to show any improvements in outcome between the intervention and standard therapy arms. Again, these studies suggest that whilst it is possible to optimise some patients with minimal
intervention provided as a blanket treatment, calibrated cardiac output monitoring provides an individualised approach to physiological management and using all the required therapies available will increase the chances of appropriately optimising patients.

The National Institute for Health and Clinical Excellence (NICE) now recommend the use of minimally invasive cardiac output monitoring in critically ill patients (Ghosh, Arthur et al. 2011) and a Cochrane review advises that given the heterogenous nature of previous trials in this field, further research is required to identify specific components of peri-operative optimisation which are beneficial in specific clinical instances (Grocott, Dushianthan et al. 2013). Therefore, Chapter 3 is an RCT comparing the clinical outcomes of peri-operative haemodynamic optimisation (Goal Directed Therapy, GDT) with standard peri-operative therapy (ST) following SPKT.
Biological Markers in Clinical Practice

A biomarker is a quantifiable and reproducible clinical sign (biochemical or clinical) observed in a patient which relates to their medical condition. For the biomarker to be relevant, it has to be clinically useful and validated in that context (Strimbu and Tavel 2010). Biomarkers are commonly used to aid in the diagnosis, management and prognosis prediction of diseases and provide targets for new pharmacological therapies. Furthermore, research continues to delineate the utility of specific biomarkers in specific clinical scenarios.

Prior to being able to use biomarkers advantageously in any clinical context, their baseline values need to be defined and in acutely evolving physiological states, their temporal evolution requires clear delineation.

Inflammation is a complex evolutionary process and the identification of specific inflammatory marker (IM) profiles is a distilled version of the genuine natural processes. However, the knowledge gleaned from these complex cases have clinical utility and the biomarker profiles in conditions involving major acute physiological insults such as sepsis, trauma and major surgery (Smith and Giannoudis 1998, Gabay and Kushner 1999, Desborough 2000, Faix 2013), and chronic medical diseases such as diabetes and end-stage renal failure (Stenvinkel and Alvestrand 2002, Chatzigeorgiou, Harokopos et al. 2010) have been described.
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Acute production of pro-inflammatory cytokines leads to increased vascular permeability causing capillary leak and tissue oedema. In severe cases this causes significant third space fluid loss resulting in peripheral and pulmonary oedema, hypotension, tachycardia, multi-organ failure and death. The vascular endothelium becomes pro-coagulant, attracting platelets and leucocytes and activation of the clotting and fibrinolysis cascades initially lead to formation of micro-thrombi and then clotting abnormalities and bleeding. Activation of the kinin system causes vasodilation and worsening of hypotension and end organ perfusion and activation of the complement system leads to lysis and cell death.

Inflammatory markers in Sepsis

Sepsis is the presence of the Systemic Inflammatory Response Syndrome (SIRS) secondary to a known infective organism. Analysis of IM and cytokine profiles have formed the basis of research into possible treatment regimens for severe sepsis. The theory being that if the cascade of inflammation can be halted, then the deleterious effects of severe inflammation can also be halted. Studies have investigated the profile of IM levels in severe sepsis (Pinsky, Vincent et al. 1993, Kellum, Kong et al. 2007, Rivers, Kruse et al. 2007) and identified cytokines as playing an integral role in this process.

Cytokines are low-molecular weight proteins, produced by many immune cells that can be categorised into two broad groups, T-helper 1 (Th1) and T-helper 2 (Th2) cells, which evoke the phagocyte-dependent and antibody mediated responses to inflammation respectively. These responses include release of lymphokines, interleukins (IL) and chemokines, which are characteristics of an inflammatory response. The effects of cytokines are regulated in two ways; either by the antagonistic action of each other (pro- and anti- inflammatory cytokines) and/ or via cytokine receptor antagonists.
In some cases, cytokines (IL-6 and 8) are now being used in routine laboratory tests to diagnose infection and inflammation (Herzum and Renz 2008). However, patient demographics and pathologies affect IM cascades and the levels and peaks of IM production. Therefore, care must be taken when applying knowledge of IM from one scenario to another.

Acute Phase Proteins (APPs) also rise in response to infection or inflammation and have a short half-life. C-Reactive Protein (CRP) is an APP often measured in clinical medicine as a marker of inflammation or infection. It is synthesised in the liver in response to IL-1, IL-6 and TNF-α (Gabay and Kushner 1999) and correlates well with levels of these cytokines (Oberhoffer, Vogelsang et al. 1999). It rises within six hours of the insult and continues to rise to a peak level at 48-72 hours. However, if the source of insult persists, CRP will remain elevated until the source is removed. CRP binds to polysaccharides on the surface of micro-organisms and dying cells to form complexes which activate the complement pathway leading to phagocytosis and cell death (Mold, Gewurz et al. 1999).

Finally, the most common biomarker of sepsis to be measured in hospital medicine is White Cell Count (WCC). Leucocytosis is present in almost every episode of sepsis. Leucocytes are responsible for modulating inflammatory, infectious and fibrotic responses, killing and disposing of foreign micro-organisms and production of antibodies and cytokines, as discussed above. Their roles are so encompassing that measuring levels of leucocytes for sepsis is highly sensitive but poorly specific. Also, due to the necessity of their functions, using therapies to manipulate disease progression is almost impossible.
Inflammatory Markers in Surgery

Surgery is associated with a systemic acute rise in IM levels. The first cytokines to be released post-surgery are IL-1, IL-6 and TNF-a and the levels of these cytokines are proportional to the extent of surgery (Desborough 2000). This in turn causes a rise in CRP and leucocytosis. In addition, cytokines are produced and released locally at the site of surgical trauma (Syk, Mangell et al. 2002). Anaesthesia per se is unlikely to influence levels of cytokine production, further emphasising its release in relation to tissue trauma (Desborough 2000). Nevertheless, IL-6 production can be reduced by anti-inflammatory agents (e.g. steroid and Indomethacin) (Schulze, Sommer et al. 1992) and under physiological stress, IMs stimulate release of cortisol which acts as a negative feedback on cytokine release itself, therefore reducing serum concentrations of IMs (Jameson, Desborough et al. 1997) and tempering the inflammatory insult.

Inflammatory Markers in End-Stage Renal Disease and IDDM

There are a number of causes for inflammation in end-stage renal disease (ESRD). These causes are different to the ones mentioned above as they are associated with chronic inflammation as a result of ESRD and the mode of dialysis (Stenvinkel 2002). Reduced clearance of cytokines, chronic disease (heart failure, atherosclerosis and primary inflammatory diseases) and persistent sub-clinical infections are partly due to the cause of inflammation in all ESRD patients. In addition to this, patients on haemodialysis (HD) may suffer from fistula complications and patients on peritoneal dialysis (PD) may suffer from superficial infections of the catheter track and peritonitis. Increased CRP correlates to increased mortality in HD patients (Bologa, Levine et al. 1998, Kimmel, Phillips et al. 1998, Stenvinkel, Andersson et al. 1999, Zoccali, Benedetto et al. 2000).
Despite the causes of inflammation being chronic as opposed to acute, the biomarkers of clinical utility are again CRP, IL-1, IL-6 and TNF-α (Stenvinkel 2002). Raised levels of all these IMs are associated with higher rates of cardiovascular disease and mortality in ESRD patients, but because of the chronic nature of the disease processes, therapies can be instituted to reduce the long-term IM load. Angiotensin-converting enzyme inhibitors reduce TNF-α and CRP, and Aspirin reduces CRP and IL-6 in ESRD patients (Ikonomidis, Andreotti et al. 1999, Stenvinkel, Andersson et al. 1999, Kronish, Rieckmann et al. 2010).

CRP, IL-6 and TNF-α are also raised in patients with IDDM (Mitrovic, Ilic et al. 2011). It is likely that all the Th1 and Th2 cytokines are raised in IDDM, but the Th1 group tends to have higher levels within the first six months of disease diagnosis compared to the Th2 group (Chatzigeorgiou, Harokopos et al. 2010, Snell-Bergeon, West et al. 2010). CRP is independently associated with increased risk of ischaemic heart disease in diabetes (Aseganokar, Marathe et al. 2011) and is a predictor of cardiovascular morbidity and mortality (Ridker, Hennekens et al. 2000).

For patients undergoing PT these findings are particularly concerning as IL-1 and TNF-α are cytotoxic to pancreatic islet cells and destroy B-cells (Mandrup-Poulsen, Helqvist et al. 1990) and IL-6 and TNF-α induce insulin resistance in muscle cells and hepatocytes (Dandona, Aljada et al. 2004).

Given these findings it would seem particularly advantageous to investigate the pattern of these markers in the peri-operative period following transplantation.
Inflammatory Markers in Transplant Surgery

The vast majority of studies investigating the role of IMs in transplant surgery have done so with a view to investigating the role of IMs in rejection. This is particularly important in transplantation because the IMs commonly used in clinical practice (CRP, WCC, platelets) are sensitive, but not specific to rejection. A serum marker specific for rejection has not been discovered and the benefits of finding one are great. To date, findings suggest that a panel of IMs may be useful in the differentiation of graft rejection and infection, but no specific one can differentiate the two conditions.

Cytokines play a role in graft rejection. An early generalised increase in IMs post-lung transplantation have a higher occurrence of graft rejection (Bharat, Narayanan et al. 2007). The “10th Congress of the European Society for Organ Transplantation: Pancreas Transplantation” [2011] discussed the roles of cytokines in acute renal graft rejection. It concluded that IL-2 and IFN-γ are both important in precipitating graft rejection. It also concluded that IL-4 levels increase prior to a clinical episode of rejection and that patients with high levels of post-operative IL-10 and TNF-α have poorer graft survival following renal transplantation.

In PT, IL-4 and IFN-γ have been implicated in graft rejection in animal work (Han, Zhang et al. 2006), but there is a paucity of data in human subjects.

In addition, there is very little literature investigating the role of IMs in the peri-operative period specific to whole-organ transplant surgery. Raised IL-6 levels post lung transplantation are related to an increased incidence of primary graft dysfunction (Moreno, Mir et al. 2008). In renal transplantation, metabolic stresses to the kidney have led to a
production of pro-inflammatory cytokines (IL-6, IL-8 and TNF-α) (Fougeray, Bouvier et al. 2011).

In relation to inflammation of the pancreas (pancreatitis), clinically this may progress to SIRS and death in up to 5% of individuals. Patients with pancreatitis suffer from a generalised inflammatory response resulting in the rise of plasma concentrations of pro-(IL-1B, IL-6 and TNF-α) and anti-inflammatory (IL-1RA and IL-10) cytokines (Brivet, Emilie et al. 1999). Raised levels of IL-6, IL-8 and CRP are also early markers of organ failure following pancreatitis (Malmstrom, Hansen et al. 2012).

Of particular concern in relation to PT are findings during islet transplantation which suggest that Islet β-cells themselves produce pro-inflammatory cytokines IL-6 and IL-8 following implantation (Barbe-Tuana, Klein et al. 2006). Recognising the deleterious effects of pro-inflammatory cytokines during islet cell transplant, centres have now introduced the use of anti-inflammatory therapies (Etanercept) to minimise these effects, leading to improved rates of insulin independence when compared to other induction regimens (100% insulin independence, compared to 20% insulin independence at 18 months (Faradji, Tharavanij et al. 2008)) But, these studies are small, observational, single-centre experience and the results must be interpreted with this in mind. There are multiple factors which changed between these two groups and the use of an anti-inflammatory agent was only one such change in the protocol. However, defining the inflammatory sequelae following islet transplantation enabled the initiation of appropriate therapies which may have clinically beneficial effects.
In PT, despite the multiple co-morbidities affecting our patients, the considerable physiological insult inflicted upon them during surgery and the life-long immunosuppression they face, the peri-operative biomarker profiles have not been delineated. Therefore, Chapters 4 and 5 initially, identify the potentially relevant inflammatory and diabetes markers in the early post-operative period following SPKT (Chapter 4) followed by determining the peri-operative patterns of inflammatory and diabetes markers of potential clinical importance and finally establish a correlation of these biomarkers with clinical factors involved in SPKT (Chapter 5).
Contrast Enhanced Ultrasonography

Contrast Enhanced Ultrasonography (CEUS) involves the use of traditional ultrasound (US) in combination with microbubble contrast technology to provide a clear interface between the contrast and surrounding tissue. The contrast enhances the reflection of US waves, providing clearer differentiation between tissues and vasculature, organs and pathology. It is used as a diagnostic tool to help enhance regular US and doppler and in certain scenarios has the potential to replace computer tomography (CT) and magnetic resonance imaging (MRI).

US contrast agents were first developed and came to market in 2000. Levovist was the first US contrast agent to market and was widely utilised for use in characterising liver lesions (Bertolotto, Dalla Palma et al. 2000, Burns, Wilson et al. 2000), but is now no-longer manufactured. Sonovue was initially developed for use in cardiac imaging, but quickly replaced Levovist for use in Liver CEUS and is now the most common US contrast agent in use.

US Contrast Agents

All US contrast agents are composed of two elements:

1. Shell: this determines the “residence time”, the time the micro-bubble is available in the circulation. This is dependent upon the affiliation of the shell to water and the elasticity of the shell. Hydrophobic and elastic shells have a longer half-life than hydrophilic, stiff shells.

2. Gas core: this determines the echogenicity of the micro-bubble. Each bubble is gas filled and the heavier the gas the more echogenic the bubble is, because heavier gases are less likely to leak out of the bubble when compressed by US waves.
SonoVue is composed of a hydrophobic phospholipid shell filled with sulphur hexafluoride (SF6). Each SonoVue kit contains 25mg of lyophilised powder, per vial, to be reconstituted into solution with sodium chloride. In this form, each millilitre of reconstituted contrast agent contains 8µl of SF6 microbubbles. The mean diameter of each bubble is 2.5µm, considerably smaller than a red blood cell (mean 8µm). The half-life of Sonovue is approximately 6 minutes and is excreted via exhalation (Schneider 1999). These characteristics increase the stability and resistance to external pressure (US waves), therefore slowing the diffusion of the gas into blood and thereby reducing the volume of gas required per bubble. Ultimately, this minimises potential adverse effects of the contrast (Greis 2004).

Uses of CEUS

Historically CEUS has predominantly been used in conjunction with SonoVue to characterise both normal and pathological liver morphology and architecture. The first guidelines published for the use of SonoVue were almost entirely based on its use in hepatobiliary imaging (Albrecht, Blomley et al. 2004). More recent guidelines have emphasised the non-hepatobiliary applications for SonoVue (Claudon, Cosgrove et al. 2008) which has led to “an exponential increase in the clinical context in which it is used” (Piscaglia, Nolsoe et al. 2012).

Furthermore, software packages are now available to analyse flow and perfusion patterns observed with CEUS, therefore allowing quantitative assessment of organ perfusion and potentially correlating these quantitative measures with clinical outcomes. Specifically,
these have been used in renal transplantation and will be discussed later in the chapter.

CEUS is used in surgical contexts including the evaluation of severity of inflammatory bowel disease (Migaleddu, Scanu et al. 2009), the characterisation of focal splenic lesions (Gorg and Bert 2005) and in scanning for abdominal trauma, as an adjunct to FAST (Focused Assessment with Sonography in Trauma, (Catalano, Aiani et al. 2009)). Vascular uses of CEUS include the diagnosis of carotid artery disease, particularly the detection of plaque ulceration, flow and thrombus (Clevert, Sommer et al. 2011) and the technique has a sensitivity and specificity of 100% and 93% respectively in the detection of endoleaks following endo-vascular abdominal aortic aneurysm repair (EVAR) in experienced hands (Clevert, Minaifar et al. 2008).

CEUS use in renal disease is particularly advantageous as the brisk vascular supply ensures that kidneys enhance quickly, whilst SonoVue does not undergo renal excretion. In structural renal disease, CEUS is more sensitive than CT and MRI in characterising complex renal masses (Quaia, Bertolotto et al. 2008) and is used as a confirmatory investigation to monitor the adequacy of ablative therapies for tumour response (Meloni, Bertolotto et al. 2008). CEUS can differentiate between malignancy and thrombus in the renal vein (Ignee, Straub et al. 2010) and is superior to doppler US and comparable to CT in demonstrating renal parenchymal perfusion, non-perfused, infarcted and hypoperfused kidneys (Bertolotto, Martegani et al. 2008). In addition, CEUS can improve sensitivity of renal artery stenosis detection by 10% when compared to the use of Doppler US alone (Blebea, Zickler et al. 2003).
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CEUS has also been used in the context of native pancreatic lesion assessment. With experienced operators it is able to accurately diagnose tumours of the head of the pancreas and aid in the differentiation of benign and malignant pancreatic head lesions (Itoh, Hirooka et al. 2005). It is also able to identify and delineate necrotic areas which do not enhance on CT scanning in cases of pancreatitis (Ripolles, Martinez et al. 2010), where it can be used to follow-up chronic pancreatitis as a less harmful alternative to CT by avoiding exposure to radiation.

Regarding the use of CEUS in transplantation, the 2011 European Federation and Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines on CEUS (Piscaglia, Nolsoe et al. 2012) categorise transplant surgery as an “emerging perspective and potential future application for CEUS”. Given its previous hepatobiliary indications, CEUS has already evolved into a recognised imaging modality in liver transplantation to monitor both acute and chronic vascular and biliary complications post-operatively (Marshall, Beese et al. 2002, Berry and Sidhu 2004, Clevert, Stickel et al. 2009).

In renal transplantation, traditional qualitative assessment provided by CEUS coupled with quantitative perfusion analysis technology has allowed the calculation of relative organ perfusion (either whole organ, or specific regions of interest within the organ) and flow-related parameters (peak perfusion rates, time to peak perfusion, wash-in and wash-out curves). In this clinical setting, analysis of organ quantification methods have been used to explore possible correlations with chronic transplant dysfunction and in the differentiation of acute tubular necrosis, acute rejection and renal vein thrombosis (Schwenger, Korosoglou et al. 2006, Grzelak, Kurnatowska et al. 2011).
In contrast to the kidney, the pancreas has low volume and pressure vascular flows. This coupled with the technical challenges of implantation results in high rates of vascular complications (Troppmann 2010), resulting in the routine use of peri-operative anti-coagulation. In addition, accurate and timely diagnosis of this event continues to provide a challenge in the immediate post-operative period due to the lack of a safe, mobile, reliable and reproducible method of assessing both organ perfusion and vascular patency before an organ becomes unsalvageable. The documented use of CEUS in PT is sparse. It has been used to pre-operatively assess vasculature and potential organ viability of the allograft prior to implantation, but the clinical relevance of this use is unproven (Aboutaleb, Leen et al. 2011). In a case of possible segmental pancreatic ischaemia, CEUS was used to assess the blood supply to the pancreas allograft and confirmed patent blood-flow, resulting in conservative management and organ preservation. In this case, both CT angiography and Doppler US, would have both indicated surgical intervention and possible subsequent explanation of the graft (Boggi, Morelli et al. 2009). Most recently, and perhaps most clinically relevant, an American group has investigated the use of quantified flow-related measures in the out-patient follow-up of SPKT recipients. They identified the entire pancreas using only CEUS, despite the intraperitoneal, posterior placement of the allograft pancreas. In addition, they suggest that flow-related parameters identified on CEUS could relate to episodes of pancreatic inflammation associated with pancreatic rejection. These flow parameters identified as having changed with episodes of rejection resolved following treatment of rejection episodes (Kersting, Ludwig et al. 2013).

Given the current published wide-ranging uses of CEUS, it potentially offers a novel technique to assess the vascular status of the graft and further inform post-operative management and anti-coagulation on an individualised basis following PT. Validation of this
technique as both reliable and safe would have significant implications. Primarily, it may allow avoidance of CT angiography in the peri-operative period. CT angiography is currently the established imaging technique in suspected vascular complications following PT, but involves both the administration of nephrotoxic contrast coupled with logistic and safety concerns of moving a potentially unstable patient away from the critical care environment to radiology with potential compromise in clinical care. The cost for a CT angiogram is approximately £170, compared to £70 for a CEUS. The advantages of CEUS in this scenario are found in its portability to the bed-side, the opportunity of real-time results that avoid nephrotoxic contrast and the economic benefit. Secondarily, routine use may allow early identification of complications leading to timely interventions and avoidance of some of the morbidities associated with the complications of the procedure. A potential disadvantage is the operator dependence of all US imaging techniques, but this can be negated by using a dedicated radiology team.

Therefore, CEUS in PT remains an evolving field which is under-utilised at present. Chapter 6 presents a proof of principle observational study, investigating the feasibility of using CEUS and explores the potential advantages of CEUS in the peri-operative period following SPKT.
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Summary Statement

PT is now a well-established procedure and patient and graft survivals have very good outcomes. However, peri-operative morbidity continues to be significant, and a source of continued apprehension among clinicians and patients. Outcomes in organ transplantation are multi-factorial, and in addressing the risks involved; donor, organ, recipient and surgical factors need to be considered. Despite the significant improvements seen in PT outcome since its inception, it continues to be considered particularly high-risk. With peri-operative morbidity approaching 70% and one-year graft loss at approximately 10%, this requires quality improvement at each step of the transplantation process.

This research has identified key areas along the patient’s journey which may be enhanced, resulting in improved outcomes. Pre-operative individualised risk stratification would offer more reliable data for use in the consent process and more efficient planning of resources. Chapter 2 therefore recognises the need of a multi-system risk prediction score to help stratify SPKT recipients pre-operatively. Peri-operatively, supra-normal physiological optimisation leads to significantly improved clinical outcomes in other critically ill surgical and non-surgical patients. Therefore, Chapter 3 presents the results of a randomised clinical trial comparing Goal-Directed Therapy and Standard Therapy in SPKT recipients. In addition, the delineation of a biological marker profile in critically ill patients may aid in the prognosis prediction, monitoring and treatment of the disease, and augmentation of biological markers in these patients can result in improved outcomes. Chapters 4 and 5 therefore identify a biological marker profile in SPKT recipients and correlate these findings to clinical parameters. Finally, given that vascular complications continue to remain the most common cause of acute graft loss following PT, Chapter 6 presents an observational clinical study investigating the benefits of Contrast Enhanced Ultrasound following SPKT.
Table 1.1 Guidance for the ideal pancreas donor criteria (Odorico, Heisey et al. 1998, Humar, Ramcharan et al. 2004, Gruessner 2011, Maglione, Ploeg et al. 2013)

<table>
<thead>
<tr>
<th><strong>Age</strong></th>
<th>&lt; 45</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI</strong></td>
<td>&lt; 28</td>
</tr>
<tr>
<td><strong>Cold Ischaemic Time</strong></td>
<td>&lt; 12 hours</td>
</tr>
<tr>
<td><strong>Cause of Death</strong></td>
<td>Not cerebrovascular disease</td>
</tr>
<tr>
<td><strong>Mode of donor death</strong></td>
<td>Donation after brain death (DBD)</td>
</tr>
</tbody>
</table>
## Table 1.2 Pre-Procurement Pancreas Suitability Score (Vinkers, Rahmel et al. 2008)

<table>
<thead>
<tr>
<th>Donor Characteristic</th>
<th>1 Point</th>
<th>2 Points</th>
<th>3 Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) *</td>
<td>&lt;30</td>
<td>30-40</td>
<td>&gt; or = 40</td>
</tr>
<tr>
<td>BMI (kg/m²) *</td>
<td>&lt;20</td>
<td>20-25</td>
<td>&gt; or = 25</td>
</tr>
<tr>
<td>ICU stay (days)</td>
<td>&lt;3</td>
<td>3-7</td>
<td>&gt; or = 7</td>
</tr>
<tr>
<td>Cardiac Arrest (mins)</td>
<td>No</td>
<td>Yes, &lt; 5 mins</td>
<td>Yes, &gt; 5 mins</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>&lt;155</td>
<td>155-160</td>
<td>&gt; or = 160</td>
</tr>
<tr>
<td>Amylase (U/L) OR</td>
<td>&lt;130</td>
<td>130-390</td>
<td>&gt; or = 390</td>
</tr>
<tr>
<td>Lipase (U/L)</td>
<td>&lt;160</td>
<td>160-480</td>
<td>&lt; or = 480</td>
</tr>
<tr>
<td>Noradrenaline OR</td>
<td>No</td>
<td>&lt;0.05</td>
<td>&gt; or = 0.05</td>
</tr>
<tr>
<td>Dobutamine/ Dopamine</td>
<td>No</td>
<td>&lt;10</td>
<td>&gt; or = 10</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>18</td>
<td>27</td>
</tr>
</tbody>
</table>

*BMI, body mass index; ICU, Intensive Care Unit; * to double any point allocation.
### Elements of the Pancreas Donor Risk Index (P-DRI)

<table>
<thead>
<tr>
<th>Element</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Serum Creatinine</td>
</tr>
<tr>
<td>Body Mass Index</td>
</tr>
<tr>
<td>Height</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>Mode of death; Cerebrovascular Event</td>
</tr>
<tr>
<td>Type of Pancreas Transplant; SPKT, PTA or PAK</td>
</tr>
<tr>
<td>Type of donor; Donor after Cardiac Death or Donor after Brainstem Death</td>
</tr>
<tr>
<td>Cold Ischaemia Time</td>
</tr>
</tbody>
</table>

PAK, Pancreas after kidney; PTA, Pancreas transplant alone; SPKT, Simultaneous pancreas and kidney transplant
Chapter 1, Introduction

Table 1.4a. Criteria for High-Risk Patients as defined by Shoemaker (Shoemaker, Appel et al. 1988)

<table>
<thead>
<tr>
<th>Criteria for High-Risk Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous severe cardiopulmonary illness (acute MI, COPD, Stroke etc)</td>
</tr>
<tr>
<td>Severe multiple trauma (&gt;3 organs or &gt;2 systems)</td>
</tr>
<tr>
<td>Massive acute blood loss (&gt;8 units, Blood Volume &lt;1.5l/m², Hct &lt;20%)</td>
</tr>
<tr>
<td>Age &gt;70 years (and evidence of limited physiologic reserve of one or more vital organ)</td>
</tr>
<tr>
<td>Shock (MAP &lt;60mmHg, CVP &lt;15cmH₂O and UO &lt;20ml/hr)</td>
</tr>
<tr>
<td>Septicaemia (positive blood cultures or septic focus, WBC &gt;13,000, spiking fever to 101°F for 48 hours and haemodynamic instability)</td>
</tr>
<tr>
<td>Respiratory failure (eg. PaO₂ &lt;60% on FiO₂ &gt;0.4, Qsp/Qt &gt;30% or PaO₂ &lt;8kPa on FiO₂ &gt;0.4 (PaO₂/FiO₂ ratio &lt;20) or mechanical ventilation needed &gt; 48hours)</td>
</tr>
<tr>
<td>Acute Renal Failure (BUN &gt;50mg/dl, Cr &gt;3mg/dl or Urea &gt;20mmol/l, Cr &gt;260micro mol/l)</td>
</tr>
<tr>
<td>Late stage vascular disease affecting the aorta</td>
</tr>
</tbody>
</table>

MI, Myocardial Infarction; COPD, Chronic Obstructive Pulmonary Disease; Hct, Haematocrit; MAP, Mean Arterial Pressure; CVP, Central Venous Pressure; UO, Urine Output; WBC, White Blood Cells; PaO₂, Partial pressure of Oxygen in the blood; FiO₂, Fraction of inspired oxygen; Qsp/QT, venous admixture; BUN, Blood Urea Nitrogen

Table 1.4b Criteria for High-Risk Surgery as defined by Shoemaker (Shoemaker, Appel et al. 1988)

<table>
<thead>
<tr>
<th>Criteria for High-Risk Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive ablative surgery planned for carcinoma (oesophagectomy and total gastrectomy, prolonged surgery &gt;8 hours)</td>
</tr>
<tr>
<td>Opening of two body cavities</td>
</tr>
<tr>
<td>Acute abdominal catastrophe (eg. pancreatitis, gangrenous bowel, peritonitis, perforated viscus, GI bleeding)</td>
</tr>
</tbody>
</table>
Chapter 2, Risk Prediction in SPKT

A Prospective Cohort Study of Risk Prediction in Simultaneous Pancreas and Kidney Transplantation

H A Khambalia
Chapter 2, Risk Prediction in SPKT

2.1 Abstract

Introduction

Current risk prediction scoring systems in simultaneous pancreas and kidney transplantation (SPKT) are limited to organ factors and specific to predicting graft outcome. They do not consider recipient factors, nor inform regarding recipient morbidity. We aimed to assess the utility of commonly used general surgical risk prediction models (P-POSSUM, MODS, Charlson Score, Revised Cardiac Risk Index, ASA and Waterlow Score (WS)) and correlate to length of hospital stay (LOS) and critical care length of stay (CCLOS), important surrogate markers of patient morbidity.

Methods

All risk prediction scores were prospectively conducted on all SPKT recipients from November 2011- October 2013. All lengths of stay were recorded on a readiness to discharge basis.

Results

57 SPKT recipients were analysed: mean age was 42 years (SD 7.60), 27 (52%) were male, and mean BMI was 25.43kg/m² (SD 3.11). Mean pancreas and kidney CIT were 703mins (SD 182) and 849mins (SD 192) respectively.

Median LOS and mean CCLOS were 17 days (8- 79) and 7 days (SD 4.04) respectively. When correlated to risk prediction scores, WS was the only score to significantly correlate with
LOS and CCLOS (p<0.001 (Spearman’s correlation) and p= 0.001 (Pearson’s correlation) respectively). No other risk prediction scoring system correlated to either outcome measure (p> 0.05).

Conclusions

Pre-operative risk prediction plays an important part in planning peri-operative care. To date, no validated risk prediction scoring system exists for SPKT. This prospective study indicates that WS identifies high-risk individuals and has value in the pre-operative assessment of outcome following SPKT.
2.2 Introduction

Risk and outcome prediction scores are required prior to major surgery to aid in the counselling and consent processes and in planning intra- and post-operative care. The search for a reproducible and accurate model has been ongoing for over 30 years (Charlson, Sax et al. 1987). Several scoring systems are established and validated with variable sensitivity in the context of general surgery (predominantly abdominal and vascular), to help stratify and quantify an individual’s risk in the peri-operative period. These include Physiologic and Operative Severity Score for the Enumeration of Mortality and Morbidity (POSSUM) (Copeland, Jones et al. 1991), Multiple Organ Dysfunction Scores (MODS) (Marshall, Cook et al. 1995), Revised Cardiac Risk Index (Lee, Marcantonio et al. 1999), Charlson Score (Charlson, Pompei et al. 1987) and American Society of Anaesthesiologists Score (ASA). They are all readily available in clinical practice and are used in decision making prior to undertaking major general surgery.

Despite the number of validated risk-prediction scoring mechanisms available in major general surgery, there is a relative paucity of quantitative assessment tools in transplantation. An ideal transplant specific scoring system would require the consideration of recipient, donor and organ factors and aim to predict organ survival and/or patient morbidity/ mortality post-operatively. However, the large number of confounding factors and outcomes render existing systems relatively insensitive. Scoring systems therefore concentrate on specific confounders, with the aim of predicting a specific primary outcome. In liver transplantation the MELD score is used to score the severity of disease in the recipient (Brown, Kumar et al. 2002), whereas the Euro-transplant Liver Donor Risk Index accounts for donor factors (Braat, Blok et al. 2012).
Chapter 2, Risk Prediction in SPKT

In pancreas transplantation (PT), despite the significant long-term multi-system benefits of euglycaemia (Fiorina, La Rocca et al. 2000, Woeste, Wullstein et al. 2003, Mehra, Tavakoli et al. 2007) and in over 80% of cases simultaneous independence from renal replacement therapy, immediate risks following transplantation remain significant and unpredictable (Humar, Ramcharan et al. 2004). They include vascular complications (Troppmann 2010), graft pancreatitis (Grewal, Garland et al. 1993), enteric complications (leak, fistulae and intra-abdominal collections) (Lall, Sandrasegaran et al. 2006) and infective complications, which may occur in up to 75% of recipients (Bassetti, Salvalaggio et al. 2004). Therefore, risk prediction scores have been developed (Pre-Procurement Pancreas Suitability Score (P-PASS) (Vinkers, Rahmel et al. 2008) and The Pancreas Donor Risk Index (P-DRI) (Axelrod, Sung et al. 2010)) but are limited to analysing donor factors and specific to predicting graft outcome. They are unable to inform the clinician or patient about an individual’s post-operative morbidity and recovery time.

Retrospective studies investigating recipient factors have consistently found that high Body Mass Index (BMI) and increasing recipient age predict poorer outcome post SPKT with higher rates of morbidity in these groups (Sutherland, Gruessner et al. 2001, Gruessner, Sutherland et al. 2004, Ablorsu, Ghazanfar et al. 2008, Sampaio, Reddy et al. 2010). No cumulative score is validated which considers a multi-system approach in assessing recipient morbidity.

The Waterlow Score (WS) (Waterlow 1985) was introduced in the 1980s as a nursing tool to stratify risk for patients with respect to developing decubitus skin ulcers (pressure sores). It uses a multi-system approach and scores patients based upon a number of variables (Table
Chapter 2, Risk Prediction in SPKT

2.1a) before categorising patients into ‘at risk’, ‘high risk’ or ‘very high risk’ for developing pressure ulcers. It is routinely used by medical staff in hospitals in the United Kingdom for this purpose. Recently, evidence has emerged that a high (>20) pre-operative WS correlates with increased in-patient mortality and 30-day morbidity in a general surgical setting (Thorn, Smith et al. 2013).

As WS incorporates a multi-system approach in risk assessment, and SPKT recipients suffer with a multi-system disease, it seems logical that the scoring system may have sensitivity in risk prediction post-SPKT.

The aim of the study was therefore to prospectively assess the utility of commonly used general surgical risk prediction models (P-POSSUM, MODS, Charlson Score, Revised Cardiac Risk Index, ASA and WS), and correlate these to length of hospital and critical care unit stays following SPKT.
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2.3 Methods

The study was registered with and received approval from the Research and Development Division of The Central Manchester University Hospitals NHS Foundation Trust.

All SPKT recipients in a single centre over a two year period (November 2011-October 2013) were included for analysis. During this period, a study investigating two peri-operative optimisation strategies in SPKT recipients was also recruiting. Early graft loss (within 72 hours) was excluded from length of hospital and critical care unit stay analysis, as recovery rates of these patients differ to SPKT recipients. However, the risk prediction scores of patients with early graft losses have also been compared to those patients without early graft loss to assess potential correlations with early graft loss following SPKT. Patient mortality was excluded due to the risk of potential confounders influencing analysis with small cohorts and a lack of data regarding hospital discharge.

All risk scores (P-POSSUM, Charlson Score, MODS, Revised Cardiac Risk Index, ASA and Waterlow Scores) were calculated at the time of admission to hospital by a dedicated researcher, prior to undergoing SPKT (the components of each individual scoring system are illustrated in Tables 2.1a- e). The total number of recipient diabetic complications (history of cardiovascular disease, peripheral vascular disease, previous cerebrovascular incident, retinopathy, autonomic neuropathy, peripheral neuropathy, diabetic nephropathy and hypertension) were also noted and correlated to the outcome measures.
Chapter 2, Risk Prediction in SPKT

For those risk assessment scores requiring physiological data, the values recorded on the ward at admission were used in calculation of that score. Donor data was collected using the “Electronic Offering System” (EOS) online forms. Organ recipient and outcome data were collected prospectively. P-POSSUM and Charlson Scores were calculated using online calculators (http://www.riskprediction.org.uk/pp-index.php and http://www.biomedcentral.com/content/supplementary/1471-2407-4-94-S1.xls) respectively. All scores were correlated with a patient’s total length of hospital stay and length of critical care unit length of stay. Lengths of stay were calculated from date of admission to readiness to discharge from either hospital or critical care.

Traditionally, WS stratifies patients into ‘At Risk’ (WS > 9), ‘High Risk’ (WS > 14) or ‘Very High Risk’ (WS > 19) of developing decubitus skin ulcers. However, in the case of SPKT recipients, an individual’s absolute WS has been correlated with length of hospital and critical care unit stays. Potentially confounding factors (Recipient age, BMI, dialysis status and time on transplant register; length of surgery; pancreas and kidney cold ischaemic times (CIT) and donor age, BMI and DBD/ DCD status) were also correlated with the outcome measures to assess their effect on length of stays.

Transplant protocol

The criteria utilized by individual transplant units for acceptance onto the waiting list for pancreatic transplantation are governed by national guidelines produced by NHS Blood and Transplant and derived from European Best Practice Guidelines. Patients were allocated organs from the waiting list based on the blood group and human leucocyte antigen matching and wait time. Pancreas implantation was undertaken as described in Chapter 3.
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Statistical analysis

Statistical analyses were carried out using SPSS (IBM SPSS Statistics 20, Armonk, New York). Pearson Correlation and Spearman Rank Correlation were used to compare continuous variables (normal and skewed distribution respectively) and Kruskal-Wallis to compare categorical variables, with length of stay analysis. In addition, the risk scores of those patients who had early graft loss were compared to the risk scores of those patients who did not have early graft loss using T-test where normally distributed and Mann-Whitney-U test if skewed. A p value of <0.05 was deemed statistically significant.
Chapter 2, Risk Prediction in SPKT

2.4 Results

Recipient Demographics

All SPKT recipients (N= 57) were included in the study between November 2011- October 2013, (all primary transplants). Of these, 53 patients were recruited to a trial investigating two peri-operative management strategies and how they affect outcome (25 in the standard therapy arm and 28 in the optimisation arm). There were no missing data. There were five exclusions from length of stay analysis (four recipients had on-table pancreatectomy due to on-table thrombosis (3) and uncontrollable haemorrhage (1) and one patient died prior to discharge due to sepsis with a preceding transplant pancreatectomy). Of the remaining 52 recipients, mean age was 42.00 years (SD 7.60), there were 27 male recipients (52.0%) and mean BMI was 25.43 kg/m² (SD 3.11). The mean duration of diabetes was 26.69 years (SD 7.43) and mean time on the transplant register was 22.28 months (SD 13.03). 50 recipients were British white (96.2%), one was African and one was Indian in origin; 15 (28.8%) patients were pre-dialysis, 17 (32.7%) patients were on peritoneal dialysis at the time of SPKT and 20 (38.5%) were on haemodialysis. Table 2.2 shows the correlation of recipient factors with the outcome measures.

Donor Demographics

Of the donors, 34 (65.4%) were male and 41 (78.8%) were donors after brainstem death with mean age 34.02 years (SD 12.52) and mean BMI 24.21 kg/m² (SD 3.17). Table 2.2 shows the correlation of donor factors with the outcome measures.
Operative Demographics

Mean length of procedure was 350mins (SD 72.51), with mean pancreas and kidney cold ischaemic times (CIT) of 703mins (SD 182.16) and 849mins (SD 192.03) respectively. Table 2.2 shows the correlation of operative factors with outcome measures.

Clinical Outcomes

Median hospital LOS was 17 days (range 8-79) and mean CCLOS was 7 days (SD 4.04).

Risk Assessment Scores

In the entire cohort the ASA score was 3. Of all the risk prediction scoring systems, the Waterlow score was the only one to significantly correlate with hospital length of stay (Figure 2.1) and critical care unit length of stay (Figure 2.2) (p< 0.001, r= 0.472 (Spearman’s rank correlation) and p= 0.001, r= 0.469 respectively (Pearson Correlation) respectively).

The results of all the risk assessment scores and their correlation with length of hospital stay and critical care unit length of stay are presented in Table 2.3. These findings are not adjusted for any of the potential confounders, however with the exception of recipient BMI these were not found to be significantly associated with length of hospital or critical care unit stays (Table 2.2).
Risk Assessment Scores in Short-term Graft Failure

Four recipients had short-term graft losses. Two patients had pancreas explants intra-operatively due to bleeding (1) and portal vein thrombosis (1), while a further two had portal vein thrombosis within 72 hours of implantation.

Of these recipients; mean age was 43.25 (SD 3.58), 3 were male (75%), all were British white and on haemodialysis. Their mean BMI was 26.36 kg/m$^2$ (SD 11.85), mean duration of diabetes was 24.63 years (SD 6.45) and mean time on the transplant register was 24.86 months (SD 12.97). Of the donor factors in this cohort; all were male, mean age was 36.09 years (SD 8.53), mean BMI 22.32 kg/m$^2$ (SD 3.98) and 2 (50%) were from DBD donors. In addition, mean length of procedure was 462mins (SD 100.21), with mean pancreas and kidney CIT of 720mins (SD 57.98) and 898mins (SD 101.23) respectively. Of all these factors, only length of operation time was significantly different between this cohort and the recipients with longer graft survival ($p=0.023$).

The mean (SD)/ median (range) risk scores for this cohort per scoring system were: 14.56 (SD 5.09), Waterlow Score; 79.187 (SD 15.36), P-POSSUM Morbidity score (%); 10.034 (SD 5.367), P-POSSUM mortality score (%); 4 (0-7), MODS; 25.09 (SD 9.45), Charlson Score; 3 (3-4), Revised Cardiac Risk Index and 4 (1-7), number of diabetic complications. None of the results of these scoring systems were significantly different to any of the risk prediction scores from the recipients with longer graft survival ($p>0.05$).
Chapter 2, Risk Prediction in SPKT

2.5 Discussion

Risk predictive scoring systems aim to identify and accurately inform outcome for high-risk patients. This information can ideally be used pre-operatively in the assessment, counselling and consent processes and in the peri-operative management, to individualise a patient’s care and help reduce post-operative morbidity.

Increased lengths of critical care unit and hospital stays following surgery are surrogate markers of in-patient morbidity, and increased peri-operative morbidity leads to poorer long-term outcomes (Khuri, Henderson et al. 2005). In general surgery a number of scoring systems exist to help predict outcome of patients undergoing interventions (Charlson, Pompei et al. 1987, Copeland, Jones et al. 1991, Marshall, Cook et al. 1995, Lee, Marcantonio et al. 1999, Mak, Campbell et al. 2002).

Outcome prediction post-SPKT remains an enigma, due to the multifactorial sequela of diabetes and end-stage renal failure and the nature of dual-organ transplantation. Current SPKT scoring systems (P-PASS, P-DRI) specifically analyse donor and organ factors which influence organ outcome (30-day and 1-year survival), themselves surrogate measures of success. However, these do not necessarily accurately correlate with patient morbidity post transplantation. To date, studies have not investigated factors in SPKT affecting recipient critical care unit and hospital length of stays, surrogate markers of patient morbidity and long-term survival (Khuri, Henderson et al. 2005).
In this study we have found that WS significantly correlates with a recipients’ length of hospital and critical care unit stays (p< 0.001 and p= 0.001 for length of hospital stay and length of critical care unit stay respectively) following SPKT. This is in contrast to the number of diabetic complications and other commonly used general surgery risk prediction scoring systems (P-POSSUM morbidity/ mortality, MODS, Charlson Score, Cardiac Risk Index and ASA) which have all failed to correlate with any outcome measures. These findings are independent of donor and organ factors and are likely to be due to the benefit of the use of the multi-system approach of WS.

In SPKT, studies have previously identified recipient BMI greater than 30kg/m² as a predictor of poor outcome, (Sampaio, Reddy et al. 2010) leading to an increased risk of post- transplant complications, graft failure, graft rejection and recipient death when compared to recipient BMI less than 30kg/m². The results of this study also suggest that increased BMI is associated with increased morbidity post-SPKT, as BMI correlates significantly with our outcome measures (p= 0.002 and 0.044 respectively for recipient length of hospital and critical care unit stays). In our study, this finding is particularly noteworthy as BMI is also incorporated in the WS. However, BMI alone has a weaker association with lengths of stay in our cohort, when compared to the WS, and in addition WS assesses factors such as age, sex, co-morbidities, type of surgery, neurological deficit and continence, factors which are likely to be important to assess outcomes in SPKT recipients, due to the chronic nature of the diseases suffered by the recipients and the resulting malnutrition, inflammation and atherosclerosis syndrome suffered by them (Stenvinkel 2002). Our study also finds that the diabetic load (number of years of diabetic duration preceding transplant) significantly correlates with length of critical care unit stay (p= 0.045), however as no adjustment has been made for multiple testing, this result
Chapter 2, Risk Prediction in SPKT

should be interpreted with caution. Despite this, it would not be unreasonable to expect diabetic load to correlate with post-operative outcome, given that it may be a surrogate marker for pre-operative morbidity and may indicate lower physiological reserve in patients with greater diabetic exposure.

Potentially, these findings add weight to the growing wealth of evidence regarding frailty and its effect on outcome following transplantation. Frailty is a condition often associated with the elderly, where it can double the risk of morbidity and mortality following surgery (Afilalo, Alexander et al. 2014) leading to increased lengths of hospital stay (Makary, Segev et al. 2010). In dialysis patients, increased frailty (as defined by the Clinical Frailty Score) is associated with higher mortality (Alfaadhel, Soroka et al. 2015). However, due to the lack of the elderly undergoing SPKT, frailty is a phenomenon that has not been studied in this population, despite its independence as a risk factor from age (McAdams-DeMarco, Law et al. 2015). Frailty can be assessed by numerous scoring systems, often a mix of quantitative (grip strength, muscle mass, weight loss) and qualitative (The Clinical Frailty Score, exhaustion, low physical activity) measures. In doing so it is associated with increased risks of premature graft loss and over double the risk of mortality following kidney transplantation (independent of age) (McAdams-DeMarco, Law et al. 2015, McAdams-DeMarco, Law et al. 2015). Given the multi-system comorbidities suffered by SPKT recipients, a frailty score may have utility in predicting outcome following SPKT. Future studies could therefore analyse the relationship between frailty and the WS in SPKT recipients and assess outcomes with these two risk scoring systems.
Chapter 2, Risk Prediction in SPKT

A considerable advantage of the WS is that it is commonly used by nursing staff in their pre-operative assessment of surgical patients and can easily be transferred to an ambulatory environment to aid in pre-listing risk assessment. It provides an objective cumulative risk score based upon a combination of co-morbidities, mobilisation, nutritional factors and demographics. In comparison, all the other scoring systems assessed in this study fail to address one or more of those variables. ASA is largely subjective and the cardiac risk index and MODS scores provide a very narrow range of possible scores in PT recipients, thereby providing little differentiation between individuals. Although the cardiac risk index is not normally utilised as an aid to predict post-operative outcomes, it was felt that in a group of patients with such extensive cardiovascular disease, it potentially had utility as a risk scoring system to assess this cohort. P-POSSUM was used ahead of other POSSUM alternatives as it was felt that SPKT is best compared to major general surgical procedures and an account of the extent of the procedure should be taken when estimating the risk. P-POSSUM and the Charlson Score both take into account a patient’s co-morbid/ physiological status and provide a wide breath of scores to help differentiate level of risk, but fail to account for factors such as nutritional status, which are important in risk assessment of the diabetic and end-stage renal failure patients (Stenvinkel and Alvestrand 2002, Stenvinkel, Barany et al. 2002, Noori and Kopple 2010, Pasticci, Fantuzzi et al. 2012) and in outcome analysis following major surgery (Weimann, Braga et al. 2006, Burden, Todd et al. 2012).

In addition, and most importantly, WS identifies the highest-risk recipients, allowing for individual optimisation of post-operative care with an attempt to reduce post-operative morbidity and thereby length of hospital and CCU stays.
Chapter 2, Risk Prediction in SPKT

The low mortality associated with the procedure and low numbers of graft losses in this small cohort, unfortunately preclude any meaningful analysis of these factors as outcome measures. Despite this, a comparison was made with the scoring systems between those patients that had early graft loss compared to those patients who did not have early graft loss and no difference in their pre-operative scores were found. This suggests that these scores are unlikely to have value in predicting early graft loss, likely because of the technical nature of these losses, which are unrelated to recipient factors. But, the numbers in this group were very small and larger cohorts would need to be studied to make valid conclusions with this respect. The only difference in confounders between these two groups was the length of surgery, which would be expected given the intra-operative complications suffered in two of the four cases which suffered with early graft loss.

However, this prospective study, comparing the predictive capability of a number of commonly used general surgical risk scoring systems in SPKT does indicate that WS may have value in the prediction of outcome following the procedure. As discussed, further validation with larger cohorts is required. This would allow for more accurate stratification of patients. In addition, multivariate analysis could be conducted to determine the effect of each component of the WS on outcome and a Cox regression analysis would allow the identification of a cut-off WS to determine those patients at increased risk of graft failure and or mortality. These tools would be a valuable addition in aiding both clinicians and prospective transplant recipients in assessing individual pre-operative risk and planning for individualised post-operative care.
Table 2.1a- e. Components of the Individual Scoring Systems used in this study

Table 2.1a. Contents of the Waterlow pressure ulcer scoring system, with potential scores, per scoring category (Waterlow 1985)

<table>
<thead>
<tr>
<th>Scoring Category</th>
<th>Potential Scores, range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Mass Index</td>
<td>0- 3</td>
</tr>
<tr>
<td>Skin Integrity</td>
<td>0- 3</td>
</tr>
<tr>
<td>Sex</td>
<td>1- 2</td>
</tr>
<tr>
<td>Age</td>
<td>1- 5</td>
</tr>
<tr>
<td>Nutritional Status*</td>
<td>0- 5</td>
</tr>
<tr>
<td>Continence</td>
<td>0- 3</td>
</tr>
<tr>
<td>Mobility</td>
<td>0- 5</td>
</tr>
<tr>
<td>Special Risks, significant morbidity**</td>
<td>0- 29</td>
</tr>
<tr>
<td>Neurological Deficit</td>
<td>0- 18</td>
</tr>
<tr>
<td>Type and Length of Surgery</td>
<td>0- 13</td>
</tr>
<tr>
<td>Medications</td>
<td>0- 4</td>
</tr>
</tbody>
</table>

*assessed by extent of weight loss, if present and appetite on admission
**accounting for: terminal cachexia, multiple organ failure, single organ failure, peripheral vascular disease, anaemia (Hb <8) and smoking.
Table 2.1b. Contents of the P-POSSUM scoring system (Copeland, Jones et al. 1991)

<table>
<thead>
<tr>
<th><strong>Physiological factors</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Cardiac disease</td>
<td></td>
</tr>
<tr>
<td>Respiratory disease</td>
<td></td>
</tr>
<tr>
<td>ECG changes</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td></td>
</tr>
<tr>
<td>Pulse Rate (beats per minute)</td>
<td></td>
</tr>
<tr>
<td>Haemaglobin (g/dl)</td>
<td></td>
</tr>
<tr>
<td>White cell count (x10^9/l)</td>
<td></td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td></td>
</tr>
<tr>
<td>GCS</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Operative Factors</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of Operation</td>
<td></td>
</tr>
<tr>
<td>Number of procedures</td>
<td></td>
</tr>
<tr>
<td>Operative blood loss (mls)</td>
<td></td>
</tr>
<tr>
<td>Peritoneal contamination</td>
<td></td>
</tr>
<tr>
<td>Malignancy status</td>
<td></td>
</tr>
<tr>
<td>CEPOD category</td>
<td></td>
</tr>
</tbody>
</table>

Each result of each factor is inputted into the online scoring system. This awards weighted scores for each factor, depending on the severity of the derangement. Separate physiological and operative scores are calculated and these relate to a % risk of morbidity and mortality per each patient for that admission.
Table 2.1c. Contents of Charlson Morbidity Index Scoring System (Charlson, Pompei et al. 1987)

<table>
<thead>
<tr>
<th>Age</th>
<th>Dementia</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of myocardial infarction</td>
<td>Chronic pulmonary disease</td>
<td>Albumin</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>Connective tissue disease</td>
<td>Auto-immune condition</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>Peptic ulcer disease</td>
<td>Lymphoma/ leukaemia</td>
</tr>
<tr>
<td>(and/ or aortic aneurysm &gt;6cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>Liver disease (mild-severe)</td>
<td>Renal disease (mild-severe)</td>
</tr>
<tr>
<td>Diabetes with or without end-organ damage</td>
<td>Tumour with metastasis</td>
<td>Hemiplegia</td>
</tr>
</tbody>
</table>

The age and the presence of each factor (yes/ no) are inputted into the online calculator. A combined risk score is generated and this is used by the on-line algorithm to calculate a predicted 1 year patient survival.
Table 2.1d. Contents of the Multiple Organ Dysfunction Scoring (MODS) System (Marshall, Cook et al. 1995)

<table>
<thead>
<tr>
<th>System Assessed</th>
<th>Factor Assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory function</td>
<td>PaO$_2$/ FiO$_2$</td>
</tr>
<tr>
<td>Cardiovascular function</td>
<td>(Pulse (beats per minute) x Central venous pressure (mmHg))/ mean arterial pressure</td>
</tr>
<tr>
<td>Haematologic function</td>
<td>Platelet count</td>
</tr>
<tr>
<td>Glasgow Coma Scale</td>
<td>-</td>
</tr>
<tr>
<td>Hepatic function</td>
<td>Bilirubin</td>
</tr>
<tr>
<td>Renal function</td>
<td>Creatinine</td>
</tr>
</tbody>
</table>

Each factor assessed, depending on the result is awarded a score of between 0 and 4. Each score correlates to a risk of ICU mortality, hospital mortality and ICU length of stay, illustrated below:

<table>
<thead>
<tr>
<th>Score</th>
<th>ICU Mortality (%)</th>
<th>Hospital Mortality (%)</th>
<th>ICU Stay (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1-4</td>
<td>1-2</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>5-8</td>
<td>3-5</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>9-12</td>
<td>25</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>13-16</td>
<td>50</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>17-20</td>
<td>75</td>
<td>82</td>
<td>21</td>
</tr>
<tr>
<td>21-24</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

PaO$_2$, partial pressure of oxygen in arterial blood gas (mmHg); FiO$_2$, Fraction of inspired oxygen (%)
Chapter 2, Risk Prediction in SPKT

Table 2.1e. Contents of Revised Cardiac Risk Index Scoring System (Lee, Marcantonio et al. 1999)

<table>
<thead>
<tr>
<th>High-risk surgical procedure</th>
<th>Intraperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intrathoracic</td>
</tr>
<tr>
<td></td>
<td>Suprainguinal vascular</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of Ischaemic heart disease</th>
<th>History of myocardial infarction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>History of positive exercise test</td>
</tr>
<tr>
<td></td>
<td>Current chest pain, secondary to</td>
</tr>
<tr>
<td></td>
<td>myocardial ischaemia</td>
</tr>
<tr>
<td></td>
<td>Use of nitrate therapy</td>
</tr>
<tr>
<td></td>
<td>ECG with pathological Q waves</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of congestive heart failure</th>
<th>History of congestive heart failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pulmonary oedema</td>
</tr>
<tr>
<td></td>
<td>Paroxysmal nocturnal dyspnea</td>
</tr>
<tr>
<td></td>
<td>Bilateral rales or S3 gallop</td>
</tr>
<tr>
<td></td>
<td>Pulmonary vascular prominence on</td>
</tr>
<tr>
<td></td>
<td>chest X-ray</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of cerebrovascular disease</th>
<th>History of transient ischaemic attack or stroke</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Preoperative treatment with insulin</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative serum creatinine &gt;2.0mg/ dl</td>
<td>-</td>
</tr>
</tbody>
</table>

Each positive risk factor is assigned one point. The total number of points correlate to a risk of a major cardiac event occurring. Patients can achieve a maximum of 6 points with a risk prediction of 0.4-11% chance of suffering with a major cardiac event.
### Table 2.2. Correlation of donor, recipient and operative confounding factors with length of hospital stay and critical care unit length of stay in SPKT.

<table>
<thead>
<tr>
<th>Confounding Factor</th>
<th>Mean (SD)</th>
<th>Total length of hospital stay, p-value</th>
<th>Critical care unit length of stay, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of procedure (mins)</td>
<td>349.5 (72.51)</td>
<td>0.712 µ</td>
<td>0.424 ¥</td>
</tr>
<tr>
<td>Pancreas CIT (mins)</td>
<td>703.0 (182.16)</td>
<td>0.293 µ</td>
<td>0.248 ¥</td>
</tr>
<tr>
<td>Kidney CIT (mins)</td>
<td>849.9 (192.03)</td>
<td>0.357 µ</td>
<td>0.266 ¥</td>
</tr>
<tr>
<td>Recipient BMI (kg/m²)</td>
<td>25.43 (3.11)</td>
<td>0.002 µ</td>
<td>0.044 ¥</td>
</tr>
<tr>
<td>Recipient Age (yrs)</td>
<td>42.0 (7.60)</td>
<td>0.670 µ</td>
<td>0.633 ¥</td>
</tr>
<tr>
<td>Duration of diabetes (yrs)</td>
<td>26.69 (7.43)</td>
<td>0.428 µ</td>
<td>0.045 ¥</td>
</tr>
<tr>
<td>Time on transplant register (months)</td>
<td>22.28 (13.03)</td>
<td>0.712 µ</td>
<td>0.222 ¥</td>
</tr>
<tr>
<td>Dialysis mode N (%)</td>
<td>Pre-dialysis: 15 (28.8)</td>
<td>0.289 ¥</td>
<td>0.964 ¥</td>
</tr>
<tr>
<td></td>
<td>PD: 17 (32.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HD: 20 (38.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor Age (yrs.)</td>
<td>34.02 (12.52)</td>
<td>0.870 µ</td>
<td>0.066 ¥</td>
</tr>
<tr>
<td>Donor BMI (kg/m²)</td>
<td>24.21 (3.17)</td>
<td>0.085 µ</td>
<td>0.426 ¥</td>
</tr>
<tr>
<td>DDBD/DCD N (%)</td>
<td>DDBD: 41 (78.8)</td>
<td>0.209 ¥</td>
<td>0.705 ¥</td>
</tr>
<tr>
<td></td>
<td>DCD: 11 (21.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI, Body Mass Index; DDBD, Donor after Brainstem Death; DCD, Donor after Cardiac Death; HD, Haemodialysis; PD, Peritoneal Dialysis.

∞ Kruskal-Wallis; ¥ Pearson Correlation; µ Spearman Correlation
Figure 2.1. Correlation of Waterlow score with total Hospital Length of Stay (days; $p<0.001$, Correlation coefficient= 0.472, Spearman’s Correlation).
Figure 2.2. Correlation of Waterlow Score with Critical Care Unit Length of stay (days; $p=0.001$, Correlation Coefficient= 0.469, Pearson’s Correlation).
Table 2.3. Correlation of the studied risk assessment scores with total length of hospital and critical care unit length of stays in SPKT

<table>
<thead>
<tr>
<th>Risk Score</th>
<th>Mean (SD/ Range)</th>
<th>Total length of hospital stay, p-value</th>
<th>Critical care unit length of stay, p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waterlow score</td>
<td>13.87 (4.03)</td>
<td>&lt;0.001 µ</td>
<td>0.001 ¥</td>
</tr>
<tr>
<td>(2- 82)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-POSSUM morbidity score, % (5.468- 100%)</td>
<td>75.090 (11.752)</td>
<td>0.313 µ</td>
<td>0.359 ¥</td>
</tr>
<tr>
<td>P-POSSUM mortality score, % (0.223- 100%)</td>
<td>9.807 (6.382)</td>
<td>0.194 µ</td>
<td>0.546 ¥</td>
</tr>
<tr>
<td>MODS (0- 24)</td>
<td>4 a (0- 7)</td>
<td>0.864 °</td>
<td>0.585 °</td>
</tr>
<tr>
<td>Charlson Score</td>
<td>27.0 (24.76)</td>
<td>0.129 µ</td>
<td>0.074 ¥</td>
</tr>
<tr>
<td>(2- 99)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac risk index</td>
<td>3 a (3- 4)</td>
<td>0.816 °</td>
<td>0.061 °</td>
</tr>
<tr>
<td>(0- 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No of diabetic complications</td>
<td>3 a (1- 7)</td>
<td>0.123 °</td>
<td>0.143 °</td>
</tr>
<tr>
<td>(0- 8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ASA, Association of Anaesthesiologists; MODS, Multiple Organ Dysfunction Score; P-POSSUM, Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity.

* median; ° Kruskal-Wallis; ¥ Pearson Correlation; µ Spearman Correlation
Peri-Operative Goal-Directed Haemodynamic Optimisation Improves Short-Term Outcomes Following Simultaneous Pancreas and Kidney Transplantation: A Randomised Clinical Trial (NCT01619904)

H A Khambalia
3.1 Abstract

Background

Simultaneous pancreas and kidney transplantation (SPKT) is high-risk surgery, associated with significant peri-operative morbidity. Protocolised, peri-operative supra-physiological haemodynamic optimisation (Goal-Directed Therapy, GDT) has been shown to improve outcomes in other high-risk individuals, following major surgery.

The aim of this study was to investigate the benefits of GDT in SPKT. The primary outcome was length of hospital stay. The secondary outcome measures were patient mortality, graft survival, length of critical care unit stay, post-operative complication rates and variations in risk scoring systems pre- and post-surgery.

Methods

60 SPKT recipients were randomly allocated to either GDT or Standard therapy (ST) cohorts. The GDT cohort underwent peri-operative supra-physiological haemodynamic optimisation guided by lithium indicator dilution, to attain an indexed oxygen delivery ($\text{DO}_2\text{I}$) of greater than 600ml/min/m$^2$. The optimisation protocol was initiated at the start of surgery and continued for six hours post-operatively in the GDT arm. The ST cohort was managed according to current unit protocols, guided by each anaesthetists’/ intensivists’ clinical judgement.

Results

There were no differences in length of hospital stay between the two cohorts 18.0 days (IQR 14.0- 31.0) and 15.0 days (IQR 13.0- 22.3) in GDT and ST cohorts respectively; $p= 0.162$ (Mann-Whitney U test).

There were significant differences between the two groups in short-term outcomes. The GDT cohort had significantly lower critical care unit lengths of stay when compared to the
ST cohort (4 days (IQR 3- 5.5) and 8 days (IQR 6.0- 9.3) respectively, p< 0.001, Mann-Whitney U test). In addition, the GDT cohort had significantly lower rates of delayed graft function (6.9% and 33.3% respectively, p= 0.021, Fisher’s Exact) and shorter time to tolerating oral diet (5.0 days (IQR 4.0- 8.0) and 8.0 days (IQR 6.75- 10.0) respectively; p<0.001, Mann-Whitney U test) compared to the ST cohort.

Conclusions

In this study, we have demonstrated improved short-term outcomes following protocolised, supra-physiological haemodynamic optimisation following SPKT.
3.2 Introduction


Despite these significant overall benefits, PT is not proportionally offered to diabetic patients, as peri-operative risks following transplantation remain significant. Up to 75% of patients could have an infective morbidity (Bassetti, Salvalaggio et al. 2004) and although the majority are minor, this has implications on long-term patient and graft survival (Khuri, Henderson et al. 2005). PT is also associated with a 30% surgical re-exploration rate and up to 5% one-year mortality (Grewal, Garland et al. 1993, Bassetti, Salvalaggio et al. 2004, Lall, Sandrasegaran et al. 2006, Troppmann 2010). SPKT involves a major surgical insult and significant physiologic fluxes in the perioperative period. Given this combination of factors, and the added morbidity of immunosuppression, these patients are high-risk candidates undergoing high-risk surgery (Shoemaker, Appel et al. 1988, Boyd, Grounds et al. 1993, Wilson, Woods et al. 1999, Lobo, Salgado et al. 2000, Gan, Soppitt et al. 2002, Pearse, Dawson et al. 2005, Wakeling, McFall et al. 2005, Jhanji, Vivian-Smith et al. 2010, Mayer, Boldt et al. 2010, Pearse, Harrison et al. 2014).
SPKT has evolved since its introduction in 1966 (Kelly, Lillehei et al. 1967), with continuous improvements in outcomes (Sollinger, Stratta et al. 1988, Sollinger, Odorico et al. 1998, Humar, Kandaswamy et al. 2000). However, these improvements have concentrated on surgical technique, immunosuppression, post-operative anticoagulation and improved donor and recipient selection, thus leading to the observed higher rates of graft and patient survival (Squifflet, Van Ophem et al. 2004, Gruessner and Gruessner 2013).

Notwithstanding the unique combination of physiology and the severity of co-morbidities and complications inherent in these patients, there are still opportunities to improve outcomes, focusing on peri-operative non-surgical care. The only publications regarding peri-operative care in SPKT recipients are case-reports and small series experiences (Koehntop, Beebe et al. 2000, Halpern, Miyoshi et al. 2004, Bindi, Biancifiore et al. 2005). No effort has been made to manipulate recipient physiology with the aims of improving outcomes.

Following the advent of the Swan-Ganz catheter (Swan, Ganz et al. 1970, Ganz, Donoso et al. 1971) Shoemaker, in a series of publications in the 1970s and 1980s, identified specific flow-related physiological parameters, which recognised high-risk patients at further risk of poor outcome following major surgery, and instituted a protocol based upon achieving supra-physiological goals (commonly termed goal-directed therapy (GDT)) to improve outcomes in this cohort (Shoemaker 1972, Shoemaker, Montgomery et al. 1973, Bland, Shoemaker et al. 1978, Shoemaker and Czer 1979, Shoemaker, Appel et al. 1988, Donati, Loggi et al. 2007). However, the invasive nature of the Swan-Ganz catheter, the complications associated with it and the loss of knowledge associated with its use (Robin

In 2011 The National Institute for Health and Clinical Excellence (NICE) in the UK recognised this technology in high-risk patients, as it reduced post-operative complications and length of hospital stay (Ghosh, Arthur et al. 2011). A recent Cochrane review (Grocott, Dushianthan et al. 2013) states that GDT reduces post-operative complications and length of hospital stay and is unlikely to cause harm following major surgery, but accepts the ongoing equipoise regarding the benefits of the therapy in specific patient cohorts. Therefore, it concludes that further research is required to “disentangle the complex package of care that forms the intervention... and thereby identify which components are effective in different clinical contexts”.

In that vein, we formulated a study evaluating the benefits of a protocol-driven approach to fluid and drug administration to achieve supra-physiological goals in the peri-operative period in SPKT, using a minimally invasive flow-directed cardiac output monitor.
3.3 Methods

Study Centre

The study was undertaken at the Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust. Ethical approval was sought and received from the Central Liverpool Research Ethics Committee and Research and Development approval from the Central Manchester University Hospitals NHS Foundation Trust. It was registered on Clinical trials.gov (Registration Number NCT01619904).

Study Design

This was a randomised controlled, single-centre study. The primary outcome measure was length of hospital stay. The secondary outcome measures were patient mortality, graft survival, length of critical care unit stay, post-operative complication rates and variations in Post-Operative Morbidity Survey (POMS) (Bennett-Guerrero, Welsby et al. 1999) and Portsmouth Physiological and Operative Severity Score for the Enumeration of Mortality and Morbidity (P-POSSUM) morbidity and mortality rates between admission and at 72 hours post-operatively. Critical care unit length of stay was guided by The Intensive Care Society Standards for Levels of Critical Care for Adult Patients document, 2009 (Eddlestone J 2009) and length of hospital stay decisions were made by non-research clinical staff. Lengths of stay were calculated from date of admission to readiness to discharge from critical care and hospital.

Patients were randomised to two groups: Standard Therapy (ST) or GDT. 60 randomisations (30 per group) were conducted prospectively in a 1:1 ratio, prior to the start of the trial by
a member of the transplant clerical staff using www.sealedenvelope.com (accessed October 2011). The randomisation codes were placed in serially numbered opaque envelopes and stored in a locked office accessible only to members of the research team. The randomisation group was revealed after consent had been obtained, prior to surgery. Data were analysed on an intention-to-treat basis.

All adult SPKT recipients were eligible for inclusion in the study. The criteria for exclusion were patients unable to consent to the study, patients with a contraindication to central venous or arterial catheterization and those patients with advanced directives restricting implementation of the protocol (e.g. restricting the use of blood products).

Patients were approached in hospital by a dedicated researcher, accompanied by a member of the nursing staff, when they were asked to attend for transplantation. “Back-up” recipients were also approached and counselled with regards to the study. These recipients are those who were not the first choice for transplant, as stipulated by the National Health Service Blood and Transplant (NHS BT) waiting list, but would receive the transplant in the event of the primary recipient being considered unfit for transplantation by the operating transplant consultant. Patients on the waiting list were not approached in advance to be counselled and consented for the study due to a number of points which were discussed at the ethical review board meeting:

1. It was felt that writing to patients regarding the study could be interpreted as a sign that they were approaching the top of the waiting list and would therefore cause unnecessary distress to those patients towards the bottom of the waiting list;
2. Given the number of factors influencing a recipient’s fitness to proceed with SPKT, patients are regularly being moved between the active and suspended lists. In addition, some patients may remain suspended for many months prior to being activated. In such a fluid situation it was deemed impractical to write to all active patients on the transplant waiting list regarding the study and unfair to write to the suspended patients, to avoid confusion about their status on the transplant list;

3. It was felt that recipients for SPKT are generally well educated regarding the procedure and have often been admitted as “back-up” prior to being first on the list. They therefore understand the admission process better than would be expected and would be able to cope with new, but related information such as on-going research studies;

4. The study is multi-disciplinary in its approach, involving surgeons and anaesthetists. I felt it was appropriate for the recipients to be able to ask the anaesthetists questions during the counselling process and this would be most efficient on admission to hospital.

Therefore, it was felt that approaching them about a potential study on admission would be the most appropriate time. In addition, I stressed at the ethical review board meeting that patients would be given the maximum opportunity to consider the study, read through the Patient Information Sheet, discuss with family and friends and ask questions to either the transplant or anaesthetic teams involved prior to consenting. Counselling regarding the study took place very early in the admission process and final consent was taken as late as possible, prior to theatre. This often gave the recipients up to 12 hours to contemplate the study should they wish. This approach was discussed and agreed by the Ethical Review Board Committee. Patients were therefore counseled and consented in line with Ethical Board approval and the Helsinki Declaration.
Chapter 3, GDT in SPKT

**Intervention**

Study protocols were implemented by a dedicated researcher, following induction of anaesthesia and continued for six hours post-operatively on the intensive care unit.

All patients, independent of randomisation group, had a lithium indicator dilution cardiac output monitor (LiDCOplus, LiDCO Ltd, Cambridge, UK) attached and calibrated to continuously measure cardiac output and calculate *indexed oxygen delivery* ($DO_2I$ (ml/kg/m$^2$)) after induction of anaesthesia and prior to start of surgery, which remained in-situ for six hours post-operatively. The LiDCOplus provides continuous monitoring once calibrated, but readings were documented at specific time-points in the intra- and post-operative course for analysis.

In addition, standard monitoring for use in all patients included electrocardiography, peripheral saturation probe, invasive arterial pressure, central venous pressure and temperature monitoring. Finally, patients underwent hourly arterial and central venous (ScVO$_2$) blood gas measurements intra-operatively, as well as immediately prior to pancreas perfusion and at 30 minutes post perfusion, and then at 6, 12, 24, 48 and 72 hours post-operatively.

In the ST arm of the study the readings from the LiDCOplus were blinded to all members of the clinical team caring for the patient. The output from the LiDCOplus was recorded at regular intervals by a dedicated researcher for analysis purposes. These readings were not available to the medical team caring for the patient. Patients in the ST arm of the study
were therefore treated by each anaesthetic and critical care team with guidance from the investigating units’ Transplant Anaesthesia and Critical Care guidelines for PT (Appendices 3.1 & 3.2 respectively). In summary, the intra-operative guidance stipulates; the use of the oesophageal doppler monitoring (ODM) probe, maintenance of systolic blood pressure within 15- 20% of baseline, maintenance of CVP at 12- 16mmHg (using crystalloid, colloid and/ or albumin), maintenance of a haemoglobin greater than 8g/dl and to aim for normal biochemical markers. The post-operative guidance stipulates; the use of a cardiac output monitor “if clinically indicated”, to aim for “normal volaemic status” (CVP 7- 10mmHg, to be maintained with 250ml colloid boluses), a maintenance fluid replacement rate of “previous hourly urine output plus 30mls of crystalloid per hour”, to maintain a haemoglobin greater than 10g/dl and to consider the introduction of a vasopressor if persistently hypotensive.

The patients in the GDT arm of the study were strictly managed according to a bespoke optimisation protocol, guided by the LiDCOplus. Primary targets to be met in the protocol were a mean arterial blood pressure greater than 70mmHg (maintained by noradrenaline (NA)), haemoglobin between 10- 12g/dl (maintained via blood transfusion), saturations greater than 94% and a core temperature of 37°C. In addition, colloid fluid boluses, were used to optimise stroke volume. They were delivered by syringe bolus, via the central venous access line within five minutes of initiation at a dose of 3ml/kg. Ultimately, once these goals had been attained, the DO$_2$I was reviewed and dobutamine titrated, if required, to attain a DO$_2$I of greater than 600ml/min/m$^2$.

In June 2013, following advice from the European Medicines Agency (EMA), The Medicines and Healthcare products Regulatory Agency (MHRA), the Faculty of Intensive Care
Medicine (FICM) and the Royal College of Anaesthetists, the study institution suspended the use of hydroxyethyl starch (HES) following evidence that starch-based resuscitation products significantly increased death rates and renal failure in sepsis (Perner, Haase et al. 2012). As a substitute, 4.5% Human Albumin Solution was subsequently used as the colloid fluid of choice for resuscitation.

Due to the nature of the study and the intervention, it was not possible to blind the randomisation group which the patient was allocated to. Therefore this study was not blinded.

**Patient-Level Data**

All recipient, donor, organ, operative and outcome data and risk scoring systems were prospectively collected and collated electronically by a dedicated researcher. Haematological and biochemical profiles were taken pre-operatively, and then at time of pancreas perfusion and 30 minutes and 6, 12, 24, 48 and 72 hours post-perfusion. P-POSSUM score (Copeland, Jones et al. 1991, Mohil, Bhatnagar et al. 2004) was calculated using the online algorithm supplied by Risk Prediction in Surgery (http://www.riskprediction.org.uk/pp-index.php) at time of admission for surgery, and then at 72 hours post-transplant. Multiple Organ Dysfunction Score (MODS) (Marshall, Cook et al. 1995) was calculated immediately prior to surgery and then at 24, 48 and 72 hours post-surgery. Revised Cardiac Risk Index (Lee, Marcantonio et al. 1999) was calculated pre-operatively. Donor data were recorded and donor scores (Pancreas Donor Risk Index (P-DRI) (Axelrod, Sung et al. 2010) and Pre-Procurement Pancreas Suitability Score (P-PASS)) were calculated using data from the Electronic Offering System (EOS) form, completed by
NHS Blood and Transplant during the donor assessment. The P-DRI was calculated using the mobile phone application. Clinician perceived fattiness of the pancreas, as assessed by the implanting surgeon, was noted at time of implantation and graded 1-4, (Nil, Low, Moderate or High respectively).

Post operatively, diagnosis and management of complications were undertaken by non-research clinical staff to avoid reporting bias. POMS was conducted on days 5, 7, 10, 14, 21 and 28 post-surgery, during inpatient stay to assess rates of complications post-transplant. In addition, the following complications were also recorded: return to theatre for any cause (intra-abdominal pack changes and cystoscopy ± removal of ureteric stent excluded), kidney-delayed graft function (diagnosed as recipient requiring ≥2 dialysis sessions post-transplant), primary kidney or pancreatic graft failure, pancreatitis (diagnosed as an increase in serum amylase >3 times the normal level) and graft thrombosis.

**Transplant Protocol**

The criteria utilized by individual transplant units for acceptance onto the waiting list for PT in the UK are governed by national guidelines produced by NHS BT and derived from national consensus underpinned by European Best Practice Guidelines (Ebpg, European Renal et al. 2000). Patients were allocated organs from the waiting list, based on the blood group, human leucocyte antigen matching and wait time (PAG 2014).

Pancreata were implanted intra-peritoneally, either in the “head-up” or “head-down” position, depending on surgeon preference. All were implanted on the right side of the
pelvis with systemic venous drainage and arterial donor γ-graft to either recipient common or external iliac artery. Either enteric or bladder drainage was used, depending on surgeon preference. Kidneys tended to be implanted contralateral to the pancreas, although some were implanted ipsilateral, again dependent upon surgeon preference. Induction immunosuppression was with Alemtuzumab (Campath®, Sanofi, Paris, France), 30mg subcutaneous injection at induction of anaesthesia (repeated at 24 hours post-operatively) and Methylprednisolone (Solu-Medrone®, Pfizer, New York).

Maintenance (post-operative) immunosuppression was initially with intra-venous Mycophenolate Mofetil and Cyclosporine, but as enteric function returned these were converted to oral Mycophenolate Mofetil and Tacrolimus. In addition, post-operatively all patients were commenced on intravenous unfractionated heparin (for 3-7 days) and oral; Aspirin, Co-trimoxazole (for six months prophylaxis against Penumocystis jiroveci pneumonia) and Valganciclovir (for three months prophylaxis, if either the recipient or donor were cytomegalovirus polymerase chain reaction positive prior to transplant) with return of enteric function.

**Statistical Analysis**

Based upon an alpha level of 0.05, for a two-sided test to observe a 28% decrease in length of hospital stay with 80% power, a sample size of 27 patients per group was required. These estimations were based upon previously observed incidence of reductions in length of hospital stay following similar interventions in high-risk patients undergoing major general and vascular surgery (Gan, Soppitt et al. 2002, Donati, Loggi et al. 2007).
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Statistical analyses were carried out using SPSS (IBM SPSS Statistics 20, Armonk, New York). Continuous data are presented as mean (± Standard Deviation, SD) where normally distributed, or median (Interquartile range, IQR, 25th-75th percentile) if skewed. They have been analysed using Independent samples T-test for normally distributed data, or Mann-Whitney U test for skewed data. Categorical data are presented as absolute numbers (%) and analysed using Fisher’s exact test. Due to the number of co-variables present and small numbers of some variables and outcomes, p-values between 0.05-0.01 will be treated with caution.

Repeated measures are presented as mean (± SD) and analysed using repeated measures Analysis of Variance (ANOVA) with Bonferroni Correction or median (IQR) where skewed and analysed using repeated measures Kruskal-Wallis.
3.4 Results

60 SPKT recipients (30 randomised to each of GDT and ST cohorts) were recruited between November 2011 and March 2014 (Consort diagram, Figure 3.1).

Baseline recipient, donor and operative variables are outlined in Tables 3.1 and 3.2. The GDT group were younger (mean 39.96 years v. 44.27 years; p= 0.024) and on dialysis for a shorter period of time (mean 8.39 months v. 22.50 months; p= 0.023) compared to the ST group. However, baseline P-POSSUM morbidity and mortality scores tended to be higher in the GDT group when compared to the ST group, though not significantly (P-POSSUM morbidity 79.899% and 76.395% respectively, p= 0.061 and P-POSSUM mortality 9.723% and 8.336% respectively, 0.073). No other significant differences were apparent in any of the recipient, donor and operative variables measured.

Clinical Outcomes

Primary Outcome

There was no significant difference in our primary outcome measure. Median length of hospital stay in the GDT and ST cohorts were 18.0 days (IQR 14.0-31.0) and 15.0 days (IQR 13.0-22.3) respectively (n= 57, p= 0.162). One patient from the ST group died as an in-patient and was therefore excluded from this analysis and one patient from the GDT group remained an in-patient at one year following transplant and was therefore excluded from analysis.
Secondary Outcomes

The GDT cohort had significantly lower critical care unit length of stays when compared to the ST cohort (4 days (IQR 3-5.5) and 8 days (IQR 6.0-9.3) respectively, \( p < 0.001 \)). In post hoc analysis, the GDT cohort also had shorter time to mobilisation out of bed (2.0 days (IQR 1.0-3.0) and 4.0 days (IQR 3.0-6.25) respectively; \( p < 0.001 \)) and shorter time to tolerating oral diet (5.0 days (IQR 4.0-8.0) and 8.0 days (IQR 6.75-10.0) respectively; \( p < 0.001 \)).

Post-Operative Complications

23 patients (79.3% and 76.7% in GDT and ST groups respectively) had complications in both groups (\( p = 1.000 \)). Kidney DGF rates were significantly lower in the GDT group when compared to the ST group (2 (6.9%) and 10 (30.0%) patients respectively; \( p = 0.021 \)). There were no differences in rates of other complications between the two study groups (Table 3.3).

One-month pancreas graft survival was similar in both groups (GDT group 21 (72.4%), ST group 25 (83.3%); \( p = 0.360 \)). Causes of graft failures by study group are demonstrated in Table 3.4. Of the patients who lost their grafts due to thrombosis, pancreata were all explanted within the first 72 hours post-SPKT. One patient had persistent bleeding post-operatively resulting in pancreatectomy on day 15 post-implantation. Of the four patients who lost their grafts due to anastomotic dehiscence or pancreatitis, these were explanted between one and three weeks post-SPKT. One patient (GDT group) lost the kidney graft within the first six hours post-surgery due to a technical failure.
Peri-Operative Risk Scores

All patients in both groups were ASA three and had a revised cardiac risk index class four. Median pre-operative P-POSSUM morbidity scores for GDT and ST groups were 79.899% (IQR 71.095- 87.409) and 76.395% (IQR 65.862- 84.553) respectively, and mortality scores were 9.723% (IQR 6.088- 16.332) and 8.336% (IQR 4.831- 13.124) for GDT and ST groups respectively. The increase in P-POSSUM morbidity and mortality rates were significantly lower from baseline to 72 hours post-transplant in the GDT group when compared to the ST group (morbidity values: GDT +3.917% (IQR -5.034- +10.054), ST +17.357% (IQR +4.804- +22.248); p= 0.001. mortality values: GDT +4.108% (IQR -1.918- +11.601), ST +14.061% (IQR +4.777- +26.608); p= 0.027).

POMS values, an important quantitative indicator of peri-operative morbidity, were significantly lower for the GDT cohort compared to the ST cohort at days five (2.0 (IQR 1.0- 3.0) and 3.0 (IQR 2.0- 4.0) respectively; p= 0.010) and seven (1.0 (IQR 0.0- 2.0) and 2.0 (IQR 0.5- 4.0) respectively; p= 0.033) (Figure 3.2).

The GDT group tended to have lower MODS scores up to 72 hours post-surgery, when compared to the ST group, but these differences were not significant (Table 3.5).

11 patients required at least one episode of further surgery per group (37.9% and 36.7% in GDT and ST groups respectively, p= 1.000). Of these cases, in the GDT group only one patient (3.3%) required more than five episodes of further surgery compared to three (10%) in the ST group.
Protocol Analysis

Intra-Operative Goals

A DO$_2$I greater than 600ml/min/m$^2$ was achieved by all patients in the GDT group and 13 patients (43.3%) in the ST group (p <0.001).

There was no difference in the baseline DO$_2$I between the GDT and ST groups at the start of the protocol (431ml/kg/m$^2$ ± 132.72 and 447.55ml/kg/m$^2$ ± 109.89 respectively, p= 0.627). However, the mean DO$_2$I immediately prior to pancreas perfusion (671.11ml/kg/m$^2$ ± 188.29 and 468.69 ml/kg/m$^2$ ±139.11 in GDT and ST groups respectively) and in the period post-pancreas perfusion (at 30mins post-perfusion; 637.04 ml/kg/m$^2$ ±165.20 and 454.55 ml/kg/m$^2$ ±171.09 in GDT and ST groups respectively) were significantly higher in the GDT group compared to the ST group (p< 0.001).This is illustrated at regular time intervals in Figure 3.3a.

In addition, mean ScVO$_2$ was significantly higher in the GDT group at time of pancreas perfusion when compared to the ST group (GDT group 87.97% (± 5.49), ST group 77.931% (± 7.19); p< 0.001), but there were no differences in serum lactate at any time point between the GDT and ST groups (Tables 3.6a and b respectively).

Intra-operative Protocol

More patients received a colloid bolus in the GDT group, compared to the ST group (29 (100%) and 21 (70%) respectively; p= 0.002), and in those patients who received a colloid bolus, the GDT group tended to receive more fluid volume, but this difference was not
significant (GDT group 1500mls (IQR 1000- 2000), ST group 1250 (IQR 340- 1500); p= 0.065). All patients received intra-operative crystalloid and 28 (96.6%) and 27 patients (90.0%) received intra-operative blood in the GDT and ST cohorts respectively (p=1.000).

Significantly more patients in the GDT cohort were administered dobutamine (GDT 20 (69.0%), ST 4 (13.3%); p <0.001), but when administered, the dosages were similar in both groups (GDT group 107.01mg (IQR 76.60- 104.83), ST group 93.31mg (IQR 85.18- 102.81); p= 0.386). There were no differences in the usage of NA between the two study groups. Table 3.7 details the intra-operative protocol usage in the two cohorts.

Post-Operative Goals

Once again, all patients in the GDT group achieved a DO$_2$I greater than 600ml/min/m$^2$ compared with 17 (56.7%) in the ST group (p=0.001). Figure 3.3b illustrates hourly mean DO$_2$I recordings in the GDT and ST groups until the end of the study period. The mean DO$_2$I were significantly higher in the GDT group compared to the ST group up to the end of the study period (781.37ml/min/m$^2$ ± 373.02 and 475.59ml/min/m$^2$ ± 148.76 respectively; p <0.001). No patient in the ST group was placed on a cardiac output monitor post-operatively for clinical use.

Mean ScVO$_2$ was also significantly higher at six hours post-operatively in the GDT group compared to the ST group (80.6% ± 16.7 and 72.5% ± 9.4 respectively, p= 0.036) and this difference persisted up to 48 hours post-surgery. (Table 3.6a).
Post-Operative Protocol

Intra-venous crystalloid, dose-dependent on urine output is standard protocol post-operatively at the study centre. The GDT cohort received significantly more crystalloid in the first six hours post-transplant, compared to the ST group (1310mls (IQR 750- 1700) and 800mls (IQR 523- 1325) respectively; p= 0.034) and more patients received a colloid bolus post-operatively in the GDT group compared to the ST group (20 (69.0%) and five (16.7%) respectively p= 0.001). However, for those patients who received colloid, the ST group received significantly more than those in the GDT group (1000mls (IQR 750- 1250) and 500mls (IQR 478- 523) respectively; p= 0.042).

Although significantly more patients were given dobutamine post-operatively in the GDT group compared to the ST group (13 and 4 respectively; p< 0.001), there were no significant differences in the use of NA. Table 3.8 details the post-operative protocol usage in the GDT and ST cohorts.
3.5 Discussion


The results from this study suggest that short-term outcomes in this high-risk cohort can be improved using a protocol driven peri-operative strategy to optimise physiological parameters to defined goals. We demonstrated that patients in the GDT cohort had significantly shorter lengths of critical care unit stay when compared to the ST group. We accept that procedure-specific median critical care length of stays may be higher at our centre when compared to similar centres within the UK, but this is a reflection of local practice, guided by The Intensive Care Society Standards for Levels of Critical Care for Adult Patients (Eddlestone J 2009), which take into consideration a holistic approach to patient assessment. Additional evidence supporting this holistic improvement in patient status as a result of GDT are: i) shorter times to mobilisation out of bed, ii) shorter time to tolerating oral diet, iii) reduced rates of renal delayed graft function, and iv) superior post-operative P-POSSUM and POMS scores in the GDT cohort, compared to the ST cohort.

Patients submitted to major physiological insults suffer from unrecognised fluid deficits during the peri-operative period, resulting in a cascade of events stimulated via the action
of pro-inflammatory mediators, leading to extra-vascular fluid losses, hypotension, reduced organ perfusion and ultimately organ dysfunction and failure. Therefore, surgical groups where large fluid deficits occur will benefit more through strategies to counter this. SPKT recipients are one such group. Therefore, it would be expected, as has been highlighted in this study that this high-risk cohort would significantly benefit from supra-physiological peri-operative optimisation.

The authors accept that one-month graft survival is dramatically lower in our study than would be expected or acceptable internationally. The causes for these graft losses have been individually investigated and were found to be multi-factorial (Table 3.4), unrelated to the study protocol, but due to a combination of; technical factors, higher than recommended CIT and poor recipient and/or graft selection in those individual cases. This is highlighted in Table 3.2 that identifies the higher rate of fatty pancreata in the GDT group compared to the ST group (8 and 2 respectively) and is consistent with the higher rates of pancreatic graft losses in the GDT group compared to the ST group (83.3% and 72.4% respectively, p= 0.360). Furthermore, total rates of complications and re-operations were no different between the two study groups suggesting no positive or negative impact of GDT on these outcomes in this cohort.

Of the individual complications analysed, only rates of DGF differed between the two groups. These were significantly lower in the GDT group compared to the ST group (6.9% and 33.3% respectively, p= 0.021). We accept that the GDT group of recipients were on dialysis for a shorter period of time, when compared to the ST group, but this should not impact on allograft function post-transplantation. In our study, DGF was defined as a
recipient requiring two or more sessions of dialysis post-transplant, which has previously lead to reported rates of DGF approaching 40% in kidney alone transplantation (Koning, van Bockel et al. 1995). To our knowledge, there is nothing published about rates of kidney DGF following SPKT. The results of the ST group from this study are therefore consistent with previous reports on DGF rates published following renal transplant. However, the rates in the GDT group are significantly improved. Due to the increased morbidity associated with PT when compared to kidney transplant alone, SPKT donors are a highly selected group. One would therefore expect that kidney DGF rates following SPKT should be lower than in kidney transplant alone. Thus, we propose that the potential for recipient instability at the time of kidney perfusion following pancreas perfusion, and the associated physiological inflammatory response to dual-organ transplant, negate the advantage of having a highly selected donor. Therefore, in the GDT group, the advantages of a consistently higher DO$_2$I in the peri-operative period, leading to a physiologically stable recipient, allow for adequate kidney perfusion and maintenance of adequate perfusion peri-operatively, resulting in lower DGF.

Predicted mortality and morbidity rates, based upon P-POSSUM, in the entire cohort are consistently high and highlight the severity of disease and the poor physiological reserve of such recipients. Despite the relatively young age of the patients in this study, compared to previous similar studies (mean age 66 (Pearse, Dawson et al. 2005)), the P-POSSUM scores are comparable, suggesting that our patients are physiologically older than their chronological age suggests. However, differences between the GDT and ST groups of the changes in P-POSSUM score from pre-transplant to 72 hours post-transplant indicate a likely enhanced ability to cope with the same physiological insult in those patients who
have undergone GDT. Finally, the results of the serial POMS measurements corroborate this finding.

There were no differences seen in total complication rates, re-operation rates and POMS between the GDT and ST cohorts following one week post-transplant. These findings suggest that in this cohort, the effects of peri-operative GDT are short-lived. This is contradictory to a number of previous studies which have shown significant benefits well beyond the termination of an early goal-directed therapy protocol resulting in improved patient survival and hospital lengths of stay (Boyd, Grounds et al. 1993, Wilson, Woods et al. 1999, Lobo, Salgado et al. 2000, Gan, Soppitt et al. 2002, Venn, Steele et al. 2002, Pearse, Dawson et al. 2005, Wakeling, McFall et al. 2005, Donati, Loggi et al. 2007, Jhanji, Vivian-Smith et al. 2010, Mayer, Boldt et al. 2010). However, given the number of variables involved, and the reasons for complications and re-operations in SPKT this finding is of little surprise (Sutherland, Gruessner et al. 2001, Axelrod, Sung et al. 2010, Sampaio, Reddy et al. 2010, Troppmann 2010). In-hospital complications following SPKT result from peri-operative factors such as; surgical, donor and organ factors, cold ischaemic time, heparinisation and fluid management, as well as post-operative factors such as; immunosuppresion, re-feeding and chronic morbidity due to IDDM (peripheral vascular disease, neuropathy, gastroparesis, previous limb amputations) all of which affect length of hospital stay. In hindsight, given these variables, which peri-operative GDT is unable to optimise, powering the study for length of hospital stay was naïve. In addition, considering the multi-system co-morbidities resulting in the multi-system complications suffered by our patients’ post-SPKT, it would have been more useful to power the study using a compound morbidity index such as POMS or MODS.
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All patients in the GDT cohort achieved the goal of \( \text{DO}_2 \text{I} \) greater than 600ml/min/m\(^2\), compared to the ST group where only 13 (43.4%) and 17 (56.7%) in the intra- and post-operative period respectively reached the same target spontaneously. These proportions are similar to previous studies (Pearse, Dawson et al. 2005) and reinforce the opinion that SPKT recipients behave in a similar manner, physiologically, to other high-risk candidates for surgery.

Significantly more patients received a colloid bolus in the GDT arm when compared to the ST arm, but surprisingly, there were no differences in total volume of fluid given to the recipients intra-operatively, concurring that a GDT protocol does not result in over-resuscitation, but likely leads to a more individualised approach to cardiovascular optimisation (Pearse, Harrison et al. 2014).

During the course of the study, a trend has appeared at the investigating unit erring towards a more aggressive form of peri-operative resuscitation, akin to GDT, as clinicians have become more familiar with the study protocol. Therefore, we accept that over the course of the trial the care in the ST group may have drifted towards GDT, despite the absence of a calibrated cardiac output monitor in the ST group. Notwithstanding this, we have been able to show clinically significant differences in outcome between the two groups.

A limitation of the study includes a lack of blinding. In a study of this nature, it is not possible to conduct this adequately and remains consistent with previous publications.
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(Grocott, Dushianthan et al. 2013). The clinical decisions regarding outcome measures were made by senior medical staff, who, at the time of making the decisions would not have been actively made aware of the randomisation group. However, a way to confirm this would have been to conduct a questionnaire of the clinician making each decision relating to the outcome measures to assess their knowledge of the randomisation group.

The GDT group were also younger than the ST group (39.96 years vs 44.27 years respectively), despite our strict adherence to a well validated randomization protocol. Although this difference is statistically significant, both groups were relatively young (mean age <45 years in both groups) and a difference of 4.31 years is unlikely to be clinically significant. A linear regression between recipient age and critical care length of stay (Figure 3.4a- c) showed no significant correlation between these variables. Therefore, the age differences between the two groups, though an important consideration, should not negate the findings of this study. Furthermore, the baseline co-morbidities and risk scores tended to be worse in the GDT group compared to the ST group (as highlighted by the baseline P-POSSUM scores). Finally, since the study was not powered to assess for length of critical care unit stay or for other outcome measures, the findings should be interpreted with caution, despite being highly significant. Nevertheless, these results are entirely consistent with a number of previous publications related to GDT, and therefore, despite the small numbers, add to the argument in favour of a protocol-driven peri-operative optimisation strategy, guided by a calibrated cardiac output monitor in high-risk individuals.
3.6 Conclusion

SPKT is a niche procedure, with approximately only 2000 being conducted per annum internationally. The procedures being performed globally do not correlate with the numbers of Type 1 diabetic patients or the organs available for transplantation. Part of the reticence to SPKT is the inherent significant peri-operative morbidity. Since its inception in 1966 (Kelly, Lillehei et al. 1967) great strides have been made to improve outcomes by adjusting donor, organ and recipient selection, surgical technique and immunosuppression (Humar, Kandaswamy et al. 2000, Sutherland, Gruessner et al. 2001). The results from this study suggest that efforts should now be made to standardise and optimise intra and peri-operative care, which in turn will lead to improved peri-operative morbidity and overall outcomes.

3.7 Acknowledgements

The authors would like to thank LiDCO Ltd, who loaned the equipment and disposables for use in the study, Catherine Fullwood from The University of Manchester Biomedical Research Unit, for her statistical support and Kidneys for Life (registered charity) for their financial support.
Patients which met Inclusion Criteria  
(n= 69)

Did not consent  
n= 9

Randomised (n= 60)

Allocated to Goal-Directed Therapy Group (n= 30)

Allocated to Standard Therapy Group (n= 30)

1 Patient did not receive pancreas 1 patient remained an inpatient at 1 year

28 patients analysed

29 patients analysed

1 in-patient death

Figure 3.1. Consort diagram
### Chapter 3, GDT in SPKT

Table 3.1. Baseline Recipient and Donor variables in GDT and ST cohorts

<table>
<thead>
<tr>
<th>Recipient Variables</th>
<th>GDT Group (n=29)</th>
<th>ST Group (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>39.96 ± 7.35</td>
<td>44.27 ± 6.83</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>13 (44.8%)</td>
<td>21 (70.0%)</td>
<td>0.067</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1 African (3.4%)</td>
<td></td>
<td></td>
<td>0.343</td>
</tr>
<tr>
<td>1 Indian Asian (3.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 White (93.1%)</td>
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<td></td>
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</tr>
<tr>
<td>30 British White (100%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>25.02 ± 3.43</td>
<td>25.39 ± 2.91</td>
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<td><strong>Years diabetic</strong></td>
<td>27.11 ± 6.60</td>
<td>26.73 ± 8.35</td>
<td>0.683</td>
</tr>
<tr>
<td><strong>Number of diabetic complications</strong></td>
<td>3.89 ± 1.57</td>
<td>3.87 ± 1.53</td>
<td>0.808</td>
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<tr>
<td><strong>Daily insulin dose (Units)</strong></td>
<td>48.71 ± 14.37</td>
<td>47.07 ± 15.50</td>
<td>0.619</td>
</tr>
<tr>
<td><strong>Pre-dialysis</strong></td>
<td>13 (44.8%)</td>
<td>12 (40.0%)</td>
<td>0.795</td>
</tr>
<tr>
<td><strong>Time on dialysis (months)</strong></td>
<td>8.39 ± 11.30</td>
<td>22.50 ± 30.10</td>
<td>0.023</td>
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<td><strong>Smoking (pack years)</strong></td>
<td>0.0 IQR 0.0- 10.0</td>
<td>0.0 IQR 0- 12.8</td>
<td>0.993</td>
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<tr>
<td><strong>Time on waiting list (days)</strong></td>
<td>606 IQR 375- 688</td>
<td>606 IQR 312- 780</td>
<td>0.658</td>
</tr>
<tr>
<td><strong>P-POSSUM Morbidity (%)</strong></td>
<td>79.899 IQR 71.106- 88.287</td>
<td>75.584 IQR 64.020- 82.232</td>
<td>0.061</td>
</tr>
<tr>
<td><strong>P-POSSUM Mortality (%)</strong></td>
<td>9.723 IQR 6.087- 17.494</td>
<td>8.336 IQR 4.475- 11.423</td>
<td>0.073</td>
</tr>
<tr>
<td><strong>RCRI</strong></td>
<td>29 class IV (100%)</td>
<td>4 class III (13.3%)</td>
<td>0.083</td>
</tr>
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<td></td>
<td>26 class IV (86.7%)</td>
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<td></td>
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<tr>
<td><strong>ASA Score</strong></td>
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<td>30 class III (100%)</td>
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<td><strong>Waterlow Score</strong></td>
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</tr>
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<td><strong>MODS</strong></td>
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<td>0.647</td>
</tr>
<tr>
<td><strong>DONOR VARIABLES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>33.96 ± 12.39</td>
<td>32.55 ± 12.80</td>
<td>0.677</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>19 (65.5%)</td>
<td>21 (70.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Indian (3.4%)</td>
<td></td>
<td></td>
<td>0.571</td>
</tr>
<tr>
<td>1 Mixed White/ Asian (3.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27 British White (93.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 European Other (3.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 British White (93.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>23.31 ± 2.97</td>
<td>24.25 ± 2.29</td>
<td>0.187</td>
</tr>
<tr>
<td><strong>COD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 ICH (trauma) (43.3%)</td>
<td></td>
<td></td>
<td>0.910</td>
</tr>
<tr>
<td>7 ICH (non-trauma) (23.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Hypoxic brain injury (16.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Intra-cranial thrombosis (6.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Other (10.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DBD</strong></td>
<td>22 (73.3%)</td>
<td>23 (76.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Donor WCC (n=23)</strong></td>
<td>17.12 ± 10.16</td>
<td>12.74 ± 5.32</td>
<td>0.075</td>
</tr>
<tr>
<td><strong>Donor Amylase (n=20)</strong></td>
<td>143.25 ± 178.37</td>
<td>133.76 ± 166.50</td>
<td>0.861</td>
</tr>
<tr>
<td><strong>Time from admission to retrieval</strong></td>
<td>53 IQR 33- 84</td>
<td>51 IQR 31- 87</td>
<td>0.718</td>
</tr>
<tr>
<td><strong>P-DRI</strong></td>
<td>1.76 ± 0.85</td>
<td>1.66 ± 0.67</td>
<td>0.632</td>
</tr>
<tr>
<td><strong>P-PASS</strong></td>
<td>11.44 ± 2.39</td>
<td>10.38 ± 2.19</td>
<td>0.088</td>
</tr>
</tbody>
</table>

Values are absolute (%) or mean ± Standard Deviation, SD unless otherwise stated. * Median (Interquartile range, IQR). BMI, Body Mass Index; P-POSSUM, Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity; RCRI, Revised Cardiac Risk Index; ASA, American Society of Anaesthesiologists; COD, Cause of death; DBD, Donor after brainstem death; WCC, White cell count; P-DRI, Pancreas donor risk index; P-PASS, Pre-procurement pancreas allocation score.
Table 3.2. Operative variables in GDT and ST cohorts

<table>
<thead>
<tr>
<th>OPERATIVE VARIABLES</th>
<th>GDT group (n= 29)</th>
<th>ST group (n= 30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P CIT</td>
<td>676.00 ± 185.38</td>
<td>686.31 ± 182.30</td>
<td>0.835</td>
</tr>
<tr>
<td>K CIT</td>
<td>818.07 ± 200.68</td>
<td>830.69 ± 194.79</td>
<td>0.812</td>
</tr>
<tr>
<td>Pancreas fattiness grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Nil (41.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Mild (17.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Moderate (20.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 High (24.1%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Nil (56.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Mild (13.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Moderate (20.0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 High (6.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mismatch &gt; 3</td>
<td>18 (62.1%)</td>
<td>20 (66.67%)</td>
<td>0.789</td>
</tr>
<tr>
<td>Induction Agent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 Campath (96.6%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Simulect (3.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 Campath (96.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Simulect (3.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas Preservation Solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 UW (89.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 HTK (10.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28 UW (93.3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 HTK (6.7%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of Operation (mins)</td>
<td>332.70 ± 71.12</td>
<td>346.66 ± 78.41</td>
<td>0.490</td>
</tr>
</tbody>
</table>

Values are absolute (%) or mean (± Standard Deviation, SD) P CIT, Pancreas cold ischaemic time; K CIT, Kidney cold ischaemic time; UW, University of Wisconsin solution; HTK, Histidine-tryptophan-ketoglutarate solution
Table 3.3. Summary of complications in GDT and ST cohorts

<table>
<thead>
<tr>
<th>Complication</th>
<th>GDT</th>
<th>ST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Complications</td>
<td>23 (79.3%)</td>
<td>23 (76.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>13 (44.8%)</td>
<td>10 (33.3%)</td>
<td>0.430</td>
</tr>
<tr>
<td>DGF</td>
<td>2 (6.9%)</td>
<td>10 (33.3%)</td>
<td>0.021</td>
</tr>
<tr>
<td>Bleeding</td>
<td>5 (17.2%)</td>
<td>5 (16.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td>UTI</td>
<td>9 (31.0%)</td>
<td>6 (20.0%)</td>
<td>0.382</td>
</tr>
<tr>
<td>Wound</td>
<td>4 (13.8%)</td>
<td>6 (20.0%)</td>
<td>0.731</td>
</tr>
<tr>
<td>Respiratory</td>
<td>2 (6.9%)</td>
<td>7 (23.3%)</td>
<td>0.145</td>
</tr>
<tr>
<td>Cardiac</td>
<td>1 (3.4%)</td>
<td>1 (3.3%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Return to Theatre</td>
<td>11 (37.9%)</td>
<td>11 (36.7%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Anastomotic leak</td>
<td>2 (6.9%)</td>
<td>1 (3.3%)</td>
<td>0.612</td>
</tr>
<tr>
<td>Intra-abdominal Infection</td>
<td>3 (10.3%)</td>
<td>4 (13.3%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Values are absolute (%). GDT, Goal-directed therapy; ST, Standard therapy; DGF, Delayed graft function; UTI, Urinary tract infection
Table 3.4. Causes of graft failure in GDT and ST cohorts

<table>
<thead>
<tr>
<th>Cause</th>
<th>GDT (n= 8)</th>
<th>ST (n= 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Operative Bleeding</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Intra-Operative Bleeding</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Venous Thrombosis</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Arterial Thrombosis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anastomotic Dehiscence</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 3.2. Mean Post-operative morbidity score (POMS), days 5, 7 and 10 post-transplantation (Significant differences at days 5 and 7 (p= 0.010 and 0.033 respectively))
Table 3.5. Mean MODS values and p-values in GDT and ST cohorts, from pre-operative to 72 hrs post-operative at 24 hour intervals

<table>
<thead>
<tr>
<th>Time</th>
<th>GDT</th>
<th>ST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>3.93 +/- 1.33</td>
<td>3.77 +/- 1.43</td>
<td>0.647</td>
</tr>
<tr>
<td>24 hr MODS</td>
<td>3.03 +/- 1.426</td>
<td>3.57 +/- 0.302</td>
<td>0.192</td>
</tr>
<tr>
<td>48 hr MODS</td>
<td>2.79 +/- 1.989</td>
<td>3.20 +/- 1.730</td>
<td>0.405</td>
</tr>
<tr>
<td>72 hr MODS</td>
<td>2.34 +/- 1.876</td>
<td>2.80 +/- 1.660</td>
<td>0.329</td>
</tr>
</tbody>
</table>
Figure 3.3a. Intra-operative DO$_2$I at start and end of surgery, at pancreas perfusion and then at 5 minute intervals, up to 30 minutes post-perfusion in GDT and ST cohorts.

Figure 3.3b Post-operative DO$_2$I post-operatively at hourly intervals, up to end of intervention period (6 hours post-operation).

Figures 3.3a and b. Indexed Oxygen Delivery (DO$_2$I (ml/min/m$^2$)) readings at intra- (a) and post- (b) operative intervals.
Tables 3.6a and b. Mean Central venous oxygen saturations (a) and mean lactate levels (b) in the GDT and ST cohorts during the peri-operative period.

<table>
<thead>
<tr>
<th>Time</th>
<th>GDT</th>
<th>ST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Operative</td>
<td>84.97 ± 6.03</td>
<td>84.43 ± 6.66</td>
<td>0.576</td>
</tr>
<tr>
<td>Pre-pancreas perfusion</td>
<td>87.63 ± 5.85</td>
<td>78.09 ± 7.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30 mins post perfusion</td>
<td>88.36 ± 4.42</td>
<td>76.15 ± 6.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 hours</td>
<td>80.61 ± 16.71</td>
<td>72.50 ± 9.41</td>
<td>0.036</td>
</tr>
<tr>
<td>12 hours</td>
<td>81.01 ± 17.13</td>
<td>68.85 ± 15.94</td>
<td>0.006</td>
</tr>
<tr>
<td>24 hours</td>
<td>81.64 ± 17.00</td>
<td>72.37 ± 9.67</td>
<td>0.009</td>
</tr>
<tr>
<td>48 hours</td>
<td>82.96 ± 16.88</td>
<td>74.66 ± 8.56</td>
<td>0.015</td>
</tr>
<tr>
<td>72 hours</td>
<td>79.71 ± 22.96</td>
<td>74.72 ± 9.15</td>
<td>0.238</td>
</tr>
</tbody>
</table>

(a) Central venous oxygen saturations (ScVO₂)

<table>
<thead>
<tr>
<th>Time</th>
<th>GDT</th>
<th>ST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Operative</td>
<td>84.97 ± 6.03</td>
<td>84.43 ± 6.66</td>
<td>0.576</td>
</tr>
<tr>
<td>Pre-pancreas perfusion</td>
<td>87.63 ± 5.85</td>
<td>78.09 ± 7.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30 mins post perfusion</td>
<td>88.36 ± 4.42</td>
<td>76.15 ± 6.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 hours</td>
<td>80.61 ± 16.71</td>
<td>72.50 ± 9.41</td>
<td>0.036</td>
</tr>
<tr>
<td>12 hours</td>
<td>81.01 ± 17.13</td>
<td>68.85 ± 15.94</td>
<td>0.006</td>
</tr>
<tr>
<td>24 hours</td>
<td>81.64 ± 17.00</td>
<td>72.37 ± 9.67</td>
<td>0.009</td>
</tr>
<tr>
<td>48 hours</td>
<td>82.96 ± 16.88</td>
<td>74.66 ± 8.56</td>
<td>0.015</td>
</tr>
<tr>
<td>72 hours</td>
<td>79.71 ± 22.96</td>
<td>74.72 ± 9.15</td>
<td>0.238</td>
</tr>
</tbody>
</table>

(b) Lactate
Table 3.7. Intra-Operative fluid and drug usage in GDT and ST cohorts

<table>
<thead>
<tr>
<th></th>
<th>GDT Cohort</th>
<th>ST Cohort</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colloid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 29 (100%)</td>
<td>n= 21 (70.0%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>1500 IQR 1900- 4700</td>
<td>1250 IQR 340- 1500</td>
<td>0.065</td>
</tr>
<tr>
<td><strong>Crystalloid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 29 (100%)</td>
<td>n= 30 (100%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>2650 IQR 1900- 4700</td>
<td>3500 IQR 2904- 4000</td>
<td>0.135</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 28 (96.6%)</td>
<td>n= 27 (90.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>739 IQR 536- 1139</td>
<td>581 IQR 428- 884</td>
<td>0.216</td>
</tr>
<tr>
<td><strong>Dobutamine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Treated</td>
<td>n= 20 (69.0%)</td>
<td>n= 4 (13.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dose given (mg)</td>
<td>107.01 IQR 76.60- 104.83</td>
<td>93.31 IQR 85.18- 102.81</td>
<td>0.286</td>
</tr>
<tr>
<td><strong>NA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Treated</td>
<td>n= 14 (48.3%)</td>
<td>n= 10 (33.3%)</td>
<td>0.295</td>
</tr>
<tr>
<td>Dose given</td>
<td>0.868 IQR 0.693- 1.108</td>
<td>1.023 IQR 0.318- 1.517</td>
<td>0.931</td>
</tr>
</tbody>
</table>

Values are either absolute or medians with Interquartile range (IQR) Continuous variables tested using Mann-Whitney U-Test; Categorical variables tested using Fishers Exact. NA, Nor-adrenaline
Table 3.8. Post-Operative fluid and drug usage in GDT and ST cohorts

<table>
<thead>
<tr>
<th></th>
<th>GDT Cohort</th>
<th>ST Cohort</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colloid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 20 (66.7%)</td>
<td>n= 5 (16.7%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>500 IQR 478- 523</td>
<td>1000 IQR 750- 1250</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>Crystalloid</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n=29 (100%)</td>
<td>n= 30 (100%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>1310 IQR 750- 1700</td>
<td>800 IQR 523- 1325</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 6 (100%), 1 exclusion</td>
<td>n= 6 (100%), 1 exclusion</td>
<td>1.000</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>284 IQR 145- 379</td>
<td>291 IQR 244- 426</td>
<td>1.000</td>
</tr>
<tr>
<td><strong>Dobutamine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 13 (44.8%)</td>
<td>n= 4 (13.3%)</td>
<td>0.010</td>
</tr>
<tr>
<td>Dose given (mls)</td>
<td>45.39 IQR 23.14- 90.90</td>
<td>89.15 IQR 29.64- 103.59</td>
<td>0.477</td>
</tr>
<tr>
<td><strong>NA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number treated</td>
<td>n= 4 (13.8%)</td>
<td>n= 9 (30.0%)</td>
<td>0.209</td>
</tr>
<tr>
<td>Dose given (mg)</td>
<td>0.395 IQR 0.080- 1.318</td>
<td>0.340 IQR 0.175- 1.468</td>
<td>0.710</td>
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Values are either absolute or medians with Interquartile range (IQR). Continuous variables tested using Mann-Whitney U-Test; Categorical variables tested using Fishers Exact. NA, Nor-adrenaline
Figure 3.4a-c. Linear correlations of recipient age and length of critical care unit stay in a) the entire cohort (p = 0.173) b) the GDT cohort (p = 0.236) and c) the ST cohort (p = 0.780) (Spearman Correlation)
# Chapter 3, Appendix 3.1, ST Anaesthetic Protocol

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Anaesthetic management for Pancreas transplantation

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1. Introduction:
Pancreas transplants provide a cure for people with Type 1 Diabetes, in particular people with complications of Diabetes Mellitus (DM) like nephropathy, retinopathy and neuropathy. The goal of transplantation is to eliminate morbidity associated with labile blood glucose, stabilise or improve secondary DM complications and improve quality of life of people with Insulin dependent Diabetes Mellitus (IDDM) by restoring normal glucose metabolism. The benefits of a transplant outweigh the risks of surgery and lifelong immunosuppression.
The majority of pancreas transplants are performed in the US with rising numbers in the rest of the world. The total number of pancreas transplants done worldwide since 1966 stands at around 24000. There were 193 pancreas transplants in the UK in the year 2006-2007. There are 7 centres in the UK, which perform this surgery with Oxford being the largest (around 80 transplants done over the last year).
The Manchester Royal Infirmary (MRI) undertakes around 30-40 pancreas transplants per year.
There are 3 procedures performed with indications outlined below:

1. Simultaneous pancreas kidney (SPK): Diabetics with Chronic renal Failure (CRF) either on renal replacement therapy (RRT) or predialysis. This is the commonest of the three.
2. Pancreas after Kidney (PAK): after successful kidney transplant
3. Pancreas transplant alone (PTA): poorly controlled DM, hypoglycemic unawareness

There are other procedures like segmental transplant (tail) for live donors and islet cell transplant which are undergoing research. The selection criteria are crucial for successful outcomes. The patients are between the ages of 20-60yr and have a BMI≤30. Patients with poor cardiac reserve and untreatable Coronary Artery Disease (CAD ) are excluded. Average waiting time for a pancreas transplant is 2-3 yrs.
The surgery takes approx 5-7 hrs with surgical access achieved via a laparotomy and midline incision. The surgical technique involves placing the graft in the peritoneum and providing adequate arterial blood flow to the pancreas. Systemic venous drainage is obtained by anastomosing donor portal vein to recipient iliac vein or Inferior Vena Cava. Pancreas graft arterial revascularization typically is accomplished using the recipients’ right common or external iliac artery. Drainage of pancreatic exocrine secretions is achieved by donor duodenum being anastomosed to jejunum or bladder. The former technique is performed in majority of cases, as it is associated with significantly fewer urologic and metabolic complications, as well as fewer Urinary Tract Infections. The pancreas is implanted first. The native pancreas is not removed.
Complications immediately after surgery include rejection, thrombosis, pancreatitis, hemorrhage, anastomotic leak and infection. Late complications are usually immunological secondary to rejection.
The survival rates at 1 year for pancreas graft are 84% (SPK) and 71% (pancreas only). Patient survival is 95-100%.
Chapter 3, Appendix 3.1, ST Anaesthetic Protocol

The perioperative care of these patients is very challenging due to the nature of the disease and the associated complications and also because of the implications of major surgery. The aim is to optimise and support graft perfusion and function by providing conditions with haemodynamic and metabolic stability (glucose, potassium and acid-base balance) during the perioperative period.

The goal should be to implant the graft as soon as possible keeping the cold ischaemic times to ideally <12 hours. The bench work takes about 2 hrs and cross match takes around 4-6 hrs; patients can be sent for as soon as the cross match result is available. At the MRI we are also performing a virtual/peripheral cross match which significantly shortens the delay caused by waiting for a full cross match.

2. Preoperative Assessment:
These patients should be treated as urgent and seen as soon by on call team as possible. Surgeons usually have a backup patient who is admitted as well. They should be assessed as well. They have been extensively investigated and worked up prior to being put on the waiting list and will have been assessed annually by the transplant team regarding suitability. Hence attention to current issues like K+, last dialysis and Hb are pressing.

2.1 History:

Endocrine: Duration of DM, complications, Insulin sliding scale
Renal: End Stage Renal Failure (ESRF), RRT, Predialysis, Dry weight and time of last dialysis, to avoid heparin if within 6 hrs surgery. Urine output / Fluid restriction, Fistula site, Vascular access
Cardiac: Ischaemic Heart Disease (IHD), Hypertension (HT), Ejection fraction, CAD, inducible ischemia, previous Myocardial Infarction (MI) Their cardiac evaluation involves an echocardiogram and stress myocardial perfusion scans, those with abnormal results being referred to cardiologists for intervention and optimisation. Hence there should be no need for further assessment on day of surgery unless there is change in clinical symptoms or a new recent event.
Cardio Pulmonary Exercise (CPX) testing is being used for all new referrals.
Neuropathy: Autonomic dysfunction, orthostatic hypotension and gastroparesis
Medication: to have all drugs as normal, avoid ACE-I & All Receptor antagonists.

2.2 Investigations and Preparation:

Full Blood Count (FBC), Urea and Electrolytes (U&E,) Clotting, and Calcium on admission: especially K+. - ESRF patients can have chronically high K+ 5.5; this may be normal for them. These patients can be chronically anaemic.
12 lead ECG on admission Echo and myocardial perfusion scan results (done as part of workup)
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CPEX Reports
Ensure 4 units blood are cross matched
Discuss Epidural or Nerve Blocks and Local anaesthetic infusions,
Arterial and
Central venous line with patient.
Level 3 bed should be available for postoperative care.

3. In Anaesthetic Room:
The primary goals in this group of patients related to anaesthesia are cardiovascular stability and careful consideration to drug pharmacokinetics.

3.1 Drugs:
Induction agent: Any induction agent can be used in these patients but be aware of greater cardiovascular instability if dialysed and dehydrated.
Opioid: All opioids accumulate in renal failure; Fentanyl and Remifentanil can be used intraoperatively. Fentanyl can be used for postoperative analgesia. Avoid Morphine and Pethidine because of active metabolites.
Muscle relaxant: Cisatracurium as not dependent on renal excretion and no histamine release
Mannitol (0.5g/kg) (10% or 20%) (1st reperfusion pancreas, 2nd reperfusion kidney)
Dextrose 10%
Methyl prednisolone 500mg
Antibiotic: Augmentin 1.2g (Appendix 1)
Establish intravenous (IV) access, 16G or above. Do not use arm with fistula
Administer Immunosuppressant Alemtuzumab (MabCampath) 30mg Subcutaneously and Antibiotic during epidural insertion

3.2 Analgesia:
• Epidural: Thoracic epidural preferably above T11 as this offers the best post op analgesia and also aids in early extubation but time taken to insert an epidural and number of attempts should be reasonable so as to keep cold ischaemic times to a minimum.
• Transverse Abdominal Plane (TAP) blocks & Rectus sheath catheters: The surgeons can place multihole soaker catheters or epidural catheters in the rectus sheath under direct vision before closure. The TAP catheters or rectus sheath catheters should be either connected to an epidural pump via a 3-way tap or to two elastomeric pumps.

3.3 Induction and monitoring
Suxamethonium can be used if K+<5.5.
Check Blood glucose (BM) awake pre induction
Invasive monitoring:
Arterial line / CVP line, A-Line awake if significant cardiopulmonary disease or autonomic dysfunction.
Chapter 3, Appendix 3.1, ST Anaesthetic Protocol

Oesophageal Doppler should be used in all patients.
- Central line access – Use **quadruple lumen** lines, as these patients are difficult for venous access and need 1 line free for TPN post op.
  
  Nasogastric tube

Baseline Arterial Blood Gases (ABG), Central venous saturation (ScVO2), Thromboelastography (TEG). Please document results in anaesthetic chart for future audit purposes

**Have blood products in theatre fridge** when you start surgery. Warming equipment put warming blanket on in anaesthetic room if necessary. (Fluid warmer, warming mattress and top and bottom warming blankets)

4. Maintenance:
Aim is to keep the patient well filled, normothermic maintaining systolic blood pressure within 15 – 20 % of baseline and normal biochemical numbers so as to optimise graft perfusion and function and aid early extubation.

4.1 Monitoring:
Routine monitoring + Invasive Arterial blood pressure (IABP), Central Venous pressure (CVP), Urine output, temperature, Neuromuscular Junction (NMJ) monitor ABG hourly and simultaneous ScVO2, BMs Cardiac output monitoring: Oesophageal Doppler Monitor (ODM) or Pulmonary artery Flotation Catheter (PAFC).

A major objective of management is maximising cardiovascular performance to optimise graft perfusion and function. Flow monitoring allows us to make appropriate and rational decisions regarding fluid management or inotrope/vasopressor requirements. This applies to the intraoperative and postoperative period. This will minimise patient morbidity and graft thrombosis. ODM monitoring should be used in all patients undergoing pancreas transplants. The ODM has shown improvement in outcomes in other groups of surgical patients. Aim to optimise patients (<10% change in Systolic Diameter (SD) or Stroke Volume (SV) with fluid bolus 250ml over 10 min) prior to start of surgery, ideally in the anaesthetic room.

4.2 Maintenance drugs: A volatile/epidural, volatile/opioid or Total Intravenous Anaesthesia (TIVA) technique may be used. Use technique that provides stability and one that you are familiar with.

Volatiles: Isoflurane or desflurane should be used as they are least metabolised.

4.3 Fluid management:
Aim for CVP 12-16mmHg, this surgery tends to require significant volumes of fluid due to losses and reperfusion injury.

Crystalloids: Avoid Sodium chloride solution (NaCl) to avoid hyperchlaemiac acidosis. However K+ and lactate levels should be strictly monitored intraoperatively in oliguric patients.

Colloids:
  - Hetastarch (Volulyte 6%) has been shown to improve graft vessel
Chapter 3, Appendix 3.1, ST Anaesthetic Protocol

flow
in patients with functioning kidneys. Limit use to 1500ml/24hrs.
  o Voluven is available in main theatres if K+ is a concern.
  o Alternatives would be albumin / Blood
  o Avoid gelofusin.
If Hb <9 on admission blood transfusion should be started pre-
emptively before organ reperfusion. See Appendix 3 for hemodynamic
strategies to achieve optimal graft blood flow, oxygen delivery and graft
perfusion pressure.
(0.5-1 g/kg) of 10% mannitol. 1st dose given prior to reperfusion of
pancreas and then 2nd dose prior to kidney graft reperfusion. Increase
perfusion and acts as a free radical scavenger. Mannitol may have to
be repeated and the surgeon would communicate this.

4.4 Reperfusion:
Aim for a CVP 12-16 prior to unclamping of vessels. There can be
haemodynamic instability and arise in K+ due to reperfusion. There is also a
risk of significant blood loss in this period (2L+ in a short period of time) and
a coagulopathy. Aim to keep on top of losses and give blood as required.
Aim for Hb>8.
If blood loss high send FBC & Clotting sample including Fibrinogen &
perform TEG if staff available. (Most Emergency Anaesthetic Practitioners
can do a TEG for you) Appendix 2 provides a Treatment protocol based on
TEG values. After reperfusion graft hypoperfusion should be avoided.

Glucose monitoring:
Check BM before induction. Insulin sliding scale as per protocol. Must be
stopped on reperfusion of pancreas. BMs every 15min for 1st hr and then
every 30min until they stabilise. Major fluctuations can occur at this stage.
Dextrose 10% if BM<4. Any significant rise in BM >12 surgeons should
be made aware.

4.5 Immunosuppressant:
Alemtuzumab (MabCampath) 30mg subcutaneously at
induction. Methylprednisolone 500mg prior to unclamping needs to be
given slowly as can cause hypotension.

4.6 Anticoagulation:
Surgeons may request a bolus dose of heparin IV intraoperatively.

5. Post operatively:
Aim to extubate all patients unless cardiovascularly unstable, major
blood loss, hypothermic or severe acidosis.
All patients will be going to Intensive Care unit (ICU) but planned for
level 2 care.
Chest XR ay in recovery: Check Central line and nasogastric tube
position
Postoperative analgesia: Epidural or Fentanyl Patient controlled Analgesia (PCA) with or without continuous local anaesthetic infusion. Fluid management is aggressive to aid graft perfusion. Aim CVP 8-12 Maintenance with Normal Saline/Hartmann’s solution. Rate = last hrs urine output + 50ml. Fluid challenge with crystalloid or colloid if CVP <8. Blood if Hb<9. Gelofusin is not recommended. There is a postoperative management protocol in ICU for these patients, which include fluid management, anticoagulation and graft function monitoring.

6. Appendices:

Appendix 1: Antibiotic prophylaxis for transplants

<table>
<thead>
<tr>
<th>Procedure</th>
<th>1st line prophylaxis</th>
<th>Penicillin allergy</th>
<th>Previous / current MRSA colonised</th>
<th>Intra-op / post op doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas renal transplant / exploration of transplant</td>
<td>Co-amoxiclav 1.2g IV + metronidazole 500mg IV tds</td>
<td>Low risk: Cefuroxime 750mg IV tds</td>
<td>Oral Ciprofloxacin 500mg 1 hour pre-op</td>
<td>Add Teicoplanin 400mg IV stat</td>
</tr>
<tr>
<td>Renal transplant / exploration of renal transplant</td>
<td>Co-amoxiclav 1.2g IV + metronidazole 500mg IV tds</td>
<td>High risk: IV ciprofloxacin + IV clindamycin 450mg QDS</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Nephrectomy (native or transplant)</td>
<td>Co-amoxiclav 1.2g IV + metronidazole 500mg IV tds</td>
<td>Oral Ciprofloxacin 500mg + Oral clindamycin 450mg 1 hour pre-op</td>
<td>Add Teicoplanin 400mg IV stat</td>
<td>NA</td>
</tr>
</tbody>
</table>

** NOTE this combination of antibiotics is high risk for promotion of C. difficile infection – please discuss alternative options with microbiology if previous C. difficile disease or additional risk factors.

Please note the following points:
- Clarithromycin should not be used as it does not provide sufficient antibiotic cover and is also a powerful inhibitor of tacrolimus metabolism
- Teicoplanin does not provide sufficient antibiotic cover alone and should only be used as additional prophylaxis for patients with a history of MRSA.
Appendix 2: Treatment Protocol for TEG values

<table>
<thead>
<tr>
<th>TEG Values</th>
<th>Clinical Cause</th>
<th>Suggested Treatment (use with clinical assessment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R between 11-14 min</td>
<td>↓↓ clotting factors</td>
<td>X 2 FFP or 8 ml/kg</td>
</tr>
<tr>
<td>R greater than 14 min</td>
<td>↓↓↓ clotting factors</td>
<td>X 4 FFP or 16 ml/kg</td>
</tr>
<tr>
<td>MA between 42 - 47 mm</td>
<td>↓ platelet function</td>
<td>X 1 PLATELET POOL</td>
</tr>
<tr>
<td>MA less than 42 mm</td>
<td>↓↓ platelet function</td>
<td>X 2 PLATELET POOL</td>
</tr>
<tr>
<td>LY30 at 7.5% or greater with C.I less than 1.0</td>
<td>Primary fibrinolysis</td>
<td>Antifibrinolytic (of choice)</td>
</tr>
<tr>
<td>R less than 3 mins, MA greater than 75 mm G greater than 15 k</td>
<td>Prothrombotic state</td>
<td>Anticoagulant (of choice)</td>
</tr>
</tbody>
</table>
Appendix 3: Algorithm for Intraoperative Haemodynamic changes

Intraoperative haemodynamic strategies to optimise Kidney Pancreas allograft

Parameters to aim for

Preload
CVP 12-14
Crystalloid
Colloid
Albumin

ScVO₂ ≥ 75%
Haemoglobin >8g/dl

SBP 20% baseline
SV / SD optimised with ODM

1. Decrease concentration of inhalational anaesthetic agent.
2. Decrease Remifentanil

Regulate afterload
SVR = 900-1100 dynes/s/cm²

Small amounts of noradrenaline
PROTOCOL FOR THE CRITICAL CARE MANAGEMENT OF A PATIENT AFTER A SIMULTANEOUS PANCREAS TRANSPLANT OR A PANCREAS TRANSPLANT ALONE

Admission
The patient will be admitted to the ITU following surgery and it will be a requirement that anaesthetists, surgeons and nephrologists will each perform a daily postoperative review. This document represents a multidisciplinary approach and referral criteria for acute deterioration are outlined below.

Investigations
FBC, standard renal profile, Amylase, Magnesium, ABG, Coagulation profile, TEG, These will be done on admission and 24 hourly.
• Coagulation profile and TEG will follow the heparinisation protocol below.
• Therapeutic drug levels will follow the immunosuppressant protocol
• Monitoring PN will follow the Trust nutrition support guidelines (see appendix)

Monitoring
Continuous ECG, IABP, CVP, Oxygen saturation, blood sugar, lactate
Temperature and Urine output
Cardiac output monitoring if clinically indicated
All fluid intake and output including nasogastric aspirates, wound drainage and urine output should be monitored.
ECG and Troponin on Day 1 and 5

Blood sugar monitoring
Hourly blood sugar will be measured for the first 24 hours
2 hourly between 24-48 hours then four hourly after that.
If blood sugar > 10 mmol/l consider pancreas failure and surgical review
Chapter 3, Appendix 3.2, ST ICU Protocol

Fluid management
The goal is normal volaemic status to perfuse the transplanted organs and support normal perioperative surgical practice. This may be commonly complicated in this patient group by vasodilatation from sepsis or an epidural, surgical bleeding or bleeding from coagulopathy or oliguria following transplant. Consider autonomic dysfunction.

Maintain CVP 7-10mmHg
Fluid replacement with Hartmann’s solution
Fluid replaced equivalent to previous hourly urine output plus 30mls +/- PN
Fluid challenges of 250ml if the CVP is persistently low.
Colloid may be given in the form of Voluven
Consider the introduction of a vasopressor if persistently hypotensive
Daily fluid balance and daily weights

Blood transfusion
Maintain the Haemoglobin above 10 gm/dl.
If the patient has ischaemic heart disease transfuse to greater than 10 gm/dl

Nutrition
A nasogastric tube will be routine (free drainage). Please note that the patient may have gastroparesis or an enteric drained pancreas and is not receiving enteral nutrition. It may take some time for normal bowel motility to return
Refer to the post renal transplant protocol guidelines – refer to dietician
Follow blood test monitoring for PN as per appendix

Post operative heparinisation
- Follow the ‘individualized heparin prescription chart’
- Heparin dose based on Ideal Body Weight
- Intraoperative heparin bolus at the discretion of the surgeon
- Infusion of heparin to commence 4 hours after surgery (after TEG)
- Check APTT on admission and 2 hourly after the beginning of the infusion
- Alter infusion rate on the basis of the APTT result

Immunosuppression
Triple therapy with alemtuzumab, Tacrolimus, MMF and also prednisolone. It may vary between individuals.

- First dose Alemtuzumab (Campath®) at induction 30mg SC
- Second dose Alemtuzumab (Campath®) 30mg SC after 24 hours (Day1)
- Omit the second dose if age > 60 or at the discretion of the surgeon
- IV ciclosporin 1mg / kg BD until absorbing feed
- Stop ciclosporin when absorbing and convert to oral tacrolimus (Prograf®)
- Oral Tacrolimus (Prograf®) 0.05mg/kg BD indefinitely
- Mycophenylate Mofetil (Cellcept®) 500mg IV TDS then orally indefinitely
- **Therapeutic monitoring** Ciclosporin / Tacrolimus trough levels Mon/Wed / Fri

**Post-operative antibiotics**

- Augmentin 1.2 gm bd for 5 days
- For penicillin allergy cefuroxime and metronidazole or ciprofloxacin and clindamycin
- Septrin 480mg daily from day 1 for six months
- Valgancyclovir 900mg once daily for CMV negative patients of CMV positive donors
  - (Dose dependent on renal function) for 3 months

**Post operative Analgesia**

- Epidural or PCA with morphine or fentanyl

**Post operative medication**

- Lansoprazole 30mg or Omeprazole 20mg
- Aspirin 150mg rectally until absorbing
- Oral aspirin 75mg daily when absorbing
- Fragmin 5000iu daily once the heparin infusion has finished (alter dose in renal failure)

Treatment of hypertension oral vs IV – labetalol, metoprolol, nimodipine, doxasocin
Criteria for urgent referral

**Surgical complications**  
Haemorrhage wound drainage > 100ml hr for 3 consecutive hours

**Suspicion of Thrombosis**  
Sudden raised and sustained blood sugar (>10mmol/l), which may be accompanied by pain

**Suspicion of rejection**  
Pain or a change in the character of the pain, sepsis, raised blood sugar (>10mmol/l), altered sensorium. Deteriorating urine output, unexplained coagulopathy. Persistent pyrexia

**Renal dysfunction**  
Urine output < 0.5ml/kg / hr for 6 consecutive hours  
Anuria for more than 3 consecutive hours  
Unexpected increase in creatinine  
Potassium > 6mmol / l despite medical treatment  
Refractory acidosis pH < 7.25 despite treatment

Referral resources

**Surgical Team Lead**  
Mr Augustine and Mr Parrott and Mr Tavakoli

**Critical Care**  
Dr Greer and Dr Eddleston

**Anaesthesia**  
Dr Karmakar, Dr S Jones and Dr S Varley

**Nephrology**  
Dr Picton

**Nursing**  
Mr J Logan

**Nutrition**  
Ms O Hamer, Ms C Lyell

**Pharmacy**  
Mr A Dunne, Mr M Vincent

This Critical Care Protocol works alongside the following trust documents

- *The Anaesthetic Management of Pancreas Transplants*
- *Perioperative Heparinisation Prescription sheets for Pancreas Transplants*
- *Nasogastric feeding guidelines for Critical Care*
- *Post Pancreas Transplant Parenteral Nutrition Guideline*
- *Trust Nutrition Support Guidelines*
- *Immunosuppression protocol for adult patients undergoing pancreas transplant*
Chapter 3, Appendix 3.3, Project Protocol

Project Protocol

Effects of Goal-Directed Therapy on Inflammatory Mediators and Post-Operative Outcome in Pancreas Transplant: A Prospective, Randomised Clinical Trial


Background

Goal-Directed Therapy (GDT)

It has been shown that Goal-Directed Therapy (GDT) significantly improves clinical outcomes in patients undergoing major abdominal surgery. GDT reduces length of hospital stay Intensive Care stay and morbidity(Gan, Soppiet al. 2002, Donati, Loggi et al. 2007). Furthermore, GDT has been shown to reduce gastrointestinal complications and postoperative renal impairment (Giglio, Marucci et al. 2009).

High-risk patients, specifically those in our operative cohort (simultaneous pancreas kidney transplant recipients) can have a poor immediate post-operative course due to their inability to meet the oxygen transport demands imposed on them by major surgery. During major surgery the body goes through a number of physiological insults which result in a catabolic state (Desborough 2000) with huge fluid shifts and anaerobic metabolism. In these cases occult hypovolaemia occurs resulting in impaired tissue perfusion and increased postoperative morbidity. However, GDT aims to limit the extent of these physiological insults by targeting specific haemodynamic and oxygen transport goals during the peri-operative period. This increases end-organ oxygen delivery by reducing peri-operative hypotension and hypovolaemia and therefore improving global oxygen delivery, microvascular tissue flow and tissue oxygenation during and after major surgery (Jhanji, Vivian-Smith et al. 2010)

However, the benefits of GDT have never been assessed in patients undergoing pancreas transplant (PT). This group lends itself very well to GDT, as PT patients, due to the multisystem macro and microvascular involvement have already compromised peripheral oxygen delivery. In addition they can have a severe systemic inflammatory response (SIRS) leading to large fluid shifts and third space fluid loss in the perioperative period. In this manner they act in a way similar to
septic patients, who also undergo a SIRS response. In the case of septic patients a number of trials which have been conducted using GDT within the first few hours of presentation to hospital, demonstrate a reduced length of stay and mortality (Huang, Clermont et al. 2007) when compared to standard therapy (ST). Therefore, this study aims to investigate whether an improved outcome can also be achieved in PT patients using GDT compared to ST.

**Clinical Problems Associated With Pancreas Transplantation**

PTs are performed as emergency procedures to reduce cold ischemic times, however with a window allowing peri-operative optimisation to take place without delaying surgery and adversely affecting the post-operative outcome. More-over, it is a major abdominal procedure and the patients undergoing surgery are high risk diabetics with multiple co-morbidity. In the case of PT, all the patients have Insulin Dependent Diabetes Mellitus (IDDM), with at least one other major complication of diabetes, most commonly this is renal failure. Due to the pathology of IDDM, all patients will have an element of multi-system dysfunction and because of these complex disease processes, the peri-operative period is often turbulent. A common complication is gastrointestinal dysfunction (post-operative ileus) in as many as 50% of patients undergoing major abdominal surgery (Giglio, Marucci et al. 2009). However, GDT has been shown to reduce the incidence of gastrointestinal complications due, in part to an increase in splanchnic blood flow (Giglio, Marucci et al. 2009).

A complication specific to pancreas transplantation is post-implantation pancreatitis. It is associated with both an increased patient morbidity and a decrease in pancreas graft survival rates (Grewal, Garland et al. 1993). Pancreatitis itself has a mortality rate in the region of 1%-5%. The main factor which increases the risk of post-implantation pancreatitis is the cold ischaemic time for the organ. Of note, studies have shown that administration of post-operative steroids leads to a lower incidence of post-implantation pancreatitis compared with patients not given steroids (Grewal, Garland et al. 1993). It is felt that this is due to the anti-inflammatory effect of steroids and indicates that independent of cold ischaemic time, there are modifiable factors which can affect the rates of post-implantation pancreatitis. However, there are no studies investigating whether optimising perioperative management with GDT can reduce the rate of post-implantation pancreatitis. Given that steroids reduce the inflammatory response and subsequently reduce the rate of post-implantation pancreatitis, we hypothesise that GDT will reduce the inflammatory response and therefore reduce the post-implantation rates of pancreatitis. To test this hypothesis, we aim to determine whether levels of serum Amylase and serum Lipase post-operatively differs
between the GDT and ST groups and whether the differences reflect improved outcomes between the two groups.

**Outcome Measures**

Aside from clinical indicators such as length of stay, post-operative complications, morbidity and mortality, the measurement of inflammatory markers in serum can provide biochemical evidence of improved outcomes of GDT compared to ST. An increase in inflammatory markers, such as lactate, CRP, WCC, IL-1ra, IL-6 and TNF-alpha (14) have been associated with severity of global tissue hypoxia, organ dysfunction and mortality (Rivers, Kruse et al. 2007). The deleterious effects of inflammatory markers are particularly evident in cardiovascular disease and diabetes. Both of these diseases have a significant negative impact on our patient group. Therefore any attempt to reduce the inflammatory marker load on our patients must be seen as a positive outcome. Rivers et. al (Rivers, Kruse et al. 2007) show a significant decrease in levels of cytokines (IL-1ra, ICAM-1, TNF-alpha and IL-8) in the group that underwent early haemodynamic optimisation.

Although the relationship between serum inflammatory markers and GDT has been extensively investigated in septic patients, this relationship is not so clear in surgical patients, and even less clear in our cohort of transplant patients.

Furthermore, because our study is on surgical patients, we are in a unique position in that we are able to obtain tissue samples without further trauma to patients. There is assumed to be a surge of cytokines during re-vascularisation of the pancreas when compared to other organs. The omentum is a physiologically and metabolically active organ which produces a number of pro-inflammatory adipocytokines including tumour necrosis factor-α (TNF-a), Resistin, plasminogen activator inhibitor-1 (PAI-1) and interleukins (Liebermann-Meffert 2000). The levels of these are directly affected by systemic inflammatory insults such as infection or major surgery (15) and would react to implantation of the pancreas. We are therefore able to hypothesise that the levels of these pro-inflammatory factors in omentum will be lower in the GDT group compared to the ST group. This is due because we aim to modulate the severity of the inflammatory response with GDT compared to ST.

**Benefits of GDT**

Rivers (Rivers 2010) has described the socioeconomic benefits of GDT in septic patients and suggests that hospital-related costs decrease by 20% with a reduction in hospital stays by 5.02 days. The study also suggests that a hospital seeing 250 such patients annually, can benefit by greater than $11.98 million per year and by
saving 1,250 bed days. A separate paper by Huang et. al. (Huang, Clermont et al. 2007) acknowledges that GDT has significant start-up costs, but in the long-term it is cost-saving and when operational, has huge cost-effectiveness.

Rationale for the Study
At the Manchester Royal Infirmary (MRI) we operate on approximately 40-50 pancreas transplant patients per year. These patients post-operatively will be transferred to the Intensive Care Unit. PT patients have an average total length of hospital stay of 14 days. If the benefits of GDT that we have seen in septic patients and those undergoing other major abdominal procedures can be transferred to our cohort of patients the benefits both clinically and economically will be huge.

Study Design

Hypothesis
We predict that GDT will lead to:
3. Improved Clinical Outcomes. Reduced ICU stay, reduced length of hospital stay, lower post-operative complications including post-operative ileus (measured by time to oral intake and time to first bowel motion) and post-operative pancreatitis, greater 30-day and 90-day graft survival and reduced mortality. POSSUM scoring (Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity) and MODS (Multi-Organ Dysfunction Scoring) will also be improved in the GDT group.

4. Biochemical Outcomes. A reduction in post-operative inflammatory marker profile in the GDT group compared to the ST group.

Eligibility
The criteria for inclusion are all adult patients undergoing a pancreas transplant (simultaneous pancreas and kidney (SPK), pancreas transplant alone (PTA) or pancreas after kidney transplant (PAK)) between November 2011- November 2013.

The criteria for exclusion are patients who are unable to consent to the study or those who will be unable to meet the follow-up protocol, patients under the age of 18, patients with a contraindication to central venous catheterisation or those patients with advanced directives restricting implementation of the protocol (e.g. A Jehovah’s Witness who does not want blood products to be used in their care).
Chapter 3, Appendix 3.3, Project Protocol

Over two years we will operate on approximately 80 pancreas transplants at MRI. I predict 75% recruitment is realistic. Therefore, approximately 60 patients should be recruited for the study.

Recruitment and Consent
Patients will be recruited on the day they are called in when an organ becomes available. Unfortunately, due to the nature of transplant surgery, patients have very little notice prior to surgery and we are unable to counsel and recruit patients prior to this point.

I will therefore approach a patient once they have been admitted on the Transplant Ward prior to surgery. I will do this while accompanied by a research nurse (during office hours) or a ward nurse (out of office hours). I will first counsel the patient, give them a copy of the Patient Information Sheet, Version 1.7, 10/10/2011, and give them the opportunity to ask any questions before leaving them time to think about whether they would like to participate or not. I will then return prior to surgery and if they are happy to proceed I will sign the consent form and reveal their randomisation group.

With the unpredictable nature of Transplant Surgery, more than one possible recipient is called in for surgery for each organ. The recipients are informed whether they are the primary recipient or a back-up prior to admission by the ward staff. This practice is common because occasionally the primary recipient may not be able to go ahead with surgery and therefore a "back-up" is asked to come to the Transplant Ward to prevent wastage of an organ. In this event, I would counsel all possible recipients- primary and "back-ups", and then consent the patient once all investigations and pre-operative assessments have been completed and it is confirmed who will receive the organ. This will give all prospective patients maximal time to consider the study. This also has the advantage of informing more patients about the study, so that when they are eventually admitted for surgery they are aware of the study.

All patients will be consented in compliance with the Helsinki Declaration.

Randomisation
All patients will be randomised prospectively prior to the start of the trial, using www.sealedenvelope.com, an on-line randomisation service for clinical trials which uses a patients unique identification number and allocates them into one of two groups (A or B). A member of the Transplant Clerical Staff will perform the
randomisation. Group A is Goal-Directed Therapy and Group B is Standard Therapy. Patients and the clinical care team will be made aware of their randomisation group after consenting for the trial by opening an envelope with the randomisation group name inserted.

**Trial Intervention**

The treatment of the patients will be the same in both groups, until they are anaesthetised. The therapy (either Standard Therapy or Goal-Directed Therapy) will be initiated following induction of anaesthesia in the anaesthetic room prior to surgery and continued for six hours post-operatively.

The patients in the Standard Therapy arm of the study will be treated by each anaesthetic team in accordance with their preferred technique and aims.

The patients in the Goal-Directed Therapy arm of the study will be set a number of targets based on physiological parameters. A protocol for achieving those targets has been devised to aim for a $\text{DO}_2\text{l}$ of $600\text{ml/min/m}^2$. The cardiac output monitor to be used will be LiDCO. The manufacturer, (LiDCO Ltd, UK) will provide this technology with consumables on loan during the trial period.

**Patient Related Information to be collected/calculated prior to Surgery:**
- age
- gender
- height
- weight
- BMI
- ethnicity
- occupation (previous occupation if unable to work at time of surgery)
- time on transplant register
- number of previous transplants
- co-morbid conditions
- pre-operative cardiac stress test results
- Charlson Index (The Charlson co-morbidity index predicts the ten-year mortality for a patient who may have a range of co-morbid conditions)
- Revised cardiac risk index (predicts the risk of a major cardiac risk factor)
- current treatment regimen for Insulin Dependent Diabetes Mellitus (IDDM)
- drug history (prescription and illicit)
- alcohol use
- smoking history
- number of years on insulin
- results of pre-operative cardiopulmonary stress testing
- results of pre-operative myoview studies

Donor Related Information to be collected:
- age
- gender
- ethnicity
- BMI
- cause of death
- cardiac arrest history
- medical history
- heart beating or non-heart beating donor
- drug history (prescription and illicit)
- alcohol use
- smoking history
- length of hospital stay prior to retrieval- hospital and ICU

Organ Related Information to be collected:
- Total ischaemic time (TIT)
- Cold ischaemic time (CIT)
- Preservation fluid used
- Degree of fatty infiltration

**Outcome Measures**

**Clinical Outcomes**
The clinical outcomes to be assessed will be:
- patient mortality
- 30-day graft survival
- 90-day graft survival
- 1-year graft survival
- length of hospital stay
- length of ICU stay
- post-operative infection rates
- post-operative pancreatitis rates
- post-operative ileus (measured by time to oral intake and time to first bowel motion)
- Physiologic and Operative Severity Score for the enUmeration of Mortality and Morbidity (P-POSSUM) and Multiple Organ Dysfunction Score (MODS)
Blood Tests
All blood tests required for the analysis of inflammatory markers for the trial will be taken on ten occasions over 72 hours. Bloods will be sampled at the following time points:
- hour 0 (immediately prior to Campath (Alentuzumab) administration, in the anaesthetic room)
- pre- knife-to-skin (in theatre, to assess the immediate effect of Campath on the inflammatory markers, prior to any surgical insult)
- immediately prior to pancreas implantation
- thirty minutes post pancreas implantation (The time of re-perfusion of the pancreas is time X)
- post Kidney implantation (if simultaneous Pancreas and Kidney (SPK) Transplant) or prior to extubation if Pancreas Alone (PAT).
- the following hours post- time X- 6, 12, 24, 48 and 72.
Blood samples will be taken by the applicant, the anesthetists or the nursing staff caring for the patient.

The following will be measured by the biochemistry department at the Manchester Royal Infirmary at the same time-points as above:
- C- reactive protein (CRP)
- Full Blood Count (FBC ) (to include White cell count (WCC), Platelets, Leucocyte count, Neutrophil count and Eosinophil count)
- Urea and Electrolytes (U&Es) (to include Creatinine, Urea, Sodium and Potassium)
- Calcium (Ca2+)
- Liver Function Tests (LFTs) (to include Bilirubin, Albumin, ALT and ALP)
- Amylase
- Lipase
- Blood Glucose (For 72 hours blood glucose will also be assessed using continuous glucose monitoring. The probe will be placed in the anaesthetic room and will follow the patient through surgery and onto the intensive care unit).

The following serum inflammatory markers are to be measured by myself, using bioplex multi-assay systems:
- IL-1ra
- IL-4
- IL-6
- IL-8
- IL-10
- TNF-a
Further blood tests to be carried out are:
- PaO2
- Arterial blood pH
- Lactate
- Central venous oxygen saturation

These are already done as a matter of protocol by the anaesthetic and nursing staff at various time intervals, but will be done at the same time as the tests named above for use in the study. These further blood tests will also be done immediately post extubation and at regular intervals in the post-operative period. This will be guided by clinical need, but the results used for the purposes of the study. The samples will be taken by the anaesthetic and intensive care staff.

**Omental Biopsies**
Two omental biopsies will be taken intra-operatively. These will be done by the operating surgeon on entering the abdomen (at the start of the case) and finally, prior to closing the abdomen (at the end of the case). These will be assayed and levels of inflammatory markers will be measured.

**Clinical Measurements**
Clinical measurements are to be made at half hourly intervals intra-operatively and hourly intervals post-operatively (including while the patient is on the Intensive Care Unit). Again, these are all done as a matter of norm by the anaesthetic and nursing staff and will be used for the purposes of the study. Some of these measurements will also be used in the goal-directed study group to attain the goals set for the patients’ care (at the discretion of the anaesthetist). I will also make a note of the highest and lowest values intra-operatively and values immediately prior to and after extubation. Finally, I will calculate a mean of all the intra-operative readings taken.

The clinical measurements to be made and used for the study are:
- Temperature
- Pulse
- Systolic blood pressure
- Mean arterial pressure (MAP)
- Respiratory rate
- PaO2/ FiO2 ratio
- Central Venous Pressure (CVP)
- ScVO2

In the case of these patients the recordings peri-operatively will be done by the anaesthetist. In the post-operative period they will be carried out by the recovery and intensive care nurses.

**Follow-up**

Patients will be followed up daily until discharge and then three-monthly for one year post surgery as an out-patient. As out-patients, the patients will be followed-up in line with current practice, by the transplant team. In the out-patient clinics, basic observations (Pulse, Blood-Pressure, Temperature, Oxygen saturations) will be recorded by the nursing staff and all the blood tests which are measured by the biochemistry department (noted above) will be requested. These are already done as a matter of protocol, but, for the purposes of the study, the outcomes of the follow-up will be noted. Complications and graft function will also be assessed at this time.

However the Manchester Royal Infirmary (MRI) covers a large geographical area for Pancreas Transplant, therefore we do not routinely follow-up all Pancreas Transplant patients at the MRI for one year post-operatively. Patients are followed-up by a physician at their base hospital. If patients are discharged back to their base hospital within the study time-frame, a covering letter and follow-up proforma will be sent to the patients' physician at their base hospital on a three-monthly basis, to be filled in and returned to the Transplant Unit at MRI, for follow-up purposes.

**Statistical Analysis**

Nationally, pancreas transplants are carried out in relatively small numbers. Manchester Royal Infirmary carries out one of the highest numbers in the UK, approximately 40 per year. We feel that over two years it will be possible to recruit 75% of the patients that attend for transplant. I understand that grossly speaking this is a relatively small number, but, given the rarity of the procedure, the study will give clinically valid results. We will aim to enroll as many participants as possible to strengthen the results and provide a comprehensive pool of data, but believe 60 patients over two years is realistic. Giving 30 in each group, we will be able to detect an effect size of 0.74 in terms of inter-patient standard deviation.
with 80% power. This will provide sufficient information to derive a further clinical trial with harder clinical endpoints.

The primary outcome measure for the study will be length of hospital stay (days).

The primary analysis will compare the length of hospital stay across the two groups. Descriptive statistics will be used to examine the distribution of length of stay and the difference between the groups will be compared using a Mann Whitney test.

There are two types of secondary outcome measures: biochemical and clinical.

The biochemical outcome measures will be inflammatory maker levels in serum and omentum. In serum these consist of:
- Full Blood Count (FBC) (to include White cell count (WCC), Platelets, Leucocyte count, Neutrophil count and Eosinophil count)
- Urea and Electrolytes (U&Es) (to include creatinine, urea, sodium and potassium)
- Calcium (Ca2+)
- Liver Function Tests (LFTs) (to include Bilirubin, Albumin, Alkaline Phosphatase and Alanine Transaminase)
- Glucose
- Amylase
- Lipase
- CRP
- IL-1
- IL-4
- IL-8
- IL-10
- TNF-a
- C-peptide
- Insulin
- Glucagon
- Resistin

The secondary clinical outcomes to be assessed will be:
- patient mortality
- 30-day graft survival
- 90-day graft survival
- 1-year graft survival
- length of ICU stay
- post-operative infection rates
Chapter 3, Appendix 3.3, Project Protocol

- post-operative pancreatitis rates
- post-operative ileus rates (measured by time to oral intake and time to first bowel motion)
- POSSUM and MODS scoring system

These areas of interest will be examined more in an exploratory fashion, both to see what is feasible and also which of the variables do actually show a real change.

For the secondary analyses the clinical and biochemical outcomes will be examined via descriptive statistics, with the aid of histograms and boxplots. Non-parametric methods will be used as appropriate. The number of graft failures at 30-days, 90-days and at 1 year in each group will be examined and time to failure considered.
Effects of Goal-Directed Therapy on Inflammatory Mediators and Postoperative Outcome in Pancreas Transplant: A Prospective, Randomised Clinical Trial

Investigator: Mr. H A Khambalia

Name of Institution: Manchester Royal Infirmary

Address: Department of Transplant Surgery, Oxford Road, Manchester, M13 9WL

Contact for queries: Transplant Ward on 0161 276 5106/ 4402

Name of Patient:

Patient Randomisation Number:

You are being invited

... to take part in a research study involving patients who are about to undergo a Pancreas Transplant at the Manchester Royal Infirmary. Before you decide to take part, you need to understand why the research is being done and what it will involve.

This Information Sheet is in three sections:

• Summary of Study

• Questions and answers about the study

• Consent form

Please take time to read this Information Sheet carefully, and discuss it with your family and friends before making up your mind. If anything in this Information Sheet is not clear, or if you have more questions, please ask the doctor who gave this to you.
Summary of the Study

A Pancreas Transplant is the accepted treatment in patients with Insulin Dependent Diabetes Mellitus (IDDM) and organ failure. Simultaneous Pancreas and Kidney (SPK) transplant is done in over 90% of cases. At Manchester Royal Infirmary (MRI) approximately 40-50 cases are done per year. The purpose of the study is to assess whether there are any differences in outcome when we compare two approaches of care you receive during and immediately after your surgery.

The two options we will be comparing are

1. Standard Therapy
2. Goal-Directed Therapy

In Standard Therapy, the anaesthetist will treat you according to their preferred technique and aims, as they currently practice.

Goal-Directed Therapy involves setting targets for factors such as your blood pressure and blood oxygen levels and then working from a protocol to try and achieve those goals. We will do this by giving extra fluid into your vein, during and shortly after surgery (for six hours) which is guided by specialist monitoring equipment. The anaesthetic team will follow a strict protocol, which will help them meet these targets. After six hours post-surgery your monitoring and care will revert to that which is currently practiced in ICU.

During surgery we will take three biopsies of the fat within your abdomen (belly). We will also test your blood for levels of markers of infection and inflammation at regular intervals during and for 72 hours post-surgery. This will involve taking five extra samples of blood, each approximately 10mls (about 1 table-spoon full). Finally, 24 hours post-surgery we will take a sample of fluid from one of the drains in your tummy.

During your stay in hospital and after discharge you will be followed-up by the Transplant team as normal.

You will remain in the study for one year following surgery and will be followed up by the Transplant Team, as per norm during this time.

You will not be able to choose which therapy will be used if you decide to participate in the trial. You will be randomised into a group if you decide to participate.

All adult patients undergoing pancreas transplant at the MRI between November 2011 and November 2013 will be invited to take part in the study.
Questions and Answers

Why have I been invited to take part?

You may be suitable to take part in this study because you are about to have a pancreas transplant.

We expect 60 patients to take part in the study over a two year period from Manchester Royal Infirmary.

Do I have to take part?

No- it is up to you to decide whether or not you want to take part. Your participation in this study is entirely voluntary. If you decide to take part, you will retain this Patient Information Sheet and you will be asked to sign the Consent Form. You are still free to withdraw your consent at any time without giving a reason and to stop taking part in the study.

A decision not to take part or to withdraw at any time will not affect the standard of medical care you receive.

What will happen to me if I take part?

Firstly you will be allocated into either the Goal-Directed Therapy or Standard Therapy groups. This has been done randomly, depending on what number participant you are in the study. You will be told which group you will be randomised to once you have signed the consent form. You will not be able to choose which group you will be randomised to.

The treatment of the patients will be the same in both groups, until you are anaesthetised. The therapy (either Standard Therapy or Goal-Directed Therapy) will be initiated in the anaesthetic room prior to surgery and continued for six hours post-operatively. Also, in the anaesthetic room both groups will have a pad placed on the thigh, which will continuously monitor blood sugar levels while in surgery and for three days following surgery. This method of monitoring blood sugar is new to the MRI, so we will also continue to measure the blood sugar in the normal way until staff have become familiar with the new method of continuous blood sugar monitoring.

The patients in the Standard Therapy arm of the study will be treated by each anaesthetic team in accordance with their preferred technique. If you are allocated to this group, you will discuss this with them prior to surgery.
Goal-Directed Therapy involves setting targets for factors such as your heart rate, blood pressure and oxygen levels in your blood and then working from a protocol to try and achieve those targets. We will do this by a variety of methods including giving extra fluid or blood into your vein. The amount of fluid we give is guided by specialist monitoring equipment. This includes a special line in your neck and one in your wrist. (The insertion of both these lines are not part of the trial and are normally placed in all people receiving a Pancreas Transplant, but, they will be used in slightly different ways than would be normal). The monitoring and therapy will last for six hours after your operation. After six hours post-surgery your monitoring and care will revert to that which is currently practiced.

During surgery, the surgeons will take three biopsies of fat from your abdomen (belly), the first at the beginning of surgery and the second at the end of surgery. Blood will also be taken from the special line in your neck. The blood tests will measure the level of inflammatory markers and assess the function of your kidney(s), liver and pancreas. On each occasion approximately 10mls (a tablespoon full) of blood will be taken for use in the trial. During theatre, a small pad will also be placed on a blood vessel to the pancreas which will monitor the flow of blood from the pancreas. Again, this is a new method of monitoring in pancreas transplant, but is used in a number of other types of surgery at MRI. This monitor will allow us to more closely monitor the blood flow to the pancreas and identify any problems at an earlier stage. It will stay connected for 3 days and is easily disconnected on the ward. Finally, 24 hours after surgery we will take a sample of fluid from one of the drains in your tummy- this drain will normally be placed during the operation and is part of normal care. It will not be placed purely for the requirement of research.

Following surgery you will be transferred to the Intensive Care Unit (ICU) where the intervention will last for 6 hours. Following this 6 hour period your care will return to normal practice.

You will be followed-up as per the normal protocols following surgery. However, your clinical course will be recorded as part of the study in order to assess your outcomes. This will continue for one year following surgery. If, in that time, you are discharged back to your base hospital, we will contact your doctor at that hospital at three-monthly intervals to assess your progress.
The Patient Pathway

Patient admitted to Transplant Ward

Counseling and Consent

Randomisation

Goal-Directed Therapy

Standard Therapy

All other care at the discretion of the clinical team

Surgery

Transfer to ICU post-operatively

Intervention to last for 6 hours post-operatively

Daily review by Transplant and Research Team while in-patient

Follow-up by Transplant Team post-discharge

Information to be collected for trial on clinical outcome for 1 year post-operation
What will I have to do?

You will not be expected to do anything above and beyond what is normally expected of you following a pancreas transplant. Your active involvement in the study finishes 72 hours post-surgery, but we will continue to collect data on your progress for one year post-surgery as an out-patient in accordance with current Transplant Unit follow-up protocols.

What will happen to any samples I give?

The blood samples and fat biopsies will be taken and sent to a laboratory at the University of Manchester to be analysed. Samples will be coded and labelled with your unique study number and will not contain your name.

What other management options are there during a Pancreas Transplant procedure?

Aside from the Goal-Directed Therapy we are testing, the alternative management is Standard Therapy. This is the normal type of care practiced in our department during Pancreas Transplant procedures and will be used if you decide not to participate in the study. In this case the anaesthetic team will talk to you about their own preferred techniques prior to surgery.

What are the possible benefits of taking part?

The main benefit of this study is to future patients. It will enable a more refined and beneficial approach to patient care for future pancreas transplant patients.

The benefits for you are better supervision and monitoring following surgery, than if you were not involved in the study. We would therefore expect to diagnose potential complications of surgery at an earlier stage and therefore be able to act on them faster.

However, we cannot guarantee these benefits.

What are the possible disadvantages or risks of taking part?

Your personal data will have to be collected in order to follow your progress and in order to analyse the data and make valid conclusions. However, the personal data will be limited to the minimum required to follow you up for the duration of the study and that required for analysis. Any identifiable data will be encrypted and will not be removed from University of Manchester or Manchester Royal Infirmary computer.
systems. Following the cessation of participation in the study all identifiable data will be destroyed.

Within the first 72 hours post-surgery, you will have to give blood samples on eight occasions for analysis in the study. If you were not involved in the study and had an uncomplicated post-operative course you would be giving samples on at least five occasions. The blood will be taken from lines in your blood vessels that are already in place. The extra blood samples will amount to approximately 50mls over three days. This should not impact adversely on your care.

**What information about me will be collected?**

The information (data) collected in the study will include:

- Personal data - information that could be used to identify you such as your initials and date of birth
- Sensitive personal data - information about your health and medical history

To protect your right to privacy, there are Data Protection Laws in the UK. These laws control:

- How personal and sensitive data is collected
- How it can be used
- Where it can be passed on to

When you give this information to someone (known as the data controller) they must make sure it is only used in ways for which you have given permission. The data controller for this study is Mr. Hussein Khambalia.

**What will the information be used for?**

The information collected in this study will be used to find out if there is a benefit in Goal-Directed Therapy, when compared to Standard Therapy in patients undergoing a pancreas transplant.

The results of this study may be used in presentations or published in scientific reports. Any presentation or published report will not name or otherwise identify you.

**Will my taking part in the study be kept confidential?**

You have a right to privacy, and all the information that is collected because of this study is confidential. Except as required by law, you will not be identified by name, address, telephone number, or any other direct personal identifier.
Your data such as trial records, information about your general health, the outcome of the transplant(s) and the results of any tests carried out during the study will be collected by the researcher. Unless it is considered detrimental to your health, within the law, all this data will be kept confidential and will only be accessible to members of the research team.

You will be identified by a unique study number and information about the study number will be kept in a secure location and access limited to research study personnel only. The data will be encrypted, coded, stored and protected for 5 years following completion of the trial. However, at any time during or after the study, representatives from government health departments may ask to check the data collected to test for accuracy. You are asked to give permission for the researchers to see your medical records. They will keep the information confidential.

You are also asked to give permission for us to let your GP and Physician know that you are taking part in the study.

What happens at the end of the study?

The study ends one year after the last patient has had their pancreas transplant. When the study ends a report will be written and the results published in medical literature.

Any report that is published will not identify any participants taking part in the study.

What will happen if I do not want to carry on with the study?

If you first agree to participate and then change your mind, you are free to withdraw your consent and discontinue your participation at any time with no detriment to the clinical care you receive.

If you do not want to carry on with the study post-operatively, we will not take any further blood samples (if some are still due) and we will stop collecting data on your postoperative outcomes. Again, your care will continue as normal.

However, samples and data already taken will continue to be used in the analysis of the results.

What if there is a problem?

If you have any concern about any part of the study, you should speak with Mr. Hussein Khambalia or a member of the Transplant Team, who will do their best to answer your questions. If you remain unhappy or they are unable to resolve your
concern and you wish to complain formally, please contact a University Research Practice and Governance Coordinator on 0161 275 7583 or 0161 275 8093 or by email to researchgovernance@manchester.ac.uk. You may also contact the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research you may have grounds for a legal action for compensation against Central Manchester University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

**Who is organising the study?**

The study is being organised by the Transplant Department at Manchester Royal Infirmary.

**Has the study been approved?**

This research study has been approved by the Central Liverpool Research Ethics Committee, the committee charged to ensure that the rights for human subjects are protected in the UK.

**Where can I get more information?**

If you want more information about this study you can contact the researcher named on the front of this Information Sheet, a member of the transplant team or a member of your anaesthetic team.

You can also contact the following consultants who are not involved in the research project specifically, but will be able to advise regarding participation in the study.

- Dr. Swathi Karmarkar (Consultant Anaesthetist) 0161 276 4551
- Dr. Mike Picton (Consultant Nephrologist) 0161 276 8736
Study Number:

Patient Randomisation Number:

Title of Study: Effects of Goal-Directed Therapy on Inflammatory Mediators and Postoperative Outcome in Pancreas Transplant: A Prospective, Randomised Clinical Trial

Investigator: Mr. Hussein Khambalia

1. I confirm that I have read and understood the attached Patient Information Sheet (Version Number 1.7, dated 10/10/2011) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I agree to the collection of two pieces of fat from my abdomen (tummy) and the collection of blood samples for use within the study.

4. I understand that relevant sections of my medical notes will be looked at by individuals from the clinical team looking after me, from members of the research team, from regulatory authorities, from Ethics Committees, from individuals from the University of Manchester or from the NHS Trust, where it is relevant to my taking part in the research. I give permission for these individuals to have access to my medical records.

5. I agree to my GP and physician being informed to my participation in the study.

6. I agree to take part in the above study.

_______________________  ______________  __________________
Name of Patient         Date                Signature

_______________________  ______________  __________________
Name of person taking consent       Date                Signature

Hussein Khambalia     ______________   __________________
Researcher                  Date                Signature
A Proof of Principle Study Investigating the Temporal Evolution of Inflammatory and Diabetes Markers Following Simultaneous Pancreas and Kidney Transplantation

H A Kambalia
4.1 Abstract

Introduction

Simultaneous pancreas and kidney transplantation (SPKT) is a major physiological insult, leading to an acute inflammatory state, the nature of which has not been defined. Furthermore, neither has the temporal pattern of endocrine markers post-SPKT.

This observational study aimed to identify, as proof of concept, the early evolution of biomarker profile following SPKT and identify appropriate methods with which to measure these.

Methods

The study was run in two phases. Firstly, serum was sampled on eight occasions in the perioperative period (up to 72 hours post-surgery) from four SPKT recipients and analysed in batch using a BioPlex multi-array, bead-based system to measure a panel of 27 inflammatory markers (IMs). The following biomarkers were measured in this phase of the study: IL-1RA, -1B, -2, -4, -5, -6, -7, -8, -9, -10, -12, -13, -15 and -17, IFN-γ, TNF-α, IP-10, MIP-1a and 1b, Eotaxin, FGF Basic, RANTES and GM-CSF.

Once the results of this phase were analysed, serum was taken at the same time points from a further 18 SPKT recipients and processed using a customised BioPlex assay, designed to measure levels of four diabetes markers (Insulin, C-peptide, Glucagon and Resistin) and six cytokines (IL-1RA, -6, -8, -10 and -17 and TNF-α).
Chapter 4, Preliminary Study of Biomarkers Post-SPKT

Results

The first phase of the study found levels of 12 IMs (IL-1B, -2, -5, -7, -9, -12, -13 and -15, Eotaxin, FGF Basic, RANTES and GM-CSF) were consistently undetectable. Levels of pro-inflammatory markers (IL-4, -6, -8, -17, IFN-γ, TNF-α, IP-10, MIP-1a and 1b) rose rapidly following initiation of surgery followed by rises in levels of anti-inflammatory markers (IL-1RA and -10).

The second phase of the study confirmed intra-operative peaks in IL-6 and IL-8 and diabetes markers C-peptide, glucagon and insulin, followed by post-operative peaks in IL-10 and resistin. However, the bespoke BioPlex system failed to consistently detect levels of IL-1RA, IL-17 and TNF-α.

Conclusions

The results of this study provide valuable data and knowledge with which to proceed to a more formal assessment of inflammatory and diabetes markers. They require further validation in a larger cohort and with a more familiar method with which to measure the IMs than has been used here. This will allow us to validate the findings of this preliminary study and explore the interactions between inflammatory and diabetes markers and their correlation to clinical outcome.
Chapter 4, Preliminary Study of Biomarkers Post-SPKT

4.2 Introduction

Biomarkers have been defined by the biomarker working group as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (Biomarkers Definitions Working 2001) and therefore can include both clinical and laboratory measures. Biochemical markers have been used for over 30 years, either to provide surrogate end-points in clinical trials (Ellenberg and Hamilton 1989, Wittes, Lakatos et al. 1989) or mechanistic reasoning of a clinical intervention (Rivers, Kruse et al. 2007). In doing so, they must be clinically relevant and have been evaluated in that specific clinical scenario (Strimbu and Tavel 2010).

With the advances in high-throughput molecular technologies for the identification of unique bio-signatures and biomarkers, there is interest in identifying serum biomarkers which may aid with risk stratification in surgical patients (Mentula, Kylanpaa et al. 2005). Multi- gene/ protein/ cytokine profiling is being developed for the detection of predictive biomarkers to delineate and measure the response of specific molecular pathways which could then guide clinical decision-making. However, the clinical translation or application of validated biomarkers is still in its infancy, due to the challenges in the process of reliable biomarker measurement and development.

Cytokines are small immuno-regulatory proteins which are released by a range of cells (most commonly lymphocytes and macrophages) and facilitate the immune response to stimuli by modulating the inflammatory response. They are found in consistently measurable levels in peripheral blood, and when treated and stored appropriately remain
stable for long periods to allow for analysis and use as biomarkers in clinical trials as primary outcome measures, surrogates and explanations for specific outcomes (Zhou, Fragala et al. 2010).

Simultaneous pancreas and kidney transplantation (SPKT) aims to provide instant resolution of diabetes and end-stage renal failure (ESRF). However, it is associated with significant peri-operative morbidity. In patients listed for transplant, chronic IMs provide biochemical explanations for the co-morbidities affecting them. In ESRF, the presence of an increasingly ureamic milieu results in the Malnutrition Inflammation and Atherosclerotic (MIA) syndrome, (Stenvinkel, Wanner et al. 2002) which is exacerbated once patients start dialysis. This syndrome manifests as reduced clearance of cytokines, chronic disease processes (atherosclerosis), persistent sub-clinical infections and persistently raised pro-and anti-inflammatory markers (IL-1, -6 and -10, TNF-α and CRP) (Stenvinkel and Alvestrand 2002). Diabetes, independent of other disease processes is also associated with chronically raised levels of T-helper-1 (Th1, e.g. Interleukin (IL) -2, TNF-beta and IFN-γ) and T-helper-2 (Th2, e.g. IL-4, -6 and -10) cytokine subsets which activate cell-mediated and humoral immunity respectively (Chatzigeorgiou, Harokopos et al. 2010, Mitrovic, Illic et al. 2011).

In both diabetes and ESRF, chronically raised levels of IMs (TNF-α, IL-6 and CRP) are associated with an increased risk of ischaemic heart disease and predict morbidity and mortality secondary to cardiovascular disease (Ridker, Hennekens et al. 2000, Ridker, Rifai et al. 2000, Zoccali, Benedetto et al. 2000, Asegaonkar, Marathe et al. 2011). In patients undergoing SPKT these findings are particularly unfortunate, given that these pro-
inflammatory cytokines are also toxic to pancreatic islet cells (Kanak, Takita et al. 2014) and IL-6 and TNF-α induce insulin resistance in myocytes and hepatocytes.

Despite the knowledge regarding biomarkers in the pre-transplant SPKT recipient, there is a dearth of evidence regarding peri-operative biomarker profiles following SPKT and the effects of transplantation on the inflammatory state. Therefore, suppositions have to be made from well recognised clinical scenarios in critical care and transplantation, taking into consideration: 1) the physical insult of the transplant; 2) the ischaemia-reperfusion-injury (IRI) inflicted by two organs on the recipient and 3) the effects of immunosuppression.

Acutely, the chronological profile of cytokines in the general critical care setting have been delineated and correlated with outcome in a variety of scenarios including sepsis, trauma, major surgery, pancreatitis and haemorrhage (Pinsky, Vincent et al. 1993, Desborough 2000, Rivers, Kruse et al. 2007, Cuschieri, Bulger et al. 2010, Malmstrom, Hansen et al. 2012, Faix 2013). Levels of pro-inflammatory mediators IL-1, -6, -8 and TNF-α are consistently raised within the first six hours following insult and have variable prognostic value. In extreme circumstances, normal regulatory mechanisms which would otherwise manage the inflammation locally have become overwhelmed, leading to an uncontrolled release of inflammatory mediators (Bone 1996) and a systemic inflammatory response syndrome (SIRS). There is no biochemical definition of SIRS, it is defined clinically (Table 4.1), but when associated with sepsis, mortality rates range from 25- 80% depending on the severity of sepsis (Angus and Wax 2001). It is therefore important to understand the temporal evolution of biomarkers in these extreme disease processes, to enable their use
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to predict disease progression and provide appropriate targets for anti-inflammatory pharmacological therapies (Christaki, Anyfanti et al. 2011).

In whole-organ transplantation, the study of biological markers includes the biochemical mapping of donation after brainstem death (DBD), the impact of IRI and the role of biomarkers in rejection (van der Hoeven, Ploeg et al. 1999, Bharat, Narayanan et al. 2007, Dziodzio, Biebl et al. 2014). In these scenarios, findings suggest that DBD donation stimulates a significant inflammatory response, which can worsen the IRI in liver transplantation, and in lung transplantation, raised peri-operative IMs correlate with chronic allograft rejection.

In renal transplantation IRI propagates multiple biochemical pathways (including stimulation of; nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Complement Cascade, Cytokines and oxygen free radicals) and is considered an important factor in leading to delayed graft function, which in the long-term is associated with graft rejection and reduced long-term graft survival. Until recently, no animal models existed which investigated the biochemical sequelae following post-pancreas reperfusion, despite the acceptance that each transplanted organ is likely to illicit a unique ischaemic insult leading to a unique clinical consequence (van den Akker, Manintveld et al. 2013). The first such model, in mice, demonstrated rises in levels of IL-2, IL-6 and TNF-α at six hours post-injury and IL-1β and TNF-γ at 24 hours following distal pancreatic ischaemia (Lunsford, Baird et al. 2013). Subsequently, authors have demonstrated that hypothermic ischaemia results in a significantly lesser inflammatory response (measuring TNF-α and IL-6) when compared to normothermic ischaemia in rat pancreata (Rocha-Santos, Ferro et al. 2014)
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and that intravenous pentoxifylline (a phosphodiesterase inhibitor) reduces serum IL-6, IL-10 and TNF-α following normothermic IRI in rat pancreata (Le Campion, Jukemura et al. 2013). In another mouse model of pancreas transplantation (PT), IRI from DBD leads to raised systemic levels of pro-inflammatory cytokines (IL-1β and -6) when compared to DCD, but this effect can be ameliorated with tetrahydrobiopterin (an enzyme cofactor), leading to improved graft microcirculation and survival (Oberhuber, Ritschl et al. 2015). These animal studies provide potential novel therapeutic avenues to reduce IRI and negate the deleterious effects of pro-inflammatory cytokines.

In islet transplantation, the retrieval, isolation and re-implantation processes inflict an inflammatory insult to the islets (Kanak, Takita et al. 2014) leading to significant depletions in islet cell mass between isolation and post-infusion counts. Once again, IL-6 and TNF-α have been implicated in these pathways via stimulation from NF-κB mediated pathway (Kanak, Takita et al. 2014). Additionally, in response to these physiological stressors, islets themselves produce inflammatory mediators within pancreatic β-cells (Corbett and McDaniel 1995, Cardozo, Kruhoffer et al. 2001, Kutlu, Cardozo et al. 2003, Barbe-Tuana, Klein et al. 2006, Lawrence, Naziruddin et al. 2011). This pro-inflammatory state results in β-cell dysfunction and cell death (Kutlu, Cardozo et al. 2003) via activation of apoptotic pathways (Nilsson, Ekdahl et al. 2011). In contrast to whole-organ PT, therapies have already been trialled and are routinely used in humans to ameliorate these effects with some success (Chhabra and Brayman 2011).

It is clear that the benefits of defining the temporal evolution of IMs in a clinical situation can act as prognostic indicators and enable targeted interventions to be instituted and
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therapies to be monitored. In these situations, a panel of biomarkers, including pro- and anti-inflammatory markers, are likely to be most useful (Faix 2013).

Specific to PT, markers of diabetes in the peri-operative period are of unique interest. Given the clinical picture seen post-transplantation, assumptions are made regarding the temporal evolution of diabetes markers, but these assumptions have not been verified biochemically. Clinically, blood sugar levels fall within the first hour post-pancreas perfusion, leading to the presumption that insulin is produced shortly following organ perfusion. In addition, there is no concept of sub-optimal or pancreatic delayed graft function because there are no baseline temporal measures of diabetes markers post-PT with which to compare. Only recently, evidence has emerged that normal blood sugar levels post-SPKT do not necessarily correlate with normal oral glucose tolerance tests, suggesting inferior graft function in a small cohort of patients which may be at risk of graft failure, but is otherwise clinically undetectable (Mittal, Nagendran et al. 2014). Therefore, identifying the normal temporal evolution of diabetes markers peri-operatively, together with investigating potential confounding factors affecting diabetes marker levels peri-operatively, may help identify grafts requiring short-term supportive therapy and identify patients at high-risk of pancreatic delayed graft function, an under-recognised phenomenon.

Therefore, this point of principle observational study aims to delineate as proof of concept, the early temporal changes of inflammatory and diabetes markers post-SPKT and identify appropriate methods with which to measure these.
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4.3 Methods

Study Centre

The study was undertaken at the Manchester Royal Infirmary, Central Manchester University Hospitals NHS Trust and the Cardiovascular Research Institute, University of Manchester. It received ethical approval from the Central Liverpool Research Ethics Committee and Research and Development approval from the Central Manchester University Hospitals NHS Foundation Trust. It was registered on Clinicaltrials.gov (Registration Number NCT01619904).

Study Design

Serum was taken prospectively from SPKT recipients as part of a larger trial investigating the effects of goal-directed peri-operative optimisation on clinical outcome.

This study was designed to:

1. Provide pilot data regarding the temporal patterns of inflammatory and diabetes biomarkers following SPKT, within the first 72 hours post-surgery;

2. Assess the use of a BioPlex multi-array bead based system in measuring IMs and diabetes markers;

3. Provide an inflammatory and diabetes marker profile to be used in future studies.
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Intervention

Blood for analysis was taken in a consistent and reproducible manner, in line with published guidance (Thavasu, Longhurst et al. 1992) to allow for reliable cytokine measurement. Samples were aspirated in serum-separating tubes (SSTs), via an arterial line at the following time points; pre-operatively, immediately prior pancreas perfusion, 30 minutes post pancreas perfusion and at 6, 12, 24, 48 and 72 hours post-surgery. All samples were inverted five times and stored upright at 4°C for 30 minutes. Tubes were spun for 15 minutes in a refrigerated (4°C) centrifuge at 3,300 revolutions per minute, within 60 minutes of being aspirated. The serum was pipetted into 1ml eppendorff tubes and stored at -80°C.

Patient Level Data

For the purposes of this study, basic level patient data were stored (Recipient data: age, gender, BMI, number of years diabetic, number of diabetic comorbidities and dialysis history. Donor data: age, gender, BMI cause of death and brain death status. Operative data: pancreas and kidney cold ischaemic times and length of procedure).

Transplant Protocol

The criteria utilized by individual transplant units for acceptance onto the waiting list for SPKT in the UK are governed by national guidelines produced by NHS Blood and Transplant and derived from European Best Practice Guidelines and include full cardiological assessment of physiological reserve prior to activation (Ebpg, European Renal et al. 2000). Patients were allocated organs from the waiting list based on the blood group and human
leucocyte antigen matching and wait time. Pancreas implantation was undertaken as previously described (Chapter 3).

**Serum Analysis of Blood Samples**

Cytokine analysis was carried out with Bio-Plex micro-array multi-bead based system (BioRad Life Science Group, USA) using specific antibodies per marker, each linked to a unique combination of two fluorescent dyes. This system is advantageous as it enables detection and quantification of multiple mediators simultaneously, using a small volume of serum. It was therefore the first-line technique of choice to screen for multiple cytokines which may be present in the serum of SPKT recipients (Leng, McElhaney et al. 2008). The limits of detection using this technique were as follows (pg/ml): IL-1B, 0.6; IL-1RA, 5.5; IL-2, 1.6; IL-4, 0.7; IL-5, 0.6; IL-6, 2.6; IL-7, 1.1; IL-8, 1.0; IL-9, 2.5; IL-10, 0.3; IL-12 (p70), 3.5; IL-13, 0.7; IL-15, 2.4 and IL-17A, 3.3; Eotoxin, 2.5; Basic FGF, 0.9; G-CSF, 1.7; GM-CSF, 0.2; IFN-γ, 6.4; IP-10, 6.1; MCP-1, 1.1; MIP-1α, 1.6; MIP-1β, 2.4; PDGF-BB, 2.9; RANTES, 1.8; TNF-α 6.0 and VEGF, 3.1). Beyond this level the software imputes likely doses, by extrapolating the calibration curves.

**[Phase I] 27-plex Cytokine Assay**

The first experiment was conducted using four patient samples on a standard BioPlex 27-plex cytokine plate (96 wells). The following markers were measured in duplicate: IL -1B, -1RA, -2, -4, -5, -6, -7, -8, -9, -10, 12p70, -13, 15 and -17A, Eotoxin, Basic FGF, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α and VEGF. The plates and solutions were stored at 4°C until ready for use and brought to room temperature prior
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to use. The assay plates included eight calibration wells and a blank (each in duplicate) to produce a standard calibration curve, specific for each biomarker.

One sample per patient per time point was thawed on ice prior to performing the assays.

The assay was prepared in three stages according to the manufacturer’s instructions.

1. Initial Preparation: The vacuum and system were calibrated. A single vial of standards was reconstituted in 500µl of BioPlex standard diluent and incubated on ice for 30 minutes. An 8 point standard dilution series was performed and a blank prepared for the calibration curve. Plasma samples were diluted 1:4 with sample diluents. Finally, the beads were reconstituted and protected from light in a dark room.

2. Preparing the Assay: The filter plate was pre-wet with 100µl of assay buffer and 50µl of beads were added to each well on the assay plate. These were washed twice with 100µl of wash buffer, vacuuming between washes. 50µl of samples and standards were added to each plate and covered and incubated on a shaker at 300 revolutions per minute, in a dark room at room temperature for 30 minutes. Once incubated, the wells were washed 3 times with 100µl wash buffer, vacuuming between washes. 25µl of detection antibody was added per well. The plate was recovered and incubated on a shaker at 300 revolutions per minute, in a dark room at room temperature for 30 minutes. While samples were being incubated, the software protocol and the Streptavidin-PE were prepared.
60µl of Streptavidin-PE was added to 5.94ml assay buffer and placed in a dark room. The samples were again washed 3 times with 100µl wash buffer and vacuumed between washes. 50µl of diluted Streptavidn-PE was added to each well and the plate was covered and incubated on a shaker at 300 revolutions per minute, in a dark room at room temperature for 10 minutes. Finally, the plate was washed a further 3 times with 100µl buffer and vacuumed between washes. The beads were re-suspended in 125ul assay buffer and shaken at 1,100 revolutions per minute at room temperature for 30 seconds.

3. The calibration curve was measured and used to calculate cytokine levels using BioPlex Manager 5.0.

[Phase II] Bespoke cytokine and diabetes plates

Following analysis of the 27-plex cytokine assay (n= 4) and confirmation of the presence of specific cytokines in our samples, a customised plate was designed for the next group of 18 patients to yield a more clinically relevant analysis of cytokines and markers for our patient cohort. Four bespoke plates were commissioned to measure specific cytokines (IL- 1RA, -6, -8, -10 and -17 and TNF-α) and diabetes markers (insulin, C-peptide, glucagon and resistin) in duplicate. The limits of detection of sensitivity for the cytokines have been stated above [Part 1], for the diabetes markers the assay sensitivities are: insulin, 1.0pg/ml; c-peptide, 14.5pg/ml; glucagon, 4.9pg/ml and resistin, 1.3pg/ml.
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The plates and solutions were stored at 4°C until ready for use and brought to room temperature prior to use.

One sample per patient, per time point was thawed on ice prior to performing the assays. The assays were prepared as previously described, with minor adjustments in the protocol (outlined below), due to the use of a bespoke plate.

**Initial Preparation:** The detection beads for the cytokine and diabetes markers were reconstituted with assay buffer prior to adding to the plate.

**Preparing the Assay:** The detection antibodies for each cytokine and diabetes marker were reconstituted using antibody diluent and 25μl of the reconstituted detection antibody prior to adding to each well.

**Statistical Analysis**

The results from both sets of experiments have been analyzed and presented in a descriptive manner using SPSS (IBM SPSS Statistics 20, Armonk, New York). Bar charts have been used to highlight the temporal evolution of specific biomarkers.
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4.4 Results

[Phase I] 27-plex Cytokine Assay

Patient characteristics

Four patients were recruited to this phase of the study. The recipient, donor and operative variables are detailed in Table 4.2 All patients underwent SPKT with alemtuzumab induction. Three were pre-dialysis and one patient had been on haemodialysis for 30 months at time of surgery.

Biochemical Outcomes

The following mediators were detected in less than 20% of samples and therefore not included in the analysis: IL -1B, -2, -5, -7, -9, -12, -13 and -15, Eotaxin, FGF Basic, RANTES and GM-CSF. The levels of these factors have therefore not been presented.

Figures 4.1 a- o compare mean levels of mediators consistently present in the four patients over the eight time-points in the perioperative period (pre-operation, at time of pancreas perfusion, and 30 minutes and 6, 12, 24, 48, 72 hours post-surgery). They indicate that levels of pro-inflammatory mediators (IL-4, -6, -8, -17, IFN-γ, TNF-α, IP-10, MIP-1a and 1b) rise rapidly, following initiation of surgery and peak intra-operatively. MCP-1 also peaked intra-operatively and was the first of the anti-inflammatory cytokines to do so. Levels of anti-inflammatory cytokines IL-1RA and -10 followed the rise in levels of pro-inflammatory mediators and MCP-1 by peaking at six hours post-surgery. Finally, levels of all growth factors (GCSF, VEG-F and PDGF) increased by 75- 100% prior pancreas implantation and remained elevated for the duration of the study period.
[Phase II] Bespoke cytokine and diabetes plates

Patient characteristics

Eighteen patients were recruited to this phase of the study. The recipient, donor and operative variables are summarised in Table 4.3.

Biochemical Outcomes

The following mediators were detected in less than 20% of samples and therefore not included in the analysis: IL-1RA and -17 and TNF-α. The levels of these factors have therefore not been presented.

Figures 4.2 a- g compare mean levels of mediators consistently present in the eighteen patients over the eight time-points in the perioperative period (pre-operation, at time of pancreas perfusion, and 30 minutes and 6, 12, 24, 48, 72 hours post-surgery). These results confirm the earlier findings of acute rises in the pro-inflammatory cytokines intraoperatively (IL-6 and IL-8) followed by a delayed rise in the anti-inflammatory marker (IL-10) at six hours post-operatively. The diabetes markers indicate rises of C-peptide, glucagon and insulin within 30 minutes of pancreas allograft perfusion, followed by a rise in resistin at six hours.
4.5 Discussion

A number of studies have been published investigating the inflammatory response in either chronically ill patients or acutely, critically ill patients. This is the first study of its kind to look specifically at a unique cohort of patients who have suffered with the ravages of chronic disease (IDDM and ESRF) compounded by an acute, major physiological insult (SPKT).

Phase I

A panel of pro-inflammatory mediators (IL-6, 8 and 17, IFN-γ, TNF-α, IP-10 and MIP-1a and b), anti-inflammatory mediators (IL-1RA, 4, 10 and MCP-1) and growth factors (GCSF, VEGF and PDGF) were analysed to highlight the most assessable and clinically reliable markers for use in future biomarker studies in this cohort. The small numbers enrolled to this aspect of the study limit the clinically relevant conclusions which can be ascertained, but this was not the aim of the study.

IL-6 and TNF-α are commonly credited with initiating the inflammatory response in critically ill patients (Faix 2013) and stimulate the production of acute phase proteins by the liver. IL-6 is most reliably measured in plasma and released in response to surgery, where it is produced at the surgical site (Ueo, Inoue et al. 1994) and levels are proportional to the degree of surgical insult (Cruickshank, Fraser et al. 1990). It also has independent prognostic value in sepsis (Pinsky, Vincent et al. 1993, Patel, Deen et al. 1994), trauma (Cuschieri, Bulger et al. 2010) and pancreatitis (Mentula, Kylanpaa et al. 2005). When released in response to sepsis, TNF-α mediates the initial SIRS response (Faix 2013) and predicts poor outcome (Pinsky, Vincent et al. 1993). It is also released independent of
sepsis, early following trauma and hypovolaemia (Ayala, Perrin et al. 1990, Ferguson, Taheri et al. 1997) and in response to pancreatitis (Montravers, Chollet-Martin et al. 1995). IL-8 is a chemokine which is also released in response to tissue damage (Cruickshank, Fraser et al. 1990) and raised following trauma, although peaks later than TNF-α (Ferguson, Taheri et al. 1997). In combination with IL-6 and IL-10, IL-8 provides a predictive panel of biomarkers for mortality in severe sepsis (Andaluz-Ojeda, Bobillo et al. 2012).

Of the other pro-inflammatory mediators: MIP-1a and b are chemokines and modulate the inflammatory process via their actions on IL-1, IL-6 and TNF-α; MIP-10 is secreted in response to IFN-γ and its main actions are not directly related to the inflammatory process and IL-17 is a late responder, but is involved with an inflammatory response to allergy, autoimmune disease and transplant rejection (Zhou, Fragala et al. 2010).

All pro-inflammatory markers peaked intra-operatively, either immediately prior to pancreas perfusion or 30 minutes post-perfusion (except IFN-γ), before returning to steady state within six hours post-surgery. The effect was however variable with the largest peak for IFN-γ at 72 hours post-surgery in one patient who experienced multiple vascular complications post-transplant. However, interestingly, this is not highlighted in the other pro-inflammatory markers. IFN-γ is released by T-helper 1 and Natural Killer cells and responds to infection by stimulating macrophages (Zhou, Fragala et al. 2010), whilst the other major contributors to inflammation (IL-6, -8 and TNF-α) are mainly synthesised and released by macrophages.
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Of the anti-inflammatory markers, MCP-1 peaked first. It is a chemokine which promotes
the production of IL-10 and may initiate the immunosuppressive phase of the SIRS
response, the “compensatory anti-inflammatory response syndrome” (CARS), described by
Bone et al (Bone, Grodzin et al. 1997). It precedes, but matches the peak in IL-10, a potent
anti-inflammatory cytokine. When used as part of a panel of cytokines IL-10 has prognostic
value in acute pancreatitis (Mentula, Kylanpaa et al. 2005) and sepsis (Andaluz-Ojeda,
Bobillo et al. 2012). IL-1RA is the antagonist to IL-1, and its peak coincides with that for IL-
10 (at six hours), soon after the height of the inflammatory insult. IL-4 levels were very low
pre-surgery (<8pg/ml) and remained low for the duration of the study period (<14pg/ml).

The levels of growth factors (GCSF, VEG-F and PDGF) suggest an acute elevation in reaction
to the initial surgical insult with prolonged raised levels, as would be expected as a
response to major surgery. Further long-term studies are needed to assess the long term
impact of SPKT on the inflammatory state. Furthermore, this study could not establish the
relationship between post-transplant outcome and inflammatory markers due to the small
numbers enrolled.

Phase II

Given the results of Phase I, a decision was made to customise a bespoke plate to analyse
more explicitly, biomarkers of likely clinical relevance in this cohort taking into
consideration findings of studies previously described, which investigated biomarkers in
islet cell transplantation (Barbe-Tuana, Klein et al. 2006, Kanak, Takita et al. 2014,
Naziruddin, Iwahashi et al. 2014). In addition to this, biomarkers were chosen which may
illustrate the potential benefits of goal-directed therapy. Rivers et al (Rivers, Kruse et al.
2007) noted significant differences between specific biomarkers within the first 72 hours of presentation of sepsis in varying haemodynamic optimisation strategies and it was our aim to similarly highlight the benefits of goal-directed therapy using a biomarker profile following SPKT. Finally, levels of diabetes markers (Resistin, C-peptide, Glucagon and Insulin) were measured to delineate the temporal evolution of these post-SPKT and validate the BioPlex multi-bead system for use in measuring these biomarkers.

The results of the IMs suggested only IL-6, -8 and 10 were consistently measureable using the bespoke BioPlex plate. Similar temporal patterns of these interleukins were detected in Phase I as in Phase II, within the first 24 hours of surgery. Following this, mean levels of IL-6 in the larger cohort increased again at 24 hours, due to three patients in the cohort who had developed significant peri-operative complications. This finding is consistent with the clinical picture, where catastrophic post-operative complications led to an inflammatory state secondary to an acute rise in pro-inflammatory biomarkers.

Levels of IL-1RA, IL-17 and TNF-α were not measurable using the bespoke plate in Phase II. The possible explanations for the discrepancies between the two phases of the study were discussed in detail with the manufacturer and a methodological explanation could not be found. The discrepancy in the findings highlights a number of issues regarding biomarker studies of this nature: i) the uniqueness and variability of patient samples, hence repeat testing should be carried out (as was conducted in this study); ii) the degradation of proteins following sample isolation, storage and analysis is possible, but the handling and storage of the samples in this study aimed to minimise this risk; iii) commercially-customised BioPlex kits can vary in sensitivity, especially when using different batches of
beads and standards, as was the case between the two phases of this study; iv) when an individual protein or biomarker is identified, larger patient groups need to be recruited for validation, ideally with the use of a second established technique such as individual ELISAs, western blotting or immunoprecipitation. This will be undertaken in the next phase (Chapter 5).

This is also the first study of its kind to delineate levels of diabetes markers in the immediate period post-SPKT. Of the diabetes markers measured, all increased within six hours after pancreas perfusion. Resistin is an adipokine and leads to insulin resistance. It has been linked to inflammation (Kusminski, da Silva et al. 2007), resulting in increased levels of pro-inflammatory cytokines (including IL-6), which may explain the rise in resistin levels six hours post-transplantation. C-peptide is a by-product, formed when pro-insulin is cleaved to form insulin. It is not affected by exogenous insulin administration and is therefore a more reliable biomarker of pancreatic endocrine function than insulin. Figures 4.2 e and g show pre-operative exogenous insulin administration has been measured in the assays, despite immeasurable levels of C-peptide at the same time points. Of note, insulin levels fall from a mean high of 2,577pg/ml 30 minutes post perfusion to a mean of 1,328pg/ml at 72 hours. This may represent an initial surge in insulin following pancreas perfusion, which clinically is also noted in islet transplantation. Finally, Figure 4.2f indicates that a diabetic patient’s pancreas continues to produce glucagon, but at lower levels than an immediately transplanted allograft. The results of the diabetes markers highlight the almost instantaneous effect of pancreas perfusion on systemic diabetes markers.
When the absolute marker levels are likened to physiological levels in either “normal” individuals, or those suffering with other disease processes, these comparisons have to be made with caution, given the various techniques which can be used to measure biomarker levels, the sensitivity of these techniques and the impact individual physiological states have on biomarker levels. Therefore, in these circumstances, greater emphasis is placed upon biomarker trends rather than absolute levels. Furthermore, to our knowledge, this is the first time diabetes markers have been measured peri-operatively following SPKT and therefore reliable comparisons with other physiological states are difficult to make. Despite this, our data suggest some interesting comparisons. One paper has used BioPlex recently to measure diabetes markers in; control patients, type 2 diabetic patients and those with or without concurrent Hepatitis C (to delineate biomarker patterns in those with type 2 diabetes with or without an additional chronic inflammatory disease) (Costantini, Capone et al. 2012). When our results are compared to these, resistin levels post-operatively are considerably higher in our cohort (mean 20,845pg/ml) when compared to the readings from this paper (median approximately 5000pg/ml). We believe this illustrates the considerable pro-inflammatory effect of SPKT in our patients and the corresponding role of resistin in the inflammatory process. Insulin and C-Peptide levels between the two papers are comparable to the group with Hepatitis C, which tend to be raised when compared to the normal controls. Again, this is consistent with previous findings of pro-inflammatory markers which lead to peripheral insulin resistance, therefore causing a simultaneous rise in blood sugars and insulin. In addition, the findings of raised insulin and C-peptide immediately post-perfusion are consistent with data from islet transplantation and could be caused by the uncontrolled release of insulin from damaged islet cells due to the retrieval, storage and re-implantation process (Naziruddin, Iwahashi et al. 2014). Given this finding it would be interesting to measure the longer-term levels of insulin and C-peptide in
our cohort and make comparisons with chronic inflammatory states and normal controls.

Finally, levels of Glucagon in our study are comparable to BioPlex company literature (unpublished data), but tend to be ten times higher than the levels published by Costantini. This discrepancy may be due to a reactionary production of glucagon in response to the raised insulin levels observed, or again as result of islet-cell damage prior to re-implantation (as described above), but importantly this perhaps illustrates the danger with comparing serum levels of biomarkers between different laboratories and studies.
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4.6 Conclusion

The knowledge gleaned from Phases I and II enables us to proceed with a more comprehensive analysis of inflammatory and diabetes markers, in a more directed manner and in a larger cohort of SPKT recipients.

The BioPlex method of measuring IMs initially detected a number of potentially clinically useful biomarkers which should be analysed in a larger cohort. But, when using a bespoke BioPlex plate, in this scenario, our experience was that it was unreliable in measuring some clinically interesting biomarkers. Therefore, the next stage of analysis will use a more established method of biomarker detection, Enzyme Linked Immunosorbent Assay (ELISA) to measure the most clinically relevant cytokine levels. Given that the diabetes markers appear consistently and reliably measured in the assays and levels are comparable to previously recorded physiological levels of corresponding markers, the BioPlex will continue to be used to measure these.
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Table 4.1. The systemic inflammatory response syndrome can be diagnosed if two or more of the following criteria are met (Muckart and Bhagwanjee 1997)

<table>
<thead>
<tr>
<th>Criterion</th>
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<tbody>
<tr>
<td>Temperature &gt; 38°C or &lt; 36°C</td>
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<tr>
<td>Tachycardia &gt; 90 beats/min</td>
</tr>
<tr>
<td>Respiratory rate &gt; 20/min <strong>OR</strong> pCO&lt;sub&gt;2&lt;/sub&gt; &lt; 32mmHg</td>
</tr>
<tr>
<td>WCC &gt; 12 x 10&lt;sup&gt;9&lt;/sup&gt;/L or &lt;4 x 10&lt;sup&gt;9&lt;/sup&gt;/L</td>
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Table 4.2. Recipient, Donor and Operative variables for patients to have undergone the first phase of cytokine analysis (n= 4)

<table>
<thead>
<tr>
<th>RECIPIENT VARIABLES</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>40.25 ± 6.60</td>
</tr>
<tr>
<td>Male</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.65 ± 3.74</td>
</tr>
<tr>
<td>Years diabetic</td>
<td>28.25 ± 11.18</td>
</tr>
<tr>
<td>Number of diabetic complications</td>
<td>4.75 ± 1.50</td>
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<tr>
<td>Daily Insulin Dose (Units)</td>
<td>42.50 ± 4.45</td>
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<thead>
<tr>
<th>DONOR VARIABLES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.25 ± 11.73</td>
</tr>
<tr>
<td>Male</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>BMI</td>
<td>23.20 ± 2.23</td>
</tr>
<tr>
<td>COD</td>
<td>1 ICH, trauma</td>
</tr>
<tr>
<td></td>
<td>1 ICH, non-trauma</td>
</tr>
<tr>
<td></td>
<td>2 hypoxic brain injury</td>
</tr>
<tr>
<td>DBD</td>
<td>3 (75%)</td>
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</table>

<table>
<thead>
<tr>
<th>OPERATIVE VARIABLES</th>
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</tr>
</thead>
<tbody>
<tr>
<td>P CIT</td>
<td>799.75 ± 168.66</td>
</tr>
<tr>
<td>K CIT</td>
<td>966.75 ± 251.70</td>
</tr>
<tr>
<td>Length of Operation (mins)</td>
<td>372.00 ± 114.71</td>
</tr>
</tbody>
</table>

Values are absolute (%) or mean (± Standard Deviation, SD). BMI, Body Mass Index; COD, Cause of death; DBD, Donor after brainstem death; P CIT, Pancreas cold ischaemic time; K CIT, Kidney cold ischaemic time.
Chapter 4, Preliminary Study of Biomarkers Post-SPKT

Figure 4.1. (n= 4) Temporal profiles of biomarkers in the peri-operative period. a. IL-1RA

Figure 4.1b. IL-4

Figure 4.1c. IL-6
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Figure 4.1d. IL-8

Figure 4.1e. IL-10

Figure 4.1f. IL-17
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Figure 4.1g. GCSF

Figure 4.1h. IFN-γ

Figure 4.1i. TNF-α
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Figure 4.1j. IP-10

Figure 4.1k. MCP-1

Figure 4.1l. MIP-1a
Chapter 4, Preliminary Study of Biomarkers Post-SPKT

Figure 4.1m. MIP-1b

Figure 4.1n. VEG-F

Figure 4.1o. PDGF
Table 4.3. Recipient, Donor and Operative variables for patients to have undergone the second phase of biomarker analysis (n= 18)

<table>
<thead>
<tr>
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<tr>
<td>Age (years)</td>
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<tr>
<td>BMI</td>
<td>25.33 ± 3.15</td>
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<tr>
<td>Predialysis</td>
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<tr>
<td>Years diabetic</td>
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</tr>
<tr>
<td>Number of diabetic complications</td>
<td>3.56 ± 1.46</td>
</tr>
<tr>
<td>Daily Insulin Dose (Units)</td>
<td>48.33 ± 12.68</td>
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<table>
<thead>
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<th>DONOR VARIABLES</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>31.11 ± 12.55</td>
</tr>
<tr>
<td>Male</td>
<td>14 (77.8%)</td>
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<tr>
<td>BMI</td>
<td>23.91 ± 2.60</td>
</tr>
<tr>
<td>COD</td>
<td>11 ICH, trauma</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>2 hypoxic brain injury</td>
</tr>
<tr>
<td></td>
<td>1 other</td>
</tr>
<tr>
<td>DBD</td>
<td>14 (77.8%)</td>
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</table>

<table>
<thead>
<tr>
<th>OPERATIVE VARIABLES</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>P CIT</td>
<td>790.61 ± 188.11</td>
</tr>
<tr>
<td>K CIT</td>
<td>940.67 ± 206.47</td>
</tr>
<tr>
<td>Length of Operation (mins)</td>
<td>346.56 ± 81.78</td>
</tr>
</tbody>
</table>

Values are absolute (%) or mean (± Standard Deviation, SD). BMI, Body Mass Index; COD, Cause of death; DBD, Donor after brainstem death; P CIT, Pancreas cold ischaemic time; K CIT, Kidney cold ischaemic time.
Figure 4.2 Temporal profiles of biomarkers in peri-operative period (Bespoke plate, n= 18)

a. IL-6

Figure 4.2b. IL-8
Figure 4.2c. IL-10

Figure 4.2d. Resistin
Chapter 4, Preliminary Study of Biomarkers Post-SPKT

Figure 4.2e. C-Peptide

Figure 4.2f. Glucagon
Figure 4.2g. Insulin
The Temporal Evolution of Inflammatory and Diabetes Biomarkers Following Simultaneous Pancreas and Kidney Transplantation

H A Khambalia
5.1 Abstract

Introduction

The temporal evolutions of biomarkers in sepsis and following major surgery have been delineated, allowing for application in diagnosis, management, surveillance and treatment. Patients undergoing SPKT suffer with a systemic inflammatory response. However, the biomarker profile is not defined, despite the recognised pro-inflammatory cytokines’ detrimental effects on islet cell function.

This study aimed to determine the expression of biomarkers in the peri-operative period following SPKT and establish a correlation to clinical outcome.

Methods

The temporal patterns of pro- and anti-inflammatory cytokines (interleukin (IL) -6, -10 and TNF-α), inflammatory markers (WCC and CRP) and diabetes markers (insulin, C-peptide, glucagon and resistin) were serially measured at 8 time-points in the first 72 hours post-SPKT.

Results

46 patients were recruited to the study (November 2011- March 2014). Patterns of expression of inflammatory and diabetes markers were delineated.
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Levels of C-peptide, insulin and glucagon raised significantly 30 minutes post pancreas perfusion and were significantly negatively related to prolonged CIT (p< 0.05, linear regression model).

Levels of IL-6 and IL-10 significantly peaked at 30 minutes and six hours respectively (p< 0.05, ANOVA). CRP levels rose rapidly in the post-operative period and correlated significantly with the Post-Operative Morbidity Survey (p< 0.05, Spearman Correlation).

Conclusions

The temporal evolutions of inflammatory markers after SPKT are comparable to patterns observed following other physiological insults. This paper identifies that CIT is significantly related to early pancreatic endocrine function and that CRP provides an easily measurable predictor of recipient morbidity. The findings also provide evidence for the potential use of targeted anti-inflammatory therapies in the peri-operative period.
Chapter 5, Biomarkers in SPKT

5.2 Introduction

Biomarkers have been the focus of translational research in medicine for over a century. They are increasingly used in the diagnosis, management and surveillance of disease.

In sepsis, trauma and major surgery, where an explicit physiological insult leads to a significant systemic inflammatory response, the acute temporal evolution of biomarkers have been delineated (Ferguson, Taheri et al. 1997, Smith and Giannoudis 1998, Gabay and Kushner 1999, Leung, Lai et al. 2000, Rivers, Kruse et al. 2007, Faix 2013). In these settings, Interleukin (IL) -6 and TNF-α are often the first pro-inflammatory markers to rise, stimulating production of acute phase proteins followed by peaks in anti-inflammatory markers (Faix 2013). Biomarkers are now used to predict outcomes at an early stage of the disease process (Mentula, Kylanpaa et al. 2005, Cuschieri, Bulger et al. 2010, Andaluz-Ojeda, Bobillo et al. 2012), estimate extent of tissue damage (Glaser, Sannwald et al. 1995, Desborough 2000, Leung, Lai et al. 2000) and provide targets for novel pharmacological therapies (Bernard, Vincent et al. 2001, Stenvinkel and Alvestrand 2002, Alejandro, Barton et al. 2008).

In chronic diseases such as insulin dependent diabetes mellitus (IDDM) and end-stage renal failure (ESRF), specific pro-inflammatory markers (IL -4, -6, -8, TNF-α) are persistently raised (Stenvinkel and Alvestrand 2002, Chatzigeorgiou, Harokopos et al. 2010), leading to higher cardiovascular risk in this cohort (Kimmel, Phillips et al. 1998).
Simultaneous pancreas and kidney transplantation (SPKT) recipients suffer with multi-system morbidity, end-organ failure and at transplantation undergo high risk surgery, leading to a systemic inflammatory response. This reaction is akin to sepsis and major trauma and is caused by an exaggerated inflammatory state. Explicit clinical factors, which include a prolonged cold ischaemic time (CIT), recipient BMI greater than 30kg/m$^2$ (Sampaio, Reddy et al. 2010), recipient age greater than 45 years (Gruessner, Sutherland et al. 2004) and donor age greater than 40-45 (Gruessner, Gruessner et al. 1993, Douzdjian, Gugliuzza et al. 1995) have all been identified as clinical markers correlating to poor outcomes. However, relevant biological profiling in this clinical context has not been explored and the temporal evolution and interactions of peri-operative inflammatory markers (IMs) have not been characterised.

In solid-organ transplantation, biomarkers have been investigated in relation to donation after brainstem death (DBD), the impact of ischaemia-reperfusion-injury (IRI) and in rejection (van der Hoeven, Ploeg et al. 1999, Bharat, Narayanan et al. 2007, Dziodzio, Biebl et al. 2014). High levels of IL-1, -2, -4, -10 and TNF-α in the peri-operative period correlate with reduced long-term graft survival and increased rates of rejection. During islet cell transplantation, β-cells produce pro-inflammatory cytokines leading to β-cell dysfunction, graft toxicity and islet-cell death (Kutlu, Cardozo et al. 2003, Barbe-Tuana, Klein et al. 2006, Lawrence, Naziruddin et al. 2011, Kanak, Takita et al. 2014). This has led to the introduction of anti-TNF-α agents being used as part of induction immunosuppression in some islet transplant centres, with evidence suggesting improved rates of graft survival (Bellin, Kandaswamy et al. 2008, Faradjí, Tharavanij et al. 2008, Koh, Senior et al. 2010). In the setting of pancreatitis, the most widely available stressor model affecting the pancreas,
raised levels of inflammatory cytokines (IL-6, IL-8, TNF-α and IL-10) have been observed and aid in prognosis prediction (Mentula, Kylanpaa et al. 2005).

Additionally, measuring tissue and circulating biomarkers together add valuable novel information to the study for diagnosing further surgical complications. Although not in widespread clinical use, tissue characterisation is listed by the American Society of Echocardiography as one of the most promising fields of application in cardiovascular disease where characterising the cells and proteins expressed locally in tissue can show a strong correlation with circulating biomarkers or provide added understanding of the disease status (Picano and Paterni 2015). Human adipose tissue is a metabolically active organ. The function and metabolic activity of the fat varies, depending on the location within the body (Baker, Silva et al. 2006), but higher levels of obesity correlate with higher levels of circulating IMs (Pou, Massaro et al. 2007) leading to increased cardio-vascular risk in the obese patient.

Within the abdomen, the omentum is an apron of fat, which like other deposits of fat is a physiologically and metabolically active organ (Liebermann-Meffert 2000). In surgery it is known as the “abdominal policeman” as it is often found to encase areas of intra-abdominal inflammation and infection. The omentum is also involved in the formation of peritoneal adhesions and the production of growth factors and cytokines (Liebermann-Meffert 2000, Wilkosz, Ireland et al. 2005). It reacts to a localised inflammatory insult via macrophage production of IMs (TNF-α, Resistin, plasminogen activator inhibitor-1 (PAI-1) and multiple interleukins (Chandra and Naik 2008) resulting in a systemic effect (Vieira-Potter 2014). This role has been outlined in cases of intra-abdominal sepsis, but minimal
evidence exists as to the role of the omentum in instigating a systemic inflammatory reaction in response to an elective intra-abdominal procedure (Collins, Hogan et al. 2009).

In SPKT, assumptions are also made regarding the production of endocrine markers by the allograft pancreas. Given that serum blood sugar levels tend to fall within the first hour post-pancreatic perfusion, and demonstrable primary pancreatic graft dysfunction is exceptionally rare, it is assumed that endocrine function is instantaneous and uniform, with the distinction between “impaired” or “delayed” graft function difficult to characterize.

Therefore, this study aimed to determine the temporal evolution of biomarkers in the perioperative period following SPKT, assess the inflammatory response of omentum in relation to elective major surgery and establish a correlation of serum biomarkers to clinical outcome.
5.3 Methods

Study Centre

The study was undertaken at The Central Manchester University Hospitals NHS Trust and The University of Manchester. Appropriate ethical and Research and Development approvals were obtained. Recipient serum and omental biopsies were taken prospectively from SPKT recipients between November 2012 and March 2014.

Study Design

This study was designed to:

1. Delineate the temporal evolution of inflammatory markers (IL-6 and -10, TNF-α, C-reactive protein (CRP), White Cell Count (WCC) and Amylase) and endocrine markers (C-peptide, insulin, glucagon and resistin) in the perioperative period following SPKT;

2. Evaluate specific factors which may affect the levels of inflammatory and endocrine markers;

3. Correlate biomarker levels with clinical outcomes.

All adult SPKT recipients were eligible for inclusion in the study. Samples were obtained from patients recruited to a study investigating two peri-operative physiological optimisation techniques, Goal directed therapy (GDT) and Standard Therapy (ST). The subgroups were analysed to compare the biomarker outcomes between these two groups.
Serum samples were taken and processed for analysis on eight occasions in the peri-operative period (pre-operatively, immediately prior pancreas perfusion, 30 minutes post pancreas perfusion and at 6, 12, 24, 48 and 72 hours post-transplantation) (Chowdhury, Ghosh et al. 2010).

Two omental biopsies were taken intra-operatively, firstly on entering the abdomen and secondly prior to closing the abdomen. Specimens were processed in an automated processor and wax embedded.

**Sample Analysis**

*Serum Samples*

Serum CRP, Amylase and WCC were measured prospectively by the biochemistry and haematology departments at the investigating unit.

IL-6, IL-10 and TNF-α, were all measured in serum using ELISA development kits from R&D Systems (Abingdon, UK). Minimum detection limits were 1pg/ml, 5pg/ml and 2pg/ml respectively. Readings below these minimal levels were considered as 0 for analysis.

Insulin, C-peptide, glucagon and resistin were measured in bulk using bioplex micro-array multi-bead based system (BioRad Life Science Group, USA). Minimum detection limits were
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1.0pg/ml, 14.5pg/ml, 4.9pg/ml and 1.3pg/ml for insulin, C-peptide, glucagon, and resistin respectively. Beyond this level the software imputes likely doses, by extrapolating the calibration curves.

Omental Biopsy Analysis

H&E staining was performed to show morphology of tissue sections prior to immunohistochemistry analysis for CD68+ (M1 Macrophages) and CD206+ (M2 Macrophages) to assess for changes in omental inflammatory response during surgery. Slides were visualised on a Zeiss Axio Scope light microscope and quantified using ImageJ analysis software.

Patient Characteristics and Clinical Data

Patient, donor, organ, operative and outcome data were recorded to assess for potential confounders and factors affecting either the inflammatory or endocrine marker levels in the post-operative period, as well as to correlate biomarker levels with clinical outcomes. In addition, Multiple Organ Dysfunction Score (MODS) (Marshall, Cook et al. 1995) and Post-Operative Morbidity Survey Score (POMS) were calculated and recorded for correlation to peak biomarker levels.

Transplant Protocol

Induction immunosuppression was with alemtuzumab (Campath®, Sanofi, Paris, France), 30mg subcutaneous injection at induction of anaesthesia (repeated at 24 hours post-
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operatively) and methylprednisolone (Solu-Medrone®, Pfizer, New York). Transplant protocol, maintenance immunosuppression and standard post-operative care were as previously described (Ablorsu, Ghazanfar et al. 2008).

**Statistical Analysis**

Statistical analyses were carried out using SPSS (IBM SPSS Statistics 20, Armonk, New York). Continuous data are presented as mean (Standard Deviation) where normally distributed, or median (Interquartile range, IQR, 25\(^{\text{th}}\) - 75\(^{\text{th}}\) percentile) if skewed.

The temporal evolutions of biomarkers in the peri-operative period have been graphically presented (95% C.I.) with the variations in individual biomarker levels with time analysed with Analysis of Variance (ANOVA) testing with Bonferroni Correction (p < 0.05 considered significant). Correlations of individual IMs were also made using Pearson correlation (p and r values presented). Univariate analyses and multiple regression analysis were carried out to assess the impact of potential confounders (GDT/ST cohort, DBD/Donor after cardiac death (DCD) status, further surgery within 72 hours of SPKT and pancreas CIT) on biomarker levels at specific time-points. In addition, Mixed Model Analysis was used to assess the effect of these potential confounders, as well as; Recipient age, Pre-procurement pancreas allocation suitability score (P-PASS) and Pancreas donor risk index (P-DRI) on the individual biomarker pattern within the first 72 hours following SPKT.

Scatter plots were drawn to evaluate the relationship between biomarker levels and CIT. P< 0.05 for each independent variable was considered significant.
Finally, peak biomarker levels of IL-6, IL-10, Amylase and WCC were correlated to clinical outcomes (number of post-operative complications, length of hospital stay, length of critical care unit stay, days to mobilisation out of bed, time to tolerating normal diet, 72 hour MODS and days 5, 7 and 10 POMS) using Spearman Correlation. In the case of CRP, which did not reach peak within 72 hours, levels at 24, 48 and 72 hours were correlated with the clinical outcomes. P< 0.05 was considered significant.
5.4 Results

There were 69 recipients of SPKTs during the recruitment period. Of these, complete serum and omentum samples of 46 recipients were analysed (9 recipients did not consent, 1 did not receive the pancreas transplant after consenting, 2 recipients received alternative immunosuppression, in 5 recipients the pancreas grafts failed within 72 hours of implantation and 6 recipients did not have all samples available for analysis).

Patient Demographics

Baseline recipient, donor and operative variables are detailed in Table 5.1.

Temporal Evolution of Biomarkers

Endocrine Markers

Figures 5.1a- d outline the temporal change in levels of resistin, C-Peptide, insulin and glucagon respectively from immediately prior SPKT, to 72 hours after surgery. Levels of C-peptide, insulin and glucagon raised significantly between pre-perfusion and 30 minutes post perfusion (C-peptide, 0pg/ml- 4899.82pg/ml (3510.44) p< 0.001; Insulin, 787.20pg/ml (982.68)- 2764.71pg/ml (1469.11) p< 0.001 and Glucagon, 244.76pg/ml (132.55)- 2879.23pg/ml (2091.00) p < 0.001). Resistin levels rose significantly between 30 minutes and six hours post-SPKT (13326.04pg/ml (4550.43)- 19696.76pg/ml (7227.48) p< 0.001), following which there were no significant changes in consecutive levels.
In the mixed model analysis, there were no significant effects of; GDT/ST cohort, DBD/DCD status, recipient age, P-PASS, P-DRI and pancreas CIT on the diabetes marker profiles measured (p >0.05).

Inflammatory Markers

Levels of IL-6 (n= 45, one outlier excluded, Figure 5.2a) rose rapidly at the start of surgery, peaking at 30 minutes post-pancreas perfusion (92.27pg/ml (149.44), p= 0.001, when compared to pre-perfusion levels, 53.94pg/ml (134.43)) and tended to decrease in concentration over the following 72 hours post-transplantation.

Levels of IL-10 (Figure 5.2b) began to rise prior to pancreas perfusion, but peaked significantly at six hours post-operatively (p <0.001) when compared to pre-operative levels (2.89pg/ml (6.70) and 89.24pg/ml (89.66) respectively). Following this peak, levels fell significantly over the following six hours to 37.04pg/ml (37.39, p= 0.008) and continued to fall to reach baseline levels at 72 hours (2.89pg/ml (6.70) at baseline and 9.70pg/ml (20.76) at 72 hours, p= 0.819).

Levels of TNF-α (Figure 5.2c) increased after start of surgery, before returning to baseline values within 12 hours of surgery, but none of these temporal changes were significant at any time point (p= 0.788).
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Levels of WCC (Figure 5.2d) rose significantly between 30 minutes and 6 hours post-transplantation ($6.73 \times 10^9/L \ (2.88 \times 10^9/L) \ - \ 11.03 \times 10^9/L \ (2.80 \times 10^9/L, \ p<0.001)$ and continued to rise to a peak at 24 hours ($17.10 \times 10^9/L \ (28.07)$), though not significantly ($p>0.05$) due to the large spread of results at that time-point.

CRP levels (Figure 5.2e) rose rapidly in the post-operative period (significantly within the first 24 hours, $p<0.001$), up to 72 hours post-transplantation (30 minutes and 72 hours; 3.73mg/L (4.29)- 164.23mg/L (115.18), respectively, $p<0.001$).

Finally, levels of serum Amylase (Figure 5.2f) rose significantly between pre-pancreas perfusion levels, up to 6 hours post-surgery ($38.52U/L \ (23.93)- \ 243.81U/L \ (221.98), \ p=0.003$) and peaked at 12 hours ($299.62U/L \ (230.99))$ before falling to baseline levels by 72 hours post-transplant (pre-operative levels= $35.76U/L \ (26.76)$, 72 hour levels= $72.09 \ (48.47)$, $p=0.292$).

When comparing levels of IM’s at corresponding time-points there were no significant correlations between IM levels ($p>0.05$). However, when correlations were made between peak IM levels, highly significant results were found between TNF-α and IL-6 ($p<0.001, \ r=0.556$) and TNF-α and IL-10 ($p=0.001, \ r=0.420$).
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In mixed model analysis, there were no significant effects of; GDT/ST cohort, DBD/DCD status, recipient age, P-PASS, P-DRI and pancreas CIT on any of the IM profiles measured (p >0.05).

_Inflammatory Markers in Omentum_

Analysis of intra-operative omental biopsies indicate acutely raised levels of macrophages, M1 (CD68+) and M2 (CD206+) when comparing the biopsies at the start and the end of surgery (p= 0.003 and p< 0.001 respectively). Figure 5.3a illustrates representative patient samples highlighting CD68+ and CD206+ staining and Figure 5.3b demonstrates the quantitative analysis of these findings. There was no difference in M1 and M2 macrophage levels between the GDT and ST cohorts (p >0.05).

_Biomarker Relationship with Cold Ischaemic Time_

_Endocrine Markers_

In a simple linear regression model, C-peptide, insulin and glucagon levels significantly negatively correlated with increasing CIT at every time point, up to 72 hours post-SPKT. Figures 5.4a-c highlight the correlations between C-peptide, insulin and glucagon respectively, with CIT at 72 hours. Furthermore, when assessed as a multiple regression model, CIT continues to exert a significant influence on insulin, C-peptide and glucagon levels within the first 72 hours (Table 5.2), but not at every time-point, as suggested by the simple linear regression model.
Inflammatory Markers

In a multiple regression model, increasing CIT did not significantly influence IL-6, IL-10, TNF-α, Amylase and CRP, at any time-point within the first 72 hours post-transplantation (p >0.05). CIT did significantly influence WCC at 12 hours post-SPKT (mean level $11.77 \times 10^9$/$L (3.42), p=0.021, r=-0.353$).

Correlation of Peak Inflammatory Marker Levels with Clinical Outcome

Clinical outcomes for this patient cohort are outlined in Table 5.3. CRP levels at 24, 48 and 72 hours post-operatively (91.19mg/L (38.03), 132.14mg/L (84.73), 164.23mg/L (115.18) respectively) correlated significantly with POMS on post-operative days 5, 7 and 10, the time taken for the patient to mobilise from the bed and the total number of post-operative complications suffered by the recipient during their inpatient stay (Table 5.4).

However, peak levels of IL-6, IL-10, WCC and Amylase did not correlate with any clinically assessed outcome.
5.5 Discussion

This study has provided unique inflammatory and endocrine marker profiles related to biomarker patterns after SPKT. In our patient group we have defined the temporal evolution of serum IM profiles (IL-6, IL-10, TNF-α, WCC, CRP and Amylase) and serum endocrine marker profiles (insulin, C-peptide, glucagon and resistin) up to 72 hours post-SPKT. Concurrently, this study has shown a significant rise in localised omental expression of CD68+ and CD206+ macrophages intra-operatively. In addition, we have demonstrated the significant negative impact of prolonged CIT on pancreatic endocrine function and finally we have presented data suggesting that consistently raised levels of post-operative CRP may be a valuable biomarker of morbidity following SPKT.

The Temporal Evolution of the Biological Markers

Biomarker profiles are associated with specific outcomes in a range of clinical settings (Glaser, Sannwald et al. 1995, Desborough 2000, Leung, Lai et al. 2000, Mentula, Kylanpaa et al. 2005, Cuschieri, Bulger et al. 2010, Andaluz-Ojeda, Bobillo et al. 2012). For the first time, this paper has shown similar profiles of IM levels following SPKT. Our paper demonstrates an early rise in TNF-α, despite the administration of pre-operative anti-inflammatory therapies, peaking prior to pancreas perfusion, suggesting the biggest systemic inflammatory insult during SPKT is not at the time of graft reperfusion and unlikely to be significantly related to IRI (as is commonly perceived). Instead, the most notable pro-inflammatory insult may be surgical trauma. The successive peaks in IL-6 and IL-10 which follow are consistent with previously published data regarding the chronological evolution of IM’s following major physiological stressor events (Kellum, Kong et al. 2007, Malmstrom, Hansen et al. 2012, Kanak, Takita et al. 2014). Our data also suggests a strong correlation
between the peaks in TNF-α and IL-6 and IL-10, confirming a relationship between these three biomarkers. Surprisingly, all three cytokines return to baseline within 72 hours of surgery, despite the clinically observed persistent pro-inflammatory state.

Since macrophages are known to secrete CRP, it seems likely that CRP and indeed other biomarkers could be secreted locally into the circulation by the presence of omentum macrophages. Since these cells are classified into two broad classes based on their secretion profile and cell surface markers; analysis of the omental biopsies enabled us to identify the presence of both M1 (classical) and M2 (alternative) macrophages. Interestingly, the data confirm a significant increase in infiltration of intra-abdominal phenotypic pro-inflammatory CD68+, M1 sub-type and an increase in the alternative CD206+, M2 macrophage sub-set, which is associated with anti-inflammatory cytokine production. Whether this is a response to the immuno-suppression or simply a reparative mechanism due to surgical trauma remains to be elucidated. The data confirms the simultaneous initiation of a localised inflammatory response to surgery and a correlation of increased CRP with macrophage infiltration into the omentum.

In combination with the temporal evolution of IMs, our data show rapid rises in insulin, C-peptide and glucagon levels immediately after pancreas allograft reperfusion which plateau within 24 hours post-surgery. Naziruddin et al (Naziruddin, Iwahashi et al. 2014) postulate that high levels of circulating C-peptide immediately after islet transplantation, rather than being a sign of immediate function, may be a sign of islet cell damage, perhaps due to the instant blood-mediated inflammatory reaction described in islet cell transplantation (Bennet, Sundberg et al. 1999). Given our results, we postulate that in solid-organ PT, the
opposite is true. The strong correlations of insulin, C-peptide and glucagon with CIT suggest, that CIT negatively impacts on pancreatic endocrine function, and rather than higher levels of C-peptide being a result of islet cell damage, we propose that higher levels seen with shorter CIT are likely to be due to improved islet cell function in solid-organ PT.

Pancreatic CIT has been strongly linked to recipient morbidity previously (Humar, Kandaswamy et al. 2000), but in our opinion this is the first study which has presented biochemical evidence of the detrimental effect of prolonged CIT on pancreatic endocrine function. This variation in endocrine function observed in relation to CIT highlights the presence of reduced graft function in PT attributable to a defined and modifiable variable, a previously unreported phenomenon. This finding should encourage the implementation of specific, islet-protective, peri-operative strategies in grafts with prolonged CIT. In addition, the use of specific anti-inflammatory induction immunosuppressive agents should also be considered to help reduce early graft dysfunction and improve long-term graft survival (Barton, Rickels et al. 2012), given; firstly the inflammatory load on the recipient at the time of pancreas perfusion, and secondly, the known deleterious effects of pro-inflammatory cytokines on islet function and survival in isolated islet cell transplantation (Kutlu, Cardozo et al. 2003, Barbe-Tuana, Klein et al. 2006, Lawrence, Naziruddin et al. 2011, Kanak, Takita et al. 2014). This course of therapy has been used successfully in animal models (Yang, Chen et al. 2005) and in clinical islet transplantation (Kanak, Takita et al. 2014) and should perhaps be utilised in PT.

In this setting too, resistin is unique as an endocrine marker as it is an adipocytokine and therefore not related to pancreatic function. It is an IM, but its temporal evolution is an
amalgamation of the inflammatory cytokines and diabetes markers. It peaks at 6 hours after surgery and remains elevated for the entire study period, highlighting the persistent pro-inflammatory effects of surgery, despite falls in traditional pro-inflammatory cytokine levels. Further reflecting this persistent pro-inflammatory state, Amylase and WCC peak at 12 and 24 hours respectively, and CRP continues to rise, up to 72 hours post-SPKT.

*Correlation of Biomarkers with Clinical Outcome*

Monitoring biomarkers in a disease process provides the opportunity to modify current treatment strategies, target the negative clinical processes modulated by specific biomarkers and therefore deliver a positive influence on patient outcome.

A second advantage of exploring biomarkers and delineating their profiles in disease processes is the ability to monitor the disease or intervention progression and predict outcome. Serum is readily available and the ability to correlate serological markers to patient outcome is of tremendous benefit, clinically and economically. In transplantation the most important outcome measures are graft loss and patient mortality. Incidences of this in SPKT are now reasonably low and have plateaued over the last 10 years (Gruessner and Gruessner 2013), making these poor comparators in clinical trials and routine clinical practice. Therefore, greater importance in assessing relevance of biomarkers in clinical practice may now be focused on the objective assessment of peri-operative morbidity, especially considering the prevalent nature of morbidity in our cohort (Bassetti, Salvalaggio et al. 2004) and the influence of peri-operative morbidity on long-term outcomes following major surgery (Khuri, Henderson et al. 2005). POMS is an objective, validated assessment tool to evaluate patient morbidity following major surgery (Bennett-Guerrero, Welsby et al. 2004).
and our data demonstrate that CRP at 48 hours best correlates with a number of objective clinical outcome measures, including POMS on days 5, 7 and 10 ($r=0.461, 0.350$ and $0.359$ respectively) and the number of complications suffered by an individual as an inpatient ($r=0.394$). It is interesting that a single and commonly available biomarker, CRP is statistically related to outcomes following SPKT in this study, but this finding is entirely consistent with studies in patients with acute pancreatitis (Wilson, Heads et al. 1989) and pancreatic resections (Welsch, Frommhold et al. 2008).

**Limitations to the Study**

The study was not powered to investigate the relationships of biomarkers and clinical outcomes. The sample size is small, and the numbers of concomitant variables associated with outcomes in SPKT are large limiting the analysis which could be performed. Therefore, to conduct a detailed multiple regression analysis a larger cohort would need to be investigated. Nevertheless, in our analysis we have attempted to account for the most clinically relevant variables affecting this cohort with the aim of proposing factors affecting biomarker levels and the predictive capabilities of specific biomarkers to outcome post-SPKT.

It cannot be under-stated that the presentation of biomarker patterns in a clinical setting is a distilled version of the reality of interactions which take place in an acute inflammatory state. Inflammation is a complex, entwined evolutionary process. This study only begins to elucidate the inflammatory response to SPKT, modulated by the background of immunosuppression.
Our cohort was treated with two doses (30mg each), of subcutaneous alemtuzumab, administered 24 hours apart and a single dose of methylprednisolone at the time of anaesthetic induction. Glucocorticoids have been linked with reducing circulating pro-inflammatory cytokines (Petrovsky, McNair et al. 1998), whilst alemtuzumab is a monoclonal antibody which induces antibody mediated lysis of the cell. One would therefore have expected to see a reduction in macrophage numbers, and consequently inflammatory cytokines, following immunosuppression induction. Conversely, the opposite was noted. This may have been due to the sub-cutaneous administration of alemtuzumab, therefore likely reducing overall absorption, compared to the intra-venous route (Hale, Rebello et al. 2004), which would explain the delayed anti-inflammatory response and the persistently low IM levels measured 24 hours onwards. However, alemtuzumab has also been reported to stimulate a type 1 hypersensitivity reaction which would increase circulating pro-inflammatory cytokines. Although clinical anaphylaxis is rare, the acute pro-inflammatory tendency may be contributing to the acute pro-inflammatory state observed. Perhaps, therefore, a specific anti-inflammatory induction regimen would also negate the acute pro-inflammatory effects of alemtuzumab.
Chapter 5, Biomarkers in SPKT

5.6 Conclusion

While relieving recipients from diabetes and renal failure, SPKT precipitates a significant pro-inflammatory state. The temporal evolution of specific biomarkers, namely IL-6, IL-10 and TNF-α after SPKT are comparable to patterns observed following major surgery, trauma and sepsis. Importantly, this paper identifies; firstly, that CIT is significantly related to early pancreatic endocrine function, and secondly, that CRP provides an easily measurable predictor of recipient morbidity at an early post-operative period. Therefore, every attempt should be made to reduce CIT, not only to reduce post-operative morbidity, but also to optimise peri-operative pancreatic graft function. Finally, the findings provide evidence for the use of targeted anti-inflammatory therapies in the peri-operative period to minimise islet cell damage and improve long-term graft survival.
### Table 5.1. Baseline Recipient, Donor and Operative variables

<table>
<thead>
<tr>
<th><strong>RECIPIENT VARIABLES</strong></th>
<th><strong>N= 46</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>42.69 ± 7.02</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>26 (56.5%)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>1 Afro-Caribbean (2.2%)&lt;br&gt;45 White Caucasian (97.8%)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>25.31 ± 3.01</td>
</tr>
<tr>
<td><strong>Years diabetic</strong></td>
<td>27.09 ± 3.01</td>
</tr>
<tr>
<td><strong>Pre-dialysis</strong></td>
<td>18 (39.1%)</td>
</tr>
<tr>
<td><strong>Time on dialysis (months)</strong></td>
<td>16.58 ± 26.07</td>
</tr>
<tr>
<td><strong>Time on waiting list (days)</strong></td>
<td>646.11 ± 418.93</td>
</tr>
<tr>
<td><strong>GDT Cohort</strong></td>
<td>22 (47.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>DONOR VARIABLES</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>33.57 ± 12.37</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>32 (69.6%)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>1 Mixed, White/ Asian (2.2%)&lt;br&gt;1 Indian (2.2%)&lt;br&gt;1 European Other (2.2%)&lt;br&gt;43 White Caucasian (93.5%)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>23.78 ± 2.55</td>
</tr>
<tr>
<td><strong>DBD</strong></td>
<td>37 (80.4%)</td>
</tr>
<tr>
<td><strong>DCD</strong></td>
<td>9 (18.4%)</td>
</tr>
<tr>
<td><strong>Donor WCC (n= 18 and 18)</strong></td>
<td>12.00 IQR 8.93- 12.03</td>
</tr>
<tr>
<td><strong>Donor Amylase (n= 15 and 16)</strong></td>
<td>52.00 IQR 34.00- 133.00</td>
</tr>
<tr>
<td><strong>Time from admission to retrieval of organs</strong> (hrs)</td>
<td>52.00 IQR 29.75- 78.75</td>
</tr>
<tr>
<td><strong>P-DRI</strong></td>
<td>1.72 ± 0.77</td>
</tr>
<tr>
<td><strong>P-PASS</strong></td>
<td>10.78 ± 2.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>OPERATIVE VARIABLES</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P CIT</strong></td>
<td>694.74 ± 184.73</td>
</tr>
<tr>
<td><strong>K CIT</strong></td>
<td>836.30 ± 203.10</td>
</tr>
<tr>
<td><strong>Pancreas fattiness grade</strong></td>
<td>2.00 ± 1.12</td>
</tr>
<tr>
<td><strong>Mismatch &gt;3</strong></td>
<td>28 (60.9%)</td>
</tr>
<tr>
<td><strong>Induction Agent</strong></td>
<td>46 campath (100%)</td>
</tr>
<tr>
<td><strong>Pancreas Preservation Solution</strong></td>
<td>42 UW (91.3%)&lt;br&gt;4 HTK (8.7%)</td>
</tr>
<tr>
<td><strong>Length of Operation (mins)</strong></td>
<td>334.15 ± 72.09</td>
</tr>
</tbody>
</table>

Values are absolute (%) or mean (± Standard Deviation, SD) unless otherwise stated. *Median (Interquartile range, IQR). BMI, Body Mass Index; DCD, Donor after cardiac death; DBD, Donor after brainstem death; WCC, White cell count; P-DRI, Pancreas donor risk index; P-PASS, Pre-procurement pancreas allocation score; P CIT, Pancreas cold ischaemic time; K CIT, Kidney cold ischaemic time; UW, University of Wisconsin solution; HTK, Histidine-tryptophan-ketoglutarate solution
(a) Resistin
Figure 5.1a- d. Mean Resistin (a), C-peptide (b), Insulin (c) and Glucagon (d) levels (pg/ml 95% CI) respectively in the peri-operative period post-SPKT (Pre-surgery, pre-pancreas perfusion and then at 30 minutes and 6, 12, 24, 48 and 72 hours post-transplant, * denotes significant change in levels, p< 0.001, ANOVA).

(b) C-Peptide
Chapter 5, Biomarkers in SPKT

(c) Insulin

(d) Glucagon
(a) IL-6, *p=0.001
Figures 5.2a-f. Mean levels of IL-6, IL-10, TNF-alpha, WCC, CRP and Amylase respectively, in the peri-operative period post SPKT. Significant changes in temporal evolution within the first 72 hours post-operatively are indicated.

(b) IL-10 *p<0.001, †p = 0.008, ∞p = 0.819
Chapter 5, Biomarkers in SPKT

(c) TNF-α

Mean TNF-alpha Levels (pg/ml, 95% CI)

Time of Serum Sample

Mean WCC (x10^9/L, 95% CI)

Time of Serum Sample

d. WCC *p <0.001
Chapter 5, Biomarkers in SPKT

(e) CRP * p < 0.001

(f) Amylase * p = 0.003, ° p = 0.014, ∞ p = 0.292
Figure 5.3a. Sections from representative patient samples showing positive staining for inflammatory macrophage marker CD68 and anti-inflammatory macrophage marker CD206. Sample A, biopsied at the start of surgery, and Sample B, biopsied at the end of surgery, macrophages are indicated by arrows, bars =50µm. b) Quantification results of CD68+ and CD206+ macrophage staining in omentum (**p= 0.003 and ***p <0.001, T-test).
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Figures 5.4a-c. Linear correlation of Cold Ischaemic Time with a) C-peptide ($p=0.008$, $r=-0.384$), b) Insulin ($p=0.009$, $r=-0.382$) and c) Glucagon ($p=0.014$, $r=-0.359$) at 72 hours post-transplantation.

CIT, Cold Ischaemic Time
Chapter 5, Biomarkers in SPKT

Table 5.2. Analysis of multiple regression model, investigating the effect of CIT on serum C-peptide, Insulin and Glucagon levels in the peri-operative period following SPKT.

<table>
<thead>
<tr>
<th>Time Post-SPKT</th>
<th>C-Peptide</th>
<th>Insulin</th>
<th>Glucagon</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 minutes</td>
<td>P = 0.003</td>
<td>P = 0.025</td>
<td>P = 0.003</td>
</tr>
<tr>
<td></td>
<td>R = -0.528</td>
<td>R = -0.348</td>
<td>R = -0.479</td>
</tr>
<tr>
<td>6 hours</td>
<td>P = 0.093</td>
<td>P = 0.029</td>
<td>P = 0.082</td>
</tr>
<tr>
<td></td>
<td>R = -0.437</td>
<td>R = -0.532</td>
<td>R = -0.335</td>
</tr>
<tr>
<td>12 hours</td>
<td>P = 0.038</td>
<td>P = 0.021</td>
<td>P = 0.117</td>
</tr>
<tr>
<td></td>
<td>R = -0.466</td>
<td>R = -0.356</td>
<td>R = -0.432</td>
</tr>
<tr>
<td>24 hours</td>
<td>P = 0.006</td>
<td>P = 0.002</td>
<td>P = 0.086</td>
</tr>
<tr>
<td></td>
<td>R = -0.482</td>
<td>R = -0.483</td>
<td>R = -0.400</td>
</tr>
<tr>
<td>48 hours</td>
<td>P = 0.018</td>
<td>P = 0.009</td>
<td>P = 0.007</td>
</tr>
<tr>
<td></td>
<td>R = -0.507</td>
<td>R = -0.416</td>
<td>R = -0.528</td>
</tr>
<tr>
<td>72 hours</td>
<td>P = 0.035</td>
<td>P = 0.002</td>
<td>P = 0.033</td>
</tr>
<tr>
<td></td>
<td>R = -0.483</td>
<td>R = -0.476</td>
<td>R = -0.374</td>
</tr>
</tbody>
</table>
Table 5.3. Clinical outcomes at discharge

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>N= 46</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month pancreas survival</td>
<td>39 (84.8%)</td>
</tr>
<tr>
<td>Critical Care Unit Length of Stay (days)*</td>
<td>6.50 IQR 4.00- 8.00</td>
</tr>
<tr>
<td>Total Hospital Length of Stay (days)*</td>
<td>18.00 IQR 14.00- 29.75</td>
</tr>
<tr>
<td>Number of Complications per patient*</td>
<td>1.00 IQR 1.00- 2.00</td>
</tr>
<tr>
<td>Time to Mobilisation (days)*</td>
<td>3.98 IQR 2.00- 6.00</td>
</tr>
<tr>
<td>Time to Tolerating Normal Diet (days)*</td>
<td>7.00 IQR 5.00- 9.00</td>
</tr>
<tr>
<td>24 hour MODS</td>
<td>3.38 ±1.63</td>
</tr>
<tr>
<td>48 hour MODS</td>
<td>3.02 ± 1.79</td>
</tr>
<tr>
<td>72 hour MODS</td>
<td>2.53 ± 1.75</td>
</tr>
<tr>
<td>Post-Operative Morbidity Survey Score, Day 5*</td>
<td>3.00 IQR 2.00- 4.00</td>
</tr>
<tr>
<td>Post-Operative Morbidity Survey Score, Day 7*</td>
<td>2.00 IQR 0.00- 3.00</td>
</tr>
<tr>
<td>Post-Operative Morbidity Survey Score, Day 10*</td>
<td>1.00 IQR 0.00- 2.75</td>
</tr>
</tbody>
</table>

Values are absolute (%) or mean ± Standard Deviation (SD) if normally distributed, or *Median (Interquartile range, IQR). MODS, Multiple Organ Dysfunction Score.
Table 5.4. Correlations of clinical outcome measures and CRP at 24, 48 and 72 hours post-operatively (Spearman’s Correlation, p and r values stated).

<table>
<thead>
<tr>
<th>Clinical Outcome Measure</th>
<th>CRP 24 hours</th>
<th>CRP 48 hours</th>
<th>CRP 72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>POMS, Day 5</td>
<td>P = 0.012</td>
<td>P = 0.001</td>
<td>P = 0.041</td>
</tr>
<tr>
<td></td>
<td>R = 0.371</td>
<td>R = 0.461</td>
<td>R = 0.306</td>
</tr>
<tr>
<td>POMS, Day 7</td>
<td>P = 0.067</td>
<td>P = 0.019</td>
<td>P = 0.004</td>
</tr>
<tr>
<td></td>
<td>R = 0.273</td>
<td>R = 0.350</td>
<td>R = 0.308</td>
</tr>
<tr>
<td>POMS, Day 10</td>
<td>P = 0.024</td>
<td>P = 0.015</td>
<td>P = 0.041</td>
</tr>
<tr>
<td></td>
<td>R = 0.335</td>
<td>R = 0.359</td>
<td>R = 0.306</td>
</tr>
<tr>
<td>Time to Mobility</td>
<td>P = 0.017</td>
<td>P = 0.005</td>
<td>P = 0.201</td>
</tr>
<tr>
<td></td>
<td>R = 0.356</td>
<td>R = 0.416</td>
<td>R = 0.194</td>
</tr>
<tr>
<td>Number of Complications</td>
<td>P = 0.021</td>
<td>P = 0.007</td>
<td>P = 0.013</td>
</tr>
<tr>
<td></td>
<td>R = 0.344</td>
<td>R = 0.394</td>
<td>R = 0.367</td>
</tr>
</tbody>
</table>

POMS, Post-Operative Morbidity Survey

H A Khambalia
6.1 Abstract

Introduction

Vascular complications following simultaneous pancreas and kidney transplantation (SPKT) remain the most common causes of peri-operative graft loss. Currently, investigative options are expensive, often cumbersome, involve ionising radiation and potentially nephrotoxic contrast agents and therefore cannot be used for screening.

Contrast enhanced ultrasound (CEUS) combines conventional B-mode ultrasound with microbubble contrast technology. This study aimed to; evaluate the feasibility of conducting CEUS and investigate the potential benefits of the technique in assessing allograft perfusion and morphology following SPKT.

Methods

CEUS was carried out on the Intensive Care Unit by a dedicated transplant radiology team following SPKT. Qualitative and quantitative analysis of the images were undertaken.

Results

12 SPKT recipients were recruited to the study (10 male (83.3%), mean age 39.33 (SD 8.917) and mean BMI 25.99 (SD 3.14)). CEUS was found to aid in the identification of pancreatic and renal allograft vasculature and morphology when compared to standard B-mode and duplex US.
In addition, mean time from injection of, to visualisation of contrast within pancreatic parenchyma was 29.68s (SD 8.68s) and significantly correlated to serum amylase (145.5mmol/l (IQR 99.75- 309.5), p= 0.019 and r= 0.799, Spearman Correlation). Quantification images of implanted allografts were obtained in 4 recipients; the results have also been presented and discussed.

Conclusions

CEUS is a feasible and potentially clinically useful adjunct in the peri-operative assessment of allograft perfusion and morphology following SPKT and may negate the need for CT angiography. It appears to have utility in identifying acute inflammatory processes within the allograft pancreas. The quantification method developed requires further validation and correlation with outcomes in larger studies in this cohort.
6.2 Introduction

Pancreas transplantation (PT) is now the gold-standard treatment of choice for patients with insulin dependent diabetes mellitus (IDDM) and end-organ failure. In over 80% of instances it is performed simultaneously with a kidney transplant (simultaneous pancreas and kidney transplant, SPKT) to provide insulin and dialysis independence. Despite continual advances, complication rates are high and vascular complications remain the most common in the peri-operative period (Troppmann 2010). In the absence of a validated alternative, either computed tomography (CT) or Magnetic Resonance Imaging (MRI) remain the investigations of choice for diagnosis and monitoring of complications, despite the potential nephrotoxicity/cumbersome nature of such imaging techniques.

Recently, contrast enhanced ultrasound (CEUS) has become a well-established form of imaging the native pancreas, where it can potentially replace contrast CT in the context of differentiating between benign and malignant lesions and in assessing pancreatic necrosis following pancreatitis. (Itoh, Hirooka et al. 2005, Ripolles, Martinez et al. 2010). CEUS combines conventional B-mode US with microbubble contrast technology to enhance the reflection of US waves and therefore allow for easier delineation of target organs. The contrast agent is composed of two elements; an outer shell which controls the residence time and an inner gas core which determines the echogenicity. The contrast enhances the reflection of US waves, providing clearer differentiation between tissues and vasculature, organs and pathology.

In renal disease, CEUS is particularly advantageous because the kidneys enhance very quickly and the contrast does not undergo renal excretion. CEUS is more sensitive than CT
in the characterisation of complex renal masses, can confirm the adequacy of ablative therapies for tumor response and monitor the progression of renal masses with similar accuracy to CT and MR imaging techniques (Meloni, Bertolotto et al. 2008, Quaia, Bertolotto et al. 2008, Hoeffel, Pousset et al. 2010). It can also detect renal parenchymal perfusion deficits with results superior to Doppler US and comparable to CT imaging and differentiate between non-perfused, infarcted and hypoperfused kidneys (Bertolotto, Martegani et al. 2008).

In renal transplantation, small case series have shown CEUS is a feasible method to evaluate microvascular perfusion and a non-invasive aid to help diagnose chronic allograft nephropathy, where duplex has no correlation to clinical markers, but quantitative CEUS parameters correlate to serum creatinine and glomerular filtration rate ($r = -0.62$ and $+0.49$ respectively) (Schwenger, Korosoglou et al. 2006). In addition, in experienced hands, CEUS is able to identify acute tubular necrosis, acute rejection episodes (Benozi, Cappelli et al. 2009) and distinguish between these morbidities and renal vein thrombosis (Grzelak, Kurnatowska et al. 2011). In swine models, CEUS has been used to calculate whole organ perfusion (Hoeffel, Mule et al. 2010), but as yet this has not been validated in human subjects. Currently, nuclear medicine renograms are used in some centres to assess and quantify renal perfusion post transplantation. However, it may now be possible to quantify renal perfusion using CEUS, thereby avoiding injection of nuclear isotope.

The pancreas allograft, like the renal allograft, benefits from a well vascularised blood supply, but unlike in kidney alone transplantation, it is commonly implanted intra-abdominally, posterior to the large and small bowel, along the posterior abdominal wall, rendering standard US techniques impractical to assess organ morphology and perfusion.
Therefore, CT angiography is the gold-standard imaging technique in the event of an emergency. However, this necessitates the transfer of a critically ill patient from Intensive Care to Radiology and the administration of nephrotoxic contrast agent. This combination of factors therefore makes CEUS an ideal imaging modality and potentially highlights its unique advantages in this cohort of patients when compared to standard B-mode/ Doppler US and CT angiography. Finally, given the various complications suffered in the peri-operative period following SPKT, a safe and reiterative imaging modality with the potential to electively assess the organs and provide accurate and timely diagnosis of vascular complications is of clinical and financial benefit.

6.3 Case Report

These issues and potential benefits are highlighted by the case of a 32 year old female (KG) who presented for SPKT at the investigating unit.

KG had been diabetic for 19 years and suffered with end-stage renal failure, hypertension and hypothyroidism. She had a body mass index of 30.3kg/m² and had been on peritoneal dialysis for 20 months prior to transplantation, but produced normal volumes of urine. Both pancreas and kidney were implanted intraperitoneally, on contralateral sides. The pancreas was implanted on the right, in a “head-down” position, with arterial anastomosis via a y-graft to the common iliac artery and portal vein to inferior vena cava. The renal artery and vein were anastomosed to external iliac artery and vein respectively. Pancreas and kidney cold ischaemic times were 534 minutes and 664 minutes respectively. Post-operatively the patient was transferred to the Intensive Care Unit as per standard unit protocol.
At 12 hours post-operatively, an unexplained, sudden rise in blood glucose (12.2mmol/L from 6.7mmol/L) mandated an emergency CT angiogram, which suggested a thrombus in the arterial Y-graft (Figure 6.1). For confirmation, prior to taking the patient back to theatre for attempted salvage, a CEUS was undertaken using bolus administration of 25mg SonoVue contrast agent (Bracco, Milan, Italy), diluted to 5mls with normal saline, to assess the pancreatic vasculature. The results of this study contradicted the CT angiogram and indicated good flow within the arterial y-graft and portal venous system with good pancreatic perfusion (Figure 6.2). The patient was treated conservatively and continues to have good pancreatic function at one-year follow-up.

Following the CT angiogram, the patient became anuric and underwent a nuclear medicine renogram to assess renal perfusion at 48 hours post-transplantation. This indicated very poor perfusion (<1%), leading to a second CEUS, on this occasion of the implanted kidney. This suggested good renal blood flow, but very poor cortical perfusion, consistent with acute tubular necrosis. Again, the patient was managed conservatively, but the graft failed to recover function. She returned to theatre on day 25 for washout of an intra-abdominal collection, but a “white” transplant kidney was found and therefore removed. Histological diagnosis was of focal chronic inflammation, interstitial fibrosis and tubular atrophy of unknown cause.
6. 4 Aims

Given these issues, and the sparse knowledge of the role of CEUS in kidney and pancreas transplantation, a point of principle, feasibility study was therefore initiated to evaluate the utility of CEUS in the assessment of transplanted kidneys and pancreata in the peri-operative period following SPKT.
6.5 Methods

Study Centre

The study was undertaken at the Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust. Ethical approval was received from the North West, Haydock Research Ethics Committee and Research and Development approval from the Central Manchester University Hospitals NHS Foundation Trust. Patients were invited to take part in the study and counseled and consented in line with Ethical Board approval and the Helsinki Declaration. The study was registered on Clinicaltrials.gov (Registration Number NCT02104024).

Study Design

SPKT recipients underwent nuclear medicine renogram and CEUS examination of pancreas and kidney allografts on the Intensive care unit within 72 hours of transplant. A qualitative report was provided by the operator, with specific comments relating to time to perfusion (rating slow, moderate or prompt) and quality of perfusion (rating poor, moderate or good). The operator of each scan was blinded to the results of the other scan and all clinical data, except that required to perform and report the study.

Nuclear Medicine Renograms

Nuclear medicine renograms are undertaken as standard procedure, within the first 72 hours, at the investigating unit, to assess renal perfusion after every solitary kidney or SPKT. They were commenced using 200MBq technetium-99m mercaptoacetyltriglycine (MAG3, Meriooide), rapid bolus injection via IV peripheral line. Images of the kidney are taken at one second intervals for 60 seconds to calculate perfusion, followed by further
images at 20 second intervals for 30 minutes. Finally, single images of catheters, drains and the administration syringe were taken to calculate total excretion and activity. For analysis, isotope uptake as a percentage of administered agent was plotted against time post injection.

**Contrast Enhanced Ultrasound**

CEUS was conducted with a GE Logiq S8 US scanner by a dedicated transplant radiology team. All scans included initial analysis with standard B-mode ultrasonography, followed by duplex and CEUS. During the CEUS phase of the scan, contrast (25mg, diluted to 10ml with normal saline; SonoVue, Bracco, Italy) was infused at 2ml/min via a central venous catheter to provide replenishment images of the organ. This phase of the study involved the recording of a cine-loop picturing the arterial inflow and venous outflow of both organs, views of the renal cortex and medulla and the pancreas head (including duodenal cuff), body and tail. In addition, two other CEUS techniques for organ assessment were also employed depending on clinical indication: i) Bolus views and ii) Reperfusion views, where the contrast bubbles were destroyed with high intensity US waves, before further replenishing views were taken.

**Quantification Analysis**

Analysis and quantification of the CEUS images were carried out using VueBox™ software (V. 5.0, Bracco, Switzerland), replenishment kinetic modelling. Quantification values are expressed initially as arbitrary units (a.u.), but also as a percentage of the reference region. The reference region is a user-defined region, most commonly the arterial inflow (if available). If the arterial inflow has not been adequately delineated for quantification.
purposes, then the venous outflow can also be used as the reference region, as the contrast is not metabolised by either the kidney or the pancreas.

For assessment of both organs in each patient, a complete analysis was undertaken using VueBox™. Specifically for pancreas assessment, time to visualisation of contrast within pancreatic parenchyma and serum amylase levels on the day of CEUS assessment were noted. In assessment of the renal allograft “peak enhancement” following CEUS and “peak kidney uptake” following renogram were noted and compared.

**Assessing Complications and Patient Acceptance of the Procedure**

Patients were followed up until discharge to assess for complications, potentially related to the use of CEUS (rates of wound infections, haematuria and delayed renal graft function were noted).

Following both renogram and CEUS, patients were also asked to complete a validated pain questionnaire, rating pain scores from 1 to 10 (1 being no pain and 10 being the worst pain they have ever had) at rest and during each procedure. Time per procedure (mins) and between CEUS and renogram (mins) were also noted.
Statistical Analysis

Statistical analyses were carried out using SPSS (IBM SPSS Statistics 20, Armonk, New York). The early results of the trial are presented as descriptive data. Continuous data are presented as mean (± Standard Deviation, SD) where normally distributed, or median (Interquartile range, IQR, 25th - 75th percentile) if skewed. Pain scores were analysed using Student’s T-test. Where correlations have been compared, these have been analysed using scatter plots and either Pearson Correlation where normally distributed data or Spearman Correlation where data is skewed.
6.6 Results

**Patient Demographics**

12 SPKT recipients were recruited to the trial between September 2013 and July 2014, all were primary transplants. Of these recipients; 10 were male (83.3%), mean age was 39.33 (SD 8.917), mean BMI was 25.99 (SD 3.14) and 5 (41.7%), 6 (50.0%) and 1 (8.3%) were pre-dialysis, on haemodialysis and on peritoneal dialysis respectively.

Of the donors, 8 were male (66.7%), mean age was 32.83 (SD 10.530) and 8 (66.7%) and 4 (33.3%) were DBD and DCD donors respectively.

**Operative Details of Implantation**

One pancreas (8.3%) was placed in the “head-down” position, while 11 (91.7%) were placed “head-up”. All pancreata were placed on the right, intra-peritoneally and drained enterically with vascular anastomoses between the donor arterial Y-graft (from donor superior mesenteric artery and splenic artery) to recipient common iliac artery and the donor portal vein to recipient inferior vena cava. All renal grafts were placed on the left, intra-peritoneally, with vascular anastomoses between a single aortic patch to the external iliac artery and the renal vein to the external iliac vein. Four renal grafts (33.3%) were supplied by two arteries, while all others (8, 66.6%) were supplied by one artery, all on a single aortic patch. All ureters were anastomosed to the dome of the bladder over a double-J pigtail stent.
 Mean pancreas and kidney cold ischaemic times were 511.42mins (SD 70.80) and 616.00mins (SD 70.71) respectively.

Clinical Outcomes

There was no patient mortality at 90-day follow-up. One pancreas graft (8.3%) was lost within 90-days (day 20) due to haemorrhagic necrotising pancreatitis. There were no renal graft failures.

Analysis of Imaging Protocols

Eleven patients (91.7%) underwent CEUS within 72 hours of SPKT. The remaining patient was scanned at 80 hours post-transplant. Median time from transplant to CEUS for the entire cohort was 21.0 hours (IQR 15.0- 37.0). All CEUS were undertaken on the ICU by a dedicated transplant radiology team.

Eleven patients (91.7%) also underwent renogram post SPKT. Median time between CEUS and renogram was 75mins (IQR 48- 1350). One patient did not undergo renogram due to technical issues with the mobile gamma camera at the time of transplant.
Pancreas Imaging

B-mode ultrasound located the implanted pancreas in four (33.3%) recipients, duplex ultrasound was required to locate the pancreas in a further six (50%) recipients and bolus injection of contrast agent using CEUS was required in a further two (16.7%) recipients. Reperfusion, bolus and infusion images were taken in four (33.3%), 2 (16.7%) and 12 (100%) recipients respectively. All infusion rates started at 2mls/min. This infusion rate was adequate to qualitatively assess perfusion in all bar one recipient, where the infusion rate was doubled to 4mls/min to obtain adequate perfusion views.

Using CEUS, the portal vein and arterial y-graft were identified in 9 (75%) and 5 (41.7%) patients respectively. The head and duodenum, the body and the tail of the pancreas were identified in 10 (83.3%), 12 (100%) and 4 (33.3%) patients respectively (Table 6.1).

In four cases the pancreatic parenchyma was considered to be poorly visualised by the radiology team. In one case this was apportioned to an unfavourable combination of large body habitus, large volume gas in the right colon and placement of surgical drains and dressings restricting placement of the USS probe. In the three remaining cases, the organ poorly enhanced with contrast, which coincided with a raised serum amylase (>250mmol/l) in all three cases. A small haematoma was noted around the pancreatic head of one recipient. No other immediate complications were noted.
Mean time from injection of contrast to visualising of contrast within pancreatic parenchyma was 29.68s (SD 8.68s) and significantly correlated to amylase (145.5mmol/l (IQR 99.75-309.5), p= 0.019 and r= 0.799, Figure 6.3).

Quantitative Assessment of Pancreatic CEUS

Quantitative CEUS images were obtained in four of the 12 recipients (33.3%). Of these four recipients, only two had the arterial y-graft visualised adequately for use in quantification (BT and GK). In the remaining two, the portal vein was used as baseline for quantification analysis (CC and SU).

Figure 6.4 displays a linearized model of amplitude of contrast (y-axis (a.u.)) against time (x-axis (s)) using a replenishment quantification model of the pancreas. In this case, peak enhancement of the entire pancreas (Region of Interest (ROI) 2, Figure 6.5) was 1.49% of the arterial y-graft (ROI 1).

For recipients GK and CC relative peak enhancement values were 49.07% and 31.28%, but for SU this was not possible to calculate due to the uniform nature of the perfusion curve.

Renal Imaging

The kidney was located using B-mode US in all recipients. The renal artery was observed in 10 (83.3%) and 12 (100%) of the recipients using duplex ultrasound and CEUS respectively and the vein was observed in 4 (33.3%) and 12 (100%) of the recipients using duplex
ultrasound and CEUS respectively. Of the four grafts supplied by two renal arteries, this was not detected in any case by B-mode or duplex ultrasound, but noted in three cases (75%) following CEUS.

Both modalities of imaging suggested “prompt” perfusion of the allograft in every recipient. However, in assessing for adequacy of perfusion, the CEUS operator and renogram noted one allograft with “poor” cortical perfusion. One further patient was noted as having “poor” perfusion on renogram. Both patients identified as having “poor” perfusion developed delayed graft function.

Quantitative Assessment of Renal CEUS

Of the four patients with quantitative pancreas CEUS images, quantitative assessments of the renal allograft were obtained in three (75%). In the remaining case (GK), renal quantification images were taken, but these were inadequate for analysis due to an inadequate length of cine-loop recording between contrast bolus, reperfusion and replenishment modes.

Figure 6.6 displays a linearized model of amplitude of contrast (y-axis (a.u.)) against time (x-axis (s)) using a replenishment quantification model of the kidney. In this case, peak enhancement, of the kidney (Figure 6.7, ROI 2) in relation to the iliac artery (Figure 6.7, ROI 1) was 3.04%. Compared to the corresponding renogram of the same patient, taken 23 hours earlier, peak uptake was 3.1%. The patient suffered with delayed renal graft function.
Of the two remaining recipients who underwent renal allograft perfusion quantification and renogram, peak enhancement following CEUS and peak uptake following renogram, were 52.8% and 17.2% for SU and 3.32% and 5% for CC respectively.

**Analysis of Acceptance of CEUS**

Four recipients (33.3%) suffered with a total of four post-operative complications (2, wound infection; 2, delayed graft function).

Mean pain scores at rest and following CEUS and transcan were 4.50 (SD 2.20), 5.17 (SD 2.73) and 4.75 (SD 2.38) respectively (Figure 6.8). There were no statistically significant differences (p >0.05) in these scores between these groups.
6.7 Discussion

This study has developed a new and innovative technique to quantitatively assess the condition of implanted allografts following SPKT and potential associated complications. CEUS is well-established in general clinical practice and following liver transplantation (Zheng, Mao et al. 2010, Piscaglia, Nolsoe et al. 2012). It provides a safe, reliable, portable, reiterative and cheap mode of imaging, when compared to the alternatives (Piscaglia, Nolsoe et al. 2012). In PT, the uses of B-mode US and duplex have stalled due to the inherent difficulties of visualising an intra-peritoneal, posteriorly placed organ using these techniques. These difficulties escalate with bowel distension and higher BMI. Therefore, the use of CEUS has only been reported sporadically, with its’ benefits in this cohort only recently being noted (Boggi, Morelli et al. 2009, Kersting, Ludwig et al. 2013, Rennert, Farkas et al. 2013).

Qualitative Assessment of Pancreatic CEUS

Our results suggest that CEUS is feasible and logistically possible, on the ICU, in the peri-operative period following SPKT. Visualisation of the pancreas allograft was possible in all recipients (n= 12) using CEUS, compared to only 10 cases with B-mode/ duplex ultrasound. However, identification of the entire pancreas (head with duodenal cuff, body and tail) was not possible in every case. Due to the placement of the tail in the pelvis in “head-up” pancreata, visualisation of the tail was notably more problematic. Nevertheless, identification of distinct anatomical regions of the pancreas was not possible in any of the recipients using pure B-mode US and duplex. Therefore, we consider this particular aspect of CEUS a substantial advantage, which would allow for assessment of small regions of interest within an allograft, particularly so if there were specific concerns intra-operatively.
about certain segments of allograft perfusion (e.g. duodenum). CEUS may avoid CT angiography or re-exploration in these circumstances, as demonstrated in the Case Report presented. In addition, it may assist in conducting invasive interventions (e.g. allograft biopsies), which could be performed at the bed-side and avoid transfer of the patient to Radiology and contrast CT imaging.

Post-SPKT, the ability to identify and monitor vascular supply to and from the transplanted organ is of particular interest, given the high rates of vascular complications associated with the procedure (Troppmann 2010). In our cohort, the portal vein and arterial γ-graft were identified and assessed in 75% and 41.7% of cases respectively, using CEUS, and we would expect these rates to increase with operator experience. The results also suggest that the head and body of the allograft would be possible to visualise in most cases, and could be used to monitor the graft in long-term follow-up, such as in cases of chronic pancreatic inflammation (Kersting, Ludwig et al. 2013).

Clinically, post-SPKT, the ability to monitor for non-occlusive intra-mural thrombus, haematomas and/ or active bleeding could allow for an individualised approach to peri-operative management and anticoagulation and avoid negative re-explorations. Given that vascular complications (thrombosis and bleeding) tend to occur within the first 72 hours post-SPKT (due to hypovolaemic states, a systemic inflammatory response, intra-vascular fluid depletion, haemodynamic instability, an inherent post-operative hypercoaguable state and intra-venous post-operative anti-coagulation within this period) we aimed to conduct CEUS during this time period. In this case series, only one patient was noted to have a small haematoma, which was thought to be insignificant at that time and had no clinical
consequence. There were no instances of concern regarding acute vascular complications (thrombosis and bleeding). However, if these were noted, CEUS provides the advantage of being reiterative, cheap, portable and providing instant results and could therefore be repeated as required to monitor progression.

**Quantitative Analysis of Pancreatic CEUS**

Our study suggests a correlation between quality of perfusion of the organ and peak amylase. In this series the parenchyma enhanced poorly in four cases, three of which had an amylase greater than 250mmol/l at that time. In all other cases, the amylase at time of CEUS was less than 250mmol/l. Furthermore, absolute serum amylase concentration significantly correlated to time taken for visualisation of contrast within the pancreatic parenchyma, suggesting a correlation between a hypoperfused allograft and an inflammatory one, such as pancreatitis, and are independent of cardiac function within normal ranges (Kersting, Konopke et al. 2009). These findings corroborate those of Kersting et al (Kersting, Ludwig et al. 2013) who used quantification technology to monitor capillary perfusion in rejecting allografts prior and post treatment.

Due to the novel approach and unique circumstances of this study, perfusion quantification images were only available in the final four of the 12 SPKT recipients recruited. Prior to these recipients, software and hardware anomalies were identified and fixed. Of these recipients, two had the arterial y-graft identified sufficiently enough for use in perfusion quantification. In the remaining two recipients, the venous system was used for perfusion quantification. This in itself is acceptable because the pancreas does not metabolise the
contrast, but it does make flow-related analysis unreliable, therefore limiting the utility of the scan.

In the example of BT included in the results, an organ perfusion of only 1.49% was calculated, despite normal graft function and 90-day graft survival. Three points need to be considered when interpreting this finding. The first is that the same patient also had a relatively slow graft visualisation following CEUS administration (40 seconds) and a raised amylase, suggesting an inflammatory condition affecting the pancreas, which may have increased the intra-parenchymal pressure and therefore reduced capillary flow, therefore reducing over-all perfusion. Secondly, as with all quantification techniques, this value is relative to the arterial inflow, and not an absolute perfusion value. Finally, although this value appears negligible, there is no gold-standard measurement of allograft perfusion with which to compare this to. Nevertheless, this value is considerably lower than the two other values for peak enhancement obtained in this study.

**Qualitative Assessment of Renal CEUS**

As discussed, qualitative CEUS assessment is practiced in some renal transplant units internationally, despite the allograft being placed retroperitoneally, but in an anterior position, such that the assessment of the organ and its vasculature is easily possible with standard B-mode US. In SPKT, intra-peritoneal placement may have made identification and assessment of the organ more difficult. Despite this, the graft was located using B-mode US in all recipients. CEUS helped identify the renal vessels, where B-mode and duplex US were unable to. In addition, CEUS identified vascular anatomical anomalies and aided in the study of the integrity of the vessels where non-contrast US was unable to.
Quantitative Assessment of Renal CEUS

Only three patients had adequate cine-loop recordings taken enabling perfusion quantification of the renal allograft. In these cases, all quantification measures were taken relative to the external iliac artery, therefore allowing assessment of flow-related measures in all recipients. In these cases, when compared to the renogram, peak enhancement following CEUS seems comparable with peak uptake following renogram.

Analysis of Acceptance of CEUS

The complications suffered by the recipients, potentially related to the use of CEUS, do not demonstrate an increase in complications attributable to its use in the peri-operative period compared to those that would otherwise be expected in this cohort, suggesting no adverse effects of CEUS on short-term patient and allograft outcome. In addition, there were no significant differences in pain scores between those at rest, during CEUS and during renogram, suggesting the procedure is well tolerated.

A limitation of this study is the observer dependent nature of conducting and interpreting qualitative US images. This is inherent with B-mode and duplex US, but with greater experience in the technique, should be reduced with the quantitative assessment CEUS allows. Also, clearly the small numbers recruited and the initial issues with software and hardware limit the conclusions which can be drawn from this study. Despite this, the study does highlight some potential advantages of CEUS, a novel qualitative and quantitative method of assessing organs post transplantation.
6.8 Conclusion

This point of principle study was primarily initiated to evaluate the feasibility of conducting CEUS in SPKT recipients and this has been achieved. Secondarily, it aimed to assess the potential advantages of CEUS in this cohort, of which we have identified multiple potentials. CEUS provides a possible alternative to CT angiogram post-SPKT, but this requires studying in larger cohorts. In addition, the quantification method developed and the data produced should now be correlated to short- and long-term outcomes post-SPKT, in larger cohort studies.
Figures 6.1. CT Image series of KG, indicating site of reported thrombus in common iliac arterial segment of Y-graft (site of reported thrombus indicated with arrow).
Figure 6.2. CEUS of transplant pancreas, with (left image) and without (right image) contrast agent. Outline of pancreas, indicating flow in arterial γ-graft and pancreatic perfusion.
Table 6.1. Summary of qualitative results of CEUS imaging of pancreata

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<th>Patient Number</th>
<th>Time to CEUS (hrs)</th>
<th>Portal v</th>
<th>Y-graft</th>
<th>Head &amp; Duodenum</th>
<th>Body</th>
<th>Tail</th>
<th>Amylase at time of CEUS</th>
<th>Time to Visualising Pancreas (s)</th>
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Figure 6.3. Correlation of amylase at time of CEUS and time from injection of contrast agent to visualising organ perfusion (s); $p = 0.019$, $r = 0.799$. 

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Figure 6.4. A linearized model of amplitude of contrast (y-axis, in arbitrary units) plotted against time (s), using a replenishment quantification model for pancreas perfusion.
Figure 6.5. A display window, indicating Regions of Interest (ROI) used for quantification purposes. ROI 1, arterial y-graft; ROI 2, pancreas.
Figure 6.6. A linearized model of amplitude of contrast (y-axis, in arbitrary units) plotted against time (s), using a replenishment quantification model for renal perfusion.
Figure 6.7. A display window, indicating Regions of Interest (ROI) used for renal quantification purposes. ROI 1, external iliac artery; ROI 2, kidney.
Figure 6.8. Mean pain scores of SPKT recipients comparing those at rest, at time of CEUS and at time of renogram (no significant difference between all groups (p> 0.05)).
Chapter 6, Appendix 6.1, Project Protocol

Project Protocol

Contrast Enhanced Ultrasound Scanning for Kidney and Pancreas Transplants (Proof of Principle Study)

H Khambalia, D van Dellen, S Lee, L Kenderdine, S Augustine, Z Moinuddin, A M Summers, T Augustine

Background

Contrast Enhanced Ultrasound (CEUS)

CEUS involves the use of traditional Ultrasound (US) in combination with microbubble contrast technology to provide a clear interface between the echogenicity of the contrast and the surrounding tissue. This enhances the reflection of US waves from the contrast, providing clearer differentiation between tissues and vasculature, organs and pathology. This technology also allows for the calculation of organ perfusion. It is used as a diagnostic tool to help enhance regular US and doppler and in certain scenarios can replace Computer Tomography (CT) and Magnetic Resonance imaging (MRI).

US contrast agents were first developed and came to market in 2000. Levovist was the first US contrast agent and was developed for use in characterising liver, but is now no-longer manufactured. Sonovue was initially developed for use in cardiac imaging, but quickly replaced Levovist for use in Liver CEUS. Now, the uses for SonoVue are wide-ranging and there are over 1000 articles published for the use of SonoVue for a number of clinical applications. Altogether, only four contrast agents are licensed in Europe. As well as Levovist and SonoVue, Luminity and Optison are also used, but their use is also limited to cardiac US.

SonoVue US Contrast Agent
All US contrast agents are composed of two elements:

5. Shell: this determines the “residence time” the micro-bubble is available in the circulation. Currently composed of albumin, galactose, lipid or polymers. The shell controls the residence time in two ways- a) hydrophilic shells are taken up by the immune system more quickly than hydrophobic shells and are therefore available for a shorter period as contrast and b) the more elastic shells are able to absorb more acoustic energy from the US prior to bursting. SonoVue is composed of a phospholipid shell.

6. Gas core: this determines the echogenicity of the micro-bubble. Each bubble is gas filled but the heavier the gas, the more echogenic the bubble is, because heavier gases are less likely to leak out of the bubble when compressed by US waves. SonoVue contains a hydrophobic gas (sulphur hexafluoride, SF6)) which significantly slows the diffusion of the gas to the surrounding blood, leading to increased stability and resistance to external pressure (Greis 2004). Also, due to the long persistence of SF6 within the bubble only a small amount of gas is required. The gas is excreted by exhalation via the lungs.

**Uses of CEUS**

Historically, the overwhelming use for SonoVue and CEUS has been for Liver morphology and pathology. The first guidelines published for the use of SonoVue were almost entirely based on the use in Hepatobiliary imaging (Albrecht, Blomley et al. 2004). However, following the publication of this article, the use of SonoVue increased as clinicians realised the potential of this technique of imaging. Therefore, further guidelines were published in 2008 emphasising the uses of SonoVue in non-liver applications (Claudon, Cosgrove et al. 2008). Since then, applications for CEUS have increased exponentially (Piscaglia, Nolsoe et al. 2012).

The characterisation of focal liver lesions and differentiating between malignant and benign liver lesions has been well publicised. A number of articles have studied CEUS, CT and MR imaging and obtained comparable results when differentiating
focal liver lesions. Papers have also published the use of CEUS in the staging and monitoring of malignant liver lesions (Claudon, Cosgrove et al. 2008) and in the guidance of ablative therapy in malignant renal and liver lesions (Quaia, Bertolotto et al. 2008). Ricci et al (Claudon, Cosgrove et al. 2008) have studied and also shown that CEUS can reliably differentiate between benign and malignant portal vein thrombosis with a sensitivity of 94% and specificity of 100%.

CEUS use in kidneys is particularly advantageous because kidneys enhance very quickly and SonoVue is not excreted via the kidneys. In renal disease, CEUS is able to evaluate renal masses (Prakash, Tan et al. 2011) and distinguish between malignancy and clot in the renal vein (Ignee, Straub et al. 2010). It is also excellent at detecting renal parenchymal perfusion with results superior to Doppler US and comparable to CT imaging and at differentiating between non-perfused, infarcted and hypoperfused kidneys (Bertolotto, Martegani et al. 2008). In detecting renal artery stenosis, the use of CEUS improves sensitivity of detection by 10% when compared to the use of Doppler US alone (Blebea, Zickler et al. 2003). CEUS is more sensitive than CT in the characterisation of complex renal masses (Quaia, Bertolotto et al. 2008) and, like liver lesions, can be used to confirm the adequacy of ablative therapies for tumour response (Hoeffel, Pousset et al. 2010) and in the monitoring of renal masses (Meloni, Bertolotto et al. 2008) with similar accuracy to CT and MR imaging techniques.

CEUS has two main uses in the diagnosis of pancreatic lesions. Firstly, it is able to accurately diagnose tumours of the head of the pancreas and aid in the differentiation of benign and malignant pancreatic head lesions (Itoh, Hirooka et al. 2005). More detailed diagnosis can be gained using Contrast Enhanced Endoscopic US. Secondly, in pancreatitis it is able to identify and delineate necrotic areas which do not enhance on CT scanning (Ripolles, Martinez et al. 2010). In this situation it is also possible to use CEUS in the follow-up of chronic pancreatitis as a substitute to CT and therefore lower the radiation exposure to patients. CEUS is also able to
accurately visualise intra-hepatic vessels and microvessels in the arterial and venous phases.

Other common uses of CEUS in general surgery include the evaluation of severity of inflammatory bowel disease (Migaleddu, Scanu et al. 2009), in the characterisation of focal splenic lesions (Gorg and Bert 2005) and in scanning for abdominal trauma, as an adjunct to FAST (Focused Assessment with Sonography in Trauma) US scan (Catalano, Aiani et al. 2009). CEUS has also been used for intra-cavity injection for the diagnoses of fistulae, in the diagnoses of vesicoureteric reflux, in the imaging of fallopian tube patency and in percutaneous cholangiography.

In Vascular surgery CEUS is used in the diagnosis of carotid artery disease, including the detection of plaque ulceration, flow and thrombus (Clevert, Sommer et al. 2011) and CEUS is commonly used in the detection of endoleaks following endovascular aneurysm repair (EVAR). Some studies suggest that CEUS is more sensitive than CT angiography in the detection and monitoring of endoleaks (Clevert, Minaifar et al. 2008).

These are by no means an exhaustive list for the uses of CEUS, but merely indicate the versatility and safety of CEUS in a surgical setting. The uses of CEUS specifically in transplant surgery will now be discussed.

The 2011 EFSUMB guidelines on CEUS (Piscaglia, Nolsoe et al. 2012) categorise CEUS use in transplant surgery as an “emerging perspective and potential future application for CEUS”. CEUS use in solid organ transplant is still in its infancy and the benefits of it are still being investigated.

Given that CEUS use in hepatobiliary disorders is already well established it is not surprising that CEUS in liver transplantation is already a recognised form of imaging to monitor acute and chronic vascular and biliary complications post-operatively (Marshall, Beese et al. 2002, Berry and Sidhu 2004, Clevert, Stickel et al. 2009).
In renal transplantation, ten years ago CEUS was recognised as being able to quantitatively measure cortical and parenchymal vascularity and perfusion and efforts were made to correlate these to longer term graft function and survival (Lefevre, Correas et al. 2002). More recently, CEUS has been shown to be a feasible method in the evaluation of microvascular perfusion and these findings have correlated with chronic allograft nephropathy (Schwenger, Korosoglou et al. 2006). Since then, CEUS has been used in the differentiation of acute tubular necrosis and acute rejection episodes (Benozzi, Cappelli et al. 2009), and in distinguishing between renal vein thrombosis and acute rejection episodes or acute tubular necrosis (Grzelak, Kurnatowska et al. 2011).

In pancreas transplantation CEUS has been used as a pre-operative tool to assess the vasculature and potential viability of the transplanted pancreas. The results suggest that perfusion scores of the pre-transplanted pancreas could help predict pancreas viability post-operatively (Aboutaleb, Leen et al. 2011). There has also been one case report published where CEUS was used to assess the patency of the blood supply to the pancreas in a case of possible ischaemia to the head of the pancreas. The findings from CEUS guided management towards a non-operative route, saving the transplant. Of note, in this case the findings of both CT angiography and Doppler US would have lead to surgical intervention and possible explantation of the graft (Boggi, Morelli et al. 2009).

In summary, CEUS has a wide-range of clinical uses. Only one case report has been published about the use of CEUS in monitoring post-operative pancreas transplants, but a number of papers have recognised the use of CEUS in liver and renal transplantation. In a number of these papers the benefit of CEUS has been compared to another modality of imaging (Doppler, CT angiogram or MRI) and CEUS has been shown to be at least equivalent to these modalities.

**Advantages of CEUS**
- Immediately performed, at the bed side with instant results
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- Real-time results
- No renal metabolism of contrast
- No nephrotoxicity
- Hypersensitivity/ anaphylaxis rates lower than with current CT contrast agents
- Hypersensitivity/ anaphylaxis rates comparable to current MR contrast agents
- Avoids radiation

**Disadvantages of CEUS**
- Images are operator dependant
- Distortion of images due to fat and bowel gas is still possible (though less so, when compared to standard US/ doppler imaging)

**Undesirable Effects of SonoVue contrast (used for CEUS)**
The undesirable effects reported with SonoVue are in general, non-serious, transient and resolve spontaneously. The following is a list of all adverse reactions reported by 1,788 recipients who have taken part in clinical trials involving SonoVue.

- Bio-effects- Interactions between US waves and US contrast agents has lead to bio-effects in animal studies. These suggest that microvascular rupture could occur when microbubbles are isonated at high Mechanical Index (> 0.4) eg. in brain and eye
- Endocrine disturbances- hyperglycaemia
- Nervous system effects- Headache, paraesthesia, dizziness, insomnia, taste perversion
- Eye disorders- Blurred vision
- Vascular disorder- Vasodilation
- Respiratory/ Thoracic disorders- Phatyngitis, sinus pain
- Gastrointestinal disorders- Nausea, abdominal pain
- Skin disorders- pruritis, rash, erythema, injection site pain, bruising or burning, pain.
Musculoskeletal disorders- back pain
Cardiac disorders- In echocardiography premature ventricular contractions have been seen, again with high mechanical index (>0.4) procedures.

Contra-indications to SonoVue Use

Absolute
- Allergy to Sulphur Hexafluoride
- Respiratory disease- pulmonary hypertension, right-to-left shunts, respiratory distress syndrome
- Cardiac disease- uncontrolled systemic hypertension, patients with diagnosis of recent myocardial infarction, unstable angina, recent significant worsening of cardiac symptoms, acute cardiac failure, class III/ IV cardiac failure or severe rhythm disorder.

Relative
- Lactation
- Pregnancy
Rationale for Study

At the Manchester Royal Infirmary (MRI) we perform approximately 200 renal transplants and 30-40 pancreas transplants per year. All our renal transplant recipients undergo renal transcan 24-48 hours post-operatively to assess renal perfusion. This is a nuclear perfusion scan which gives a quantitative analysis of renal perfusion. It is a cumbersome technique at the patient’s bedside requiring injection of radioactive isotope. Each transcan costs the transplant department £349.71. This is compared with £75 per CEUS.

There are no studies which compare nuclear medicine perfusion scans with CEUS and we therefore do not know whether CEUS will provide comparable and reliable results when compared to our standard, the transcan. However, the literature would suggest that CEUS would be ideal in assessing renal perfusion. It is versatile, portable, it does not expose the patient to nephrotoxic contrasts, radiation or nuclear medicines and it is £234.71 per scan cheaper than transcans. If it is found that CEUS could be used to quantify renal perfusion, this would represent a cost saving of approximately £46,942 per year to a hospital serving approximately 200 kidney transplants per year.

In terms of the pancreas transplant recipients, we currently do not routinely scan the pancreas to assess pancreas perfusion post-operatively. This is despite, the most common post-operative complications following pancreas transplantation being vascular in nature. If we were able to show that assessment of the transplanted pancreas vasculature was possible and reliable we would be able to assess the vasculature post-operatively. This would enable us to:

- Stratify the vascular risks of the graft and manage our post-operative anti-coagulation on a patient-by-patient basis, rather than a blanket regimen which we currently follow.
- Use CEUS in the event of a possible vascular emergency post-pancreas transplant. Currently, CT angiogram is the gold standard, but this involves nephrotoxic contrast and having to move a potentially unstable patient off the ICU/ward and to radiology. The advantage of CEUS in this situation would be that it is a bed-side technique, with real-time results that avoids nephrotoxic contrast.
Study Design

Aims

Proof of principle study to:
- Evaluate the effectiveness of CEUS in assessing blood flow and morphology of transplanted kidneys and pancreata
- Compare these findings to those of Transcan and US doppler

Eligibility

The criteria for inclusion are all adult living donor kidney transplant recipients and all simultaneous pancreas and kidney (SPK) transplant recipients. The criteria for exclusion are patients who are unable to consent to the study, patients under the age of 18, patients with an absolute or relative contraindication to receiving SonoVue contrast (as explained above).

Recruitment and Consent

All participants in the trial will be counseled and consented on day of admission for transplant. For the live donor transplants, this will commonly be the day prior to surgery, but for the cadaveric transplants this is commonly the day of surgery. Unfortunately, due to the nature of transplant surgery, patients have very little notice prior to surgery and we are unable to counsel and recruit patients prior to these points.

With the unpredictable nature of transplant surgery, more than one possible recipient is called in for cadaveric transplantation for each organ. The recipients are informed whether they are the primary recipient or a back-up prior to admission by the ward staff. This practice is common because occasionally the primary recipient may not be able to go ahead with surgery and therefore a "back-up" is asked to come to the Transplant Ward to prevent wastage of an organ. In this event, all possible recipients will be counseled- primary and "back-ups", and then consent taken post-operatively from the recipient. This will give all prospective patients maximal time to consider the study. This also has the advantage of informing more
patients about the study, so that when they are eventually admitted for surgery they are aware of the study.

All patients will be consented in compliance with the Helsinki Declaration.

Statistical Analysis
This is an exploratory study of feasibility and as such the sample size is mainly dictated by pragmatic considerations. Recruitment will run for six months, during which we expect to see 15 kidney and pancreas patients and 100 kidney patients. The aim is to recruit all consenting patients in this time period.

In a study of agreement between two methods of measurement, there is no explicit way to calculate the sample size. Instead suggestions can be made on the basis of the desired accuracy. Following the methods of Bland and Altman (Bland and Altman 1986) a suggested sample size is based upon the 95% limits of agreement. Considering both this and the pragmatic concerns a sample size of 15 pancreas and kidney patients and 50 kidney patients is realistic.
A Point of Principle Study to Investigate the Use of Contrast Enhanced Ultrasound in Renal and Pancreas Transplantation

Investigator: Mr. H A Khambalia

Name of Institution: Manchester Royal Infirmary

Address: Department of Transplant Surgery, Oxford Road, Manchester, M13 9WL

Contact for queries: Transplant Ward on 0161 276 5106/ 4402

You are being invited

... to take part in a research study involving patients who are about to undergo a Kidney or Pancreas Transplant at the Manchester Royal Infirmary. Before you decide to take part, you need to understand why the research is being done and what it will involve.

This Information Sheet is in three sections:

1. Summary of Study

2. Questions and answers about the study

3. Consent form

Please take time to read this Information Sheet carefully, and discuss it with your family and friends before making up your mind. If anything in this Information Sheet is not clear, or if you have more questions, please ask the doctor who gave this to you.
Summary of the Study

The purpose of this study is to investigate the use of a special type of ultrasound scan called Contrast Enhanced Ultrasound Scan (CEUS) on patients who have had a kidney +/- pancreas transplant. CEUS is essentially a standard ultrasound scan (the same as women have when they are pregnant), but prior to starting the scan, a small amount of contrast will be given. This makes the pictures of the blood vessels much clearer and enables us to measure the blood flow to the kidney (and pancreas). CEUS has been used widely in other hospital specialties, but we have not investigated its’ benefits in transplantation. CEUS does not involve any radiation and there are no known harmful effects of this contrast on the kidney or pancreas.

Our normal practice is to conduct a “Transcan” the day after a kidney transplant to assess the blood flow and perfusion to the kidney. The Transcan involves a nuclear medicine scan the day after surgery. It is able to tell us about the blood supply to the kidney and that gives us an indication as to how we would expect it to be working. We would like to conduct both, the Transcan and CEUS on patients who have had a transplant, to see if they give us similar results.

If you are undergoing a simultaneous kidney and pancreas transplant... you will continue, as normal practice, to have the Transcan to assess the blood supply to the Kidney. We do not routinely scan the blood supply to the pancreas after surgery, as presently our only option to reliably assess the blood supply to the pancreas graft is by a CT scan. This involves radiation and an injection of contrast which may harm your kidney. As part of this study, if you are having a pancreas transplant as well, we would like to conduct a CEUS scan on your pancreas at the same time as conducting one on your kidney. If you then undergo a CT scan as well, we will compare the results of the CEUS to the CT scan.

To ensure that there are no ill-effects of the CEUS, after you have undergone the scan a member of the transplant team will ask you a few short questions about any pain you may or may not have suffered during either type of scan.

All adult patients undergoing kidney transplants or simultaneous pancreas and kidney transplants at the MRI will be invited to take part in the study.
Questions and Answers

Why have I been invited to take part?
You may be suitable to take part in this study because you are about to have a kidney or pancreas transplant.

We expect 65 patients to take part in the study from Manchester Royal Infirmary.

Do I have to take part?
No - it is up to you to decide whether or not you want to take part. Your participation in this study is entirely voluntary. If you decide to take part, you will retain this Patient Information Sheet and you will be asked to sign the Consent Form. You are still free to withdraw your consent at any time without giving a reason and to stop taking part in the study.

A decision not to take part or to withdraw at any time will not affect the standard of medical care you receive.

What will happen to me if I take part?
The day after you have had the operation if you are happy to proceed with the trial we will organise for you to have the CEUS and Transcan.

The Transcan involves a large scanning machine which comes to the ward. A nuclear medicine agent is injected through a line you already have in your vein. You need to lie flat in the bed for about 45 minutes while the scanning machine sits over the bed taking readings of how the contrast is taken up by the kidney (this will be done regardless of your involvement in the trial as this is normal practice).

The CEUS scan will involve you going to the X-ray department where one of the radiographers, specialising in CEUS will perform the scan. They will initially inject some contrast through a line you will already have in your vein. They will then spread some cold jelly on your tummy and perform the scan. The scan is the same as a pregnant woman may have, but looks at the kidney (and pancreas). It should last no more than 15 minutes. Once the scan has been completed you will return to the ward. From then on, your care will continue as normal.

If you have had a pancreas transplant as well, the Transcan and CEUS will be conducted on ICU so you will not have to go the X-ray department. If you require a CT scan, this will be organised according to normal protocol.

Once you have had the Transcan and CEUS, a member of the transplant team will go through a short questionnaire with you, asking about any pain you may or may not have felt during either scan.
We will follow up your progress on the ward. When you are discharged we will monitor your progress via the transplant clinic. You will not need to make any extra hospital visits.

What will I have to do?
You will have to attend one extra scan (the CEUS scan) in the X-ray department following your surgery. This will be done as an inpatient, often the day after surgery. Following this you will be asked a short questionnaire about any pain you may have experienced during either scan.

Following this we will continue to monitor your progress in accordance with normal transplant unit protocol.

What other scans are performed following a kidney or pancreas transplant?
Normally, following a kidney transplant we perform the Transcan (as explained above), which you will have whether you participate in the study or not.

If you have had a pancreas transplant as well... at some point you may require a CT scan. If this is the case it will be organised according to normal unit protocol.

What are the possible benefits of taking part?
The main benefit of taking part is that with CEUS, because it uses ultrasound, we are able to assess other organs in your abdomen (tummy) at the same time as performing the scan. This does not involve any further visits to the X-ray department nor does it involve any radiation or injection of contrast which may harm your transplant kidney. Another benefit is that with CEUS, the results of the scan are available immediately- therefore, if there is a problem with the blood supply to the organ we will know straight away. CEUS is also a much shorter scan and will only last approximately 10-15 minutes, when compared to Transcan, which may last 45 minutes.

If you are having a pancreas transplant as well, the benefits are as yet unknown, but the study is attempting to investigate any potential for benefit. By routinely conducting a scan of the pancreas we may be able to avoid more serious complications while checking the blood supply to the kidney at the same time.

However, we cannot guarantee these benefits.

What are the possible disadvantages or risks of taking part?
You will have to attend for an extra scan (CEUS) following your operation.
The following is a list of possible side-effects/ undesirable effects of SonoVue contrast (the contrast used in CEUS). The undesirable effects reported with SonoVue are in general, non-serious, transient and resolve spontaneously. Risks from SonoVue contrast are much lower than the possible risks of contrast for CT or MRI scanning.

- Endocrine (hormonal) disturbances- hyperglycaemia (high blood sugar)
- Nervous system effects- Headache, numbness, dizziness, insomnia, alterations in taste
- Eye disorders- Blurred vision
- Respiratory/ Thoracic disorders- Pharyngitis (sore throat), sinus pain
- Gastrointestinal disorders- Nausea (feeling of sickness), abdominal pain
- Skin disorders- rash, erythema, injection site pain, bruising or burning, pain.
- Musculoskeletal disorders- back pain

**What information about me will be collected?**
The information (data) collected in the study will include:

- Personal data- information that could be used to identify you such as your initials and date of birth
- Sensitive personal data- information about your health and medical history

To protect your right to privacy, there are Data Protection Laws in the UK. These laws control:

- How personal and sensitive data is collected
- How it can be used
- Where it can be passed on to

When you give this information to someone (known as the data controller) they must make sure it is only used in ways for which you have given permission. The data controller for this study is Mr. Hussein Khambalia.

**What will the information be used for?**
The information collected in this study will be used to investigate if there is a benefit of CEUS when compared to Transcan.

The results of this study may be used in presentations or published in scientific reports. Any presentation or published report will not name or otherwise identify you.
Will my taking part in the study be kept confidential?
You have a right to privacy, and all the information that is collected because of this study is confidential. Except as required by law, you will not be identified by name, address, telephone number, or any other direct personal identifier.

Your data such as trial records, information about your general health, the outcome of the transplant(s) and the results of any tests carried out during the study will be collected by the researcher. This is so that we may follow your progress, analyse the data and make valid conclusions. However, this data will be limited to the minimum required to follow you up for the duration of the study and that required for analysis. Any identifiable data will be encrypted and will not be removed from University of Manchester or Manchester Royal Infirmary computer systems. Following the cessation of participation in the study all identifiable data will be destroyed. Unless it is considered detrimental to your health, within the law, all this data will be kept confidential and will only be accessible to members of the research team. The utmost will be done to ensure that your data is securely protected.

You will be identified by a unique study number and information about the study number will be kept in a secure location and access limited to research study personnel only. The data will be encrypted, coded, stored and protected for 5 years following completion of the trial. However, at any time during or after the study, representatives from government health departments may ask to check the data collected to test for accuracy. You are asked to give permission for the researchers to see your medical records. They will keep the information confidential.

You are also asked to give permission for us to let your GP know that you are taking part in the study.

What happens at the end of the study?
The study ends one year after the last patient has had their transplant. When the study ends a report will be written and the results published in medical literature.

Any report that is published will not identify any participants taking part in the study.

What will happen if I do not want to carry on with the study?
If you first agree to participate and then change your mind, you are free to withdraw your consent and discontinue your participation at any time with no detriment to the clinical care you receive.

If you do not want to carry on with the study post-operatively, we will stop collecting data on your post-operative outcomes. Again, your care will continue as normal.
What if there is a problem?
If you have any concern about any part of the study, you should speak with Mr. Hussein Khambalia or a member of the Transplant Team, who will do their best to answer your questions. If you remain unhappy or they are unable to resolve your concern and you wish to complain formally, please contact a University Research Practice and Governance Co-ordinator on 0161 275 7583 or 0161 275 8093 or by email to research-governance@manchester.ac.uk. You may also contact the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research you may have grounds for a legal action for compensation against Central Manchester University Hospitals NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Who is organising the study?
The study is being organised by the Transplant and Radiology Departments at Manchester Royal Infirmary.

Has the study been approved?
This research study has been approved by the NRES Committee, North West-Haydock, the committee charged to ensure that the rights for human subjects are protected in the UK.

Where can I get more information?
If you want more information about this study you can contact the researcher named on the front of this Information Sheet, a member of the transplant team or a member of your anaesthetic team.

You can also contact the following consultants who are not involved in the research project specifically, but will be able to advise regarding participation in the study.
- Dr. Paul Taylor (Consultant Radiologist and Clinical Lead for Radiology)
- Mr. Afshin Tavakoli (Consultant Transplant Surgeon)
Chapter 6, Appendix 6.3, Consent Form

Study Number:

Title of Study:  CEUS Kidney and Pancreas Transplantation

Investigator:  Mr. Hussein Khambalia

Consent Form

1. I confirm that I have read and understood the attached Patient Information Sheet (Version Number 2.0, dated 12/12/2012) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I agree to attend a Contrast-Enhanced Ultrasound Scan following my operation.

4. I agree to have a Transcan following my operation

5. I agree to completing a short questionnaire following the scans, related to any pain I may have felt during the scans

6. I understand that relevant sections of my medical notes will be looked at by individuals from the clinical team looking after me, from members of the research team, from regulatory authorities, from Ethics Committees or from the NHS Trust, where it is relevant to my taking part in the research. I give permission for these individuals to have access to my medical records.

7. I agree to my GP being informed of my participation in the study.

8. I agree to take part in the above study.

__________________________  ____________  __________________
Name of Patient               Date            Signature

__________________________  ____________  __________________
Name of Person Taking Consent Date            Signature

Hussein Khambalia_____________  ____________  __________________
Researcher                      Date            Signature

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Pain Questionnaire for Contrast Enhanced Ultrasound Post-Transplantation

[This questionnaire is to be read by the health care professional to the patient and the patients’ responses are to be completed by the health care professional once the Contrast Enhanced Ultrasound scan and the Transcan have been completed].

Study Number

Type of Transplant Cad-Kidney(1)/ LRD-Kidney(2)/ SPK(3)/ PTA(4)/ PAK(5)

Number of days post-Transplant

On a scale of 0-10 (where 0 is no pain and 10 is the worst pain), how much pain are you in at rest?

On a scale of 0-10 (where 0 is no pain and 10 is the worst pain), how painful did you find the Ultrasound scan?

On a scale of 0-10 (where 0 is no pain and 10 is the worst pain), how painful did you find the Transcan?
General Discussion & Future Direction

H A Kambalia
Surgical and immunological aspects of pancreas transplantation have been refined since its inception in Minnesota in 1966. During the evolution of the procedure, small changes in clinical practice yielded large gains in outcome, eventually resulting in the vastly improved graft and patient survival rates now observed. Transplantation as a specialty has always been, and continues to be a field of medicine which is not risk averse, but our knowledge of an individual’s risks involved remains at best uncertain, and at worst ignorant. Risk can be described as an objective, defined element of any medical intervention. However, from an epistemological perspective, the very essence of risk assumes a “lack of knowledge” (Hansson 2014) associated with a situation and as such decisions to proceed with transplantation are associated with uncertainty at every step. Our clinical decisions are often based upon large, unverified data sets, generalizable to populations not individuals. In an era of individualised care [NICE guidelines CG138], these data sets are no longer adequate, nor are they sufficient in informing patients of their individual risks.

Given that rates of our traditional transplant related outcome measures (graft failure and patient mortality) are now consistently low and occur infrequently, it is paramount that research should move focus and concentrate on improving areas of our practice which have previously been neglected. Patient assessment and optimisation of peri-operative care to improve post-operative morbidity and further define the risks involved should be the next goal in pancreas transplantation. Therefore, this thesis has sought to improve individualised patient risk assessment and optimisation, in order to reduce peri-operative morbidity.
Pre-Operative Assessment

We firstly identified the lack of a recipient risk prediction scoring system specific to PT and therefore prospectively studied a number of readily available risk assessment scoring methods used in major surgery and critically ill patients. We applied these to SPKT recipients and correlated the results to lengths of critical care unit and hospital stay, important surrogate markers of in-patient morbidity post-operatively. The study acknowledged that a multi-system score (The Waterlow Score) best identifies patients at high-risk of longer hospital and critical care unit lengths of stay. We accept that this is not transplant specific, it does not take into account any donor or organ factors and is not intended to be the gold-standard scoring system for routine use in assessing recipients for PT. However, the paper reinforces the requirement for a multi-system scoring tool to be developed for use in PT. The P-PASS and P-DRI scoring systems are in use, but rely solely upon donor factors and are limited in predicting graft survival. Given that technical graft failures account for the majority of acute graft losses in PT, these scoring systems have limited value in risk prediction. Ideally, a multiple regression model, accounting for donor, recipient and organ factors requires development. This would necessitate large numbers, impeccable data collection and collaboration between PT units. In the UK, we are in a unique position to consider this approach, due to the established alliance of the PT units under the auspices of NHS BT.

In addition, specific peri-operative assessment tools require further evaluation in our cohort. Of note, cardiopulmonary exercise testing (CPET) could be considered. Death from cardiac disease is the most common cause of death on the transplant waiting list and remains the most common cause of long-term graft loss post-SPKT (Woeste, Wullstein et
Chapter 7, Discussion

al. 2006, van Dellen, Worthington et al. 2013). Peri-operative cardiological disturbance rates are 15.8% and 31.7% intra- and post-operatively respectively (Bindi, Biancofiore et al. 2005) and despite the recognised cardiac benefits of the procedure, SPKT recipients have a significantly higher risk of cardiovascular morbidity when compared to the normal population (Pham, Pham et al. 2007). CPET is now well established in the pre-operative assessment of high-risk patients and has been linked to outcome prediction following major vascular surgery, hepato-pancreatic surgery and renal and liver transplantation (Epstein, Freeman et al. 2004, Hartley, Pichel et al. 2012, Junejo, Mason et al. 2012, Junejo, Mason et al. 2014, Ting, Iqbal et al. 2014). Given the unique cardiovascular morbidity suffered by patients being listed for PT, the potential benefits of CPET should be investigated in this cohort.

Peri-Operative Optimisation

Secondly we conducted a randomised clinical trial, aiming to improve clinical outcomes following SPKT and therefore reduce the risks involved with the procedure. A comparison of standard peri-operative non-surgical therapy (Standard Therapy, ST) with supra-normal physiological peri-operative optimisation of the recipient (Goal Directed Therapy, GDT) was made, which resulted in improved peri-operative outcomes in the GDT group, compared to the ST group. This outcome is consistent with findings from numerous papers investigating similar optimisation strategies in patients undergoing major cardiac and non-cardiac surgery and trauma. However, it is contradictory to recent publications involving septic patients, suggesting that despite the noteworthy inflammatory response suffered by SPKT recipients and the notion that they behave in a similar manner to septic individuals, perhaps the path to the development of an oxygen debt is more advanced and
Chapter 7, Discussion

insurmountable in sepsis when compared to surgical patients (Shoemaker 1988), therefore rendering physiological optimisation ineffective.

In addition, this is the first trial of its kind to show benefit of GDT in transplantation, and given the number of confounders involved in transplantation, the results suggest a strong positive effect of the therapy on the clinical outcomes. It was naïve to power the study based upon hospital length of stay given the wide-ranging non-acute complications suffered, but the intervention was only limited to six hours post-operatively. Further studies could be conducted to identify the benefit of prolonging the optimisation period

Furthermore, and to our knowledge, again for the first time in this cohort, we conducted a biochemical analysis of recipient serum in the peri-operative period, which delineated a biological marker profile (pro- and anti- inflammatory cytokines, markers of inflammation and infection and diabetes markers) in SPKT recipients. In doing so we identified that the inflammatory marker profile following SPKT is akin to that following major surgery, trauma and sepsis. Moreover, the study opens the door for the exploration of the use of targeted anti-inflammatory therapies in the peri-operative period following SPKT, in a similar manner to which islet cell transplantation has used anti-TNF-α agents. The study of biomarkers in this cohort has also found that early pancreatic function is related to cold ischaemic time, a point previously assumed, but never demonstrated and finally we identified CRP as an early predictor of recipient morbidity following SPKT.
We accept that the numbers of markers investigated are relatively small when considering the vast array of biomarkers potentially involved as a response to multi-organ transplantation, but again, guidance was taken from studies which have already been conducted on similar cohorts. With newer fields of research available, aimed at specifically identifying biomarkers in unique circumstances such as micro-particle analysis, proteomics, metabolomics and genomics, future biomarker studies in PT should take advantage of these newer technologies.

**Post-Operative Assessment**

Finally, we identified and then investigated a requirement for a safe, reiterative and reliable method of evaluating the patency of the vascular supply to the newly implanted pancreas. Traditionally, either contrast CT, contrast MR imaging or B-mode USS (+/- duplex) have fulfilled this role, as required, in emergency situations. However, these techniques are associated with considerable disadvantages resulting in the lack of a reliable and safe screening tool. Reports of the use of contrast enhanced ultrasound (CEUS) at various stages of the pancreas transplant process have been published, including one study which found a correlation between contrast perfusion rates and pancreatic inflammation in the outpatient follow-up of implanted pancreata (Kersting, Ludwig et al. 2013). We investigated the role of CEUS in the peri-operative period as a screening tool to assess for vascular graft patency, allograft perfusion and the evaluation of peri-operative complications. In our opinion, the early results of the trial confirm that CEUS is a useful imaging modality in the peri-operative period following PT and the quantification results may provide prognostically important data. However, these findings need to be confirmed in larger prospective trials.
Furthermore, the use of newer, more sophisticated imaging techniques should also be considered in the pre-operative assessment of the pancreas allograft. Presently, assessment of the suitability of a whole-organ pancreas for transplantation is subjective and the decision to proceed with transplantation varies between clinicians, and is based upon the surgeon’s perception of the health of the organ. Factors taken into consideration are macroscopic, focusing particularly on fat content and fibrosis, vascular graft quality and cold ischaemic time (CIT). Objective assessment is often logistically impractical, due to the time constraints between organ retrieval and implantation, leading to a lack of evidence correlating objective pre-operative organ parameters with post-operative outcome. One group have used CEUS for organ assessment in this manner (Aboutaleb, Leen et al. 2011), but our experience would suggest that although CEUS may be useful at determining the vascular integrity of the organ, high-resolution US or MR imaging may be of greater value in assessing pancreatic parenchyma. Nevertheless, the requirement of an objective tool for the assessment of allograft organs pre-operatively is required and newer imaging modalities should be investigated.
Conclusion

The role of pancreas transplantation in the treatment of insulin dependent diabetes mellitus associated with end-organ failure is currently unquestioned. However, it remains unpalatable to some clinicians and patients alike, due to the potential co-morbidity associated with it. Additionally, results in islet cell transplantation are improving, although continue to be considerably inferior to those of pancreas transplantation. It is our responsibility to elude complacency and ensure continual improvements in our practice at every step of the transplant process in order to provide the best possible care for our patients.
References

H A Khambalia


References


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