Predicting and ameliorating graft function in deceased donor kidney transplantation

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Abbreviations

aHR	Adjusted hazard ratio
AKI	Acute kidney injury
AKIN	Acute Kidney Injury Network
ALG	Antilymphocyte globulin
APC	Antigen presenting cell
ATG	Antithymocyte globulin
ATP	Adenosine triphosphate
AUC	Area under the curve
BMI	Body mass index
CD	Cluster of differentiation e.g., CD1
cDCD	Controlled donation after circulatory death
CI	Confidence interval
CIT	Cold ischaemia time
CKD	Chronic kidney disease
CMV	Cytomegalovirus
CO_2	Carbon dioxide
CPR	Cardiopulmonary resuscitation
CRP	C-reactive protein
DAKT	Dual adult kidney transplant
DAMP	Damage-associated molecular pattern
DBD	Donation after brain death
DCD	Donation after circulatory death
DCGS	Death-censored graft survival
DGF	Delayed graft function
DNA	Deoxyribonucleic acid
ECD	Expanded (or extended) criteria donor

ECMO	Extracorporeal membrane oxygenation
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
EMPIRIKAL	Efficacy of Mirococept for Preventing Ischaemia-Reperfusion Injury in the Kidney ALlograft
ESOT	European Society of Organ Transplantation
ESRD	End-stage renal disease
EVNP	Ex vivo normothermic perfusion
FACS	Fluorescence-activated cell sorting
FFPE	Formalin-fixed, paraffin embedded
FSC	Forward scatter
FSC-A	Forward scatter - area
GFR	Glomerular filtration rate
GSTT	Guy's and St Thomas' NHS Foundation Trust
HIF-1	Hypoxia-inducible factor-1
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMP	Hypothermic machine perfusion
HR	Hazard ratio
HRA	Health Research Authority
IBM	International business machines
ICH	Intracranial haemorrhage
IFN-γ	Interferon gamma
IL	Interleukin
IQR	Interquartile range
IRI	Ischaemia reperfusion injury
IV	Intravenous
IVC	Inferior vena cava
KDIGO	Kidney Disease Improving Global Outcomes

KDPI	Kidney donor profile index
KDRI	Kidney donor risk index
KFTS	Kidney Fast-Track Scheme
KPA	Kidney Patients' Association
LDKT	Living-donor kidney transplantation
LDL	Low density lipoprotein
MAP	Mean arterial pressure
MDRD	Modification of Diet in Renal Disease
MHC	Major histocompatibility complex
MM	Mismatch
MMF	Mycophenolate mofetil
mRNA	Messenger ribonucleic acid
MSC	Mesenchymal stem cell
mTOR	Mammalian target of rapamycin
NF-κB	Nuclear factor-κB
NGAL	Neutrophil gelatinase-associated lipocalin
NHS	National Health Service
NHSBT	National Health Service Blood and Transplant
NICE	National Institute of Health and Care Excellence
NMP	Normothermic machine perfusion
NRP	Normothermic regional perfusion
NODAT	New onset diabetes after transplantation
NORS	National Organ Retrieval Service
O_2	Oxygen
OLT	Orthotopic liver transplantation
OKT3	Muromonab-CD3 (trade name Orthoclone OKT3)
OPTN	Organ Procurement and Transplantation Network
OR	Odds ratio

PIKB	Pre-implantation kidney biopsy
PITHIA	PreImplantation Trial of Histopathology In renal Allografts
PNF	Primary non-function
PRA	Panel reactive antibody
pRBC	Packed red blood cells
QOL	Quality of life
RBC	Red blood cell
RCT	Randomised controlled trial
REC	Research Ethics Committee
RhD	Rhesus D antigen
RINTAG	Research, Innovation, and Novel Technologies Advisory Group
RNA	Ribonucleic acid
ROC	Receiver operator curve
ROS	Reactive oxygen species
PRS	Reperfusion syndrome
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome novel coronavirus 2019
SCC	Side scatter
SCD	Standard criteria donor
SCS	Static cold storage
SRTR	Scientific Registry of Transplant Recipients
SSC-A	Side scatter - area
T_h1	T-helper 1
T_h1	T-helper 2
TLR	Toll-like receptor
TNF-a	Tumour necrosis factor alpha
UK	United Kingdom
UKKDRI	United Kingdom kidney donor risk index

UNOS	United Network for Organ Sharing
US	United States
USA	United States of America
UTI	Urinary tract infection
VIF	Variance inflation factor
WHO	World Health Organization

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Thesis summary

Background

Deceased donor kidney transplant programmes are increasingly successful, with improving long-term outcomes (1, 2). However, there remains a shortfall in the number of kidney transplants performed relative to the number of patients who would benefit from one. Expansion of the donor pool to include older donors, those with more comorbidities or with a high terminal creatinine has resulted in falling waiting lists (2-4) but greater variability in graft outcomes. The ability to predict transplant outcomes has therefore become increasingly important as the use of 'higher risk' donors results in poorer graft outcomes (5). In particular, expansion of the donor pool has resulted in inferior early graft function, with up to half of kidney transplants from donation after circulatory death donors requiring haemodialysis in the first week of transplantation (6). Ten to 20% of kidneys retrieved from deceased donors are discarded, primarily due to uncertainties about organ quality and long-term graft survival (3, 7-9). The ability to predict graft function prior to transplantation would greatly inform the decision-making process of organ allocation and recipient selection, whilst also identifying grafts that would be amenable to interventions aimed at ameliorating transplant function. Predicting graft outcomes may also lead to less organ wastage and increased transplantation rates.

This thesis focuses on three main approaches that attempt to predict graft function: histopathological analysis of the kidney prior to transplantation, analysis of known donor demographic data from registry analyses, and organ performance during ex vivo normothermic machine perfusion. The predictive ability of these tools has yet to be fully determined. Furthermore, these approaches may indirectly improve graft function, by informing decision making, or (in the case of normothermic machine perfusion) directly provide an opportunity to improve organ quality.

Aims

- To determine more reliable methods of predicting poor kidney transplant function (early and late)
- To determine means of ameliorating graft dysfunction prior to kidney transplantation

Objectives

1. Examine the predictive ability of donor kidney histology on transplant outcomes, including delayed graft function
2. Examine the national registry to determine whether the duration of delayed graft function impacts longer term graft function and survival
3. Examine the safety and feasibility of ex vivo normothermic perfusion and its efficacy ameliorating early graft function
4. Examine the use of ex vivo normothermic perfusion to reduce post-reperfusion syndrome
5. Examine the use of white cell filtration during ex vivo normothermic perfusion as a means of ameliorating early graft parameters

Results

1. Chronic donor histological changes at implantation were not associated with long-term graft and patient survival in this study. The systematic use of preimplantation kidney biopsies increased organ discard rate when an organ utilisation algorhythm was applied.
2. In donation after circulatory death donor kidney transplantation, delayed graft function lasting longer than 14 days was predictive of inferior patient and graft survival. In contrast, delayed graft function of any duration is associated with inferior outcomes in donation after brain death donor kidney transplantation .
3. Ex vivo normothermic perfusion did not reduce delayed graft function or reperfusion syndrome following deceased donor kidney transplantation. However, these studies may have been

underpowered. These studies primarily demonstrated the safety and feasibility of short duration EVNP prior to transplantation.

4. White cell filtration during EVNP with may reduce the number of donor-derived inflammatory cells in the perfusate. However this did not appear to improve early graft functional parameters

Conclusion

Early graft dysfunction can have a long-term impact on deceased donor kidney transplant function and patient survival. Methods of predicting graft dysfunction are imperfect and can mislead clinicians into unnecessary organ discard. Normothermic machine perfusion technology offers the potential for pre-transplant organ conditioning that may provide improved outcomes for patients.

Chapter 1: Background

1.1 Pre-operative assessment of deceased-donor kidneys

Accurate assessment of deceased-donor kidneys is fundamental to effective organ utilisation. Due to a shortfall between organ supply and demand, kidney transplantation cannot be immediately offered to patients who are in need of them. In response to this disparity, organs from less optimal donors have been used. This has understandably meant that early graft dysfunction and reduced organ longevity has been considered an acceptable compromise, primarily because the alternative methods of renal replacement often provide inferior quality of life and patient survival.

Accurately identifying organs that are likely to have inferior outcomes enables clinicians to longevity match donor and recipient, whereby the highest quality organs are offered to patients expected to live the longest and the lower quality organs are offered to patients with shorter predicted survival. This process attempts to optimise organ usage whilst still providing treatment benefit. Accurately predicting inferior graft function and survival also allows clinicians to identify specific groups of donor kidneys or recipients that would benefit from a specific intervention aimed at improving graft function. However, at present this is an imperfect science, with various subtleties and pitfalls. During the course of this introductory chapter, the history of organ transplantation and the background to the above issues will be outlined so that the complexities of pre-operative deceased donor kidney assessment can be more fully appreciated. Organ utilisation decisions are highly complex, with multiple interacting factors affecting a final outcome. It is therefore necessary to introduce these variables individually, before considering the broader topic of predicting and ameliorating graft dysfunction.

1.2 Chronic kidney disease in perspective

This first step in setting the scene is to consider the background condition: chronic kidney disease (CKD). Normal kidney function is necessary for the homeostasis of water, electrolyte and acid-base

balance, excretion of waste products, maintenance of blood pressure and the production of erythropoietin necessary for red blood cell production (10-16). CKD is defined as the loss of kidney function over a period of at least three months (17). This differs from acute kidney disease (termed acute kidney injury) which involves a rapid loss of renal function beyond a patient's usual baseline function. The progressive deterioration in kidney function seen in worsening CKD means that patients become increasing susceptible to the complications of chronic uraemia, hypertension and cardiovascular disease, bone demineralization and anaemia. CKD causes a significant burden on healthcare systems worldwide; nine percent of the world's population has a recorded diagnosis of CKD (18).

Kidney function can be measured in numerous ways, but most commonly by measuring the concentration of serum creatinine (19). Creatinine is an end-product of muscle catabolism and is excreted by the kidneys. The clearance of creatinine may therefore reflect kidney function. Despite its many pitfalls (19), serum creatinine is a commonly used laboratory test. In order to adjust for differences in serum creatinine between persons of different age and sex, an estimation of glomerular filtration rate can be calculated. The four-variable Modification of Diet in Renal Disease (MDRD) estimated glomerular filtration rate (eGFR) was employed in this thesis (20) (Equation 1).

Equation 1 - Modification of Diet in Renal Disease estimated glomerular filtration rate equation

$$\textit{Estimated glomerular filtration rate (mL/min per 1.73 m}^2\textit{)} = 175 \times \textit{Serum Creatinine}^{-1.154} \times \textit{age}^{-0.203} \times 1.212 \text{ (if Black ethnicity)} \times 0.742 \text{ (if female)}$$

However, it should be acknowledged that the National Institute of Clinical Excellence has since removed ethnicity from the equation due to concerns regarding its validity (21). Other methods of estimating renal function also exist (22-26). Direct measurement of the glomerular filtration rate is logistically challenging and costly (27). CKD may be classified into five stages by the estimated glomerular filtration rate (eGFR) (Table 1).

Table 1 – Stages of chronic kidney disease based on the estimated glomerular filtration rate

Chronic kidney disease stage	**Estimated glomerular filtration rate (mL/min/1.73m²)**
Stage 1	90 (with evidence of kidney disease)
Stage 2	60-89
Stage 3a **Stage 3b**	45-59 30-44
Stage 4	15-29
Stage 5 (end-stage renal disease)	<15

Adapted from Kidney Disease Improving Global Outcomes (KDIGO). Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease 2012 (reference (28)).

End-stage renal disease

CKD stage 5 may also be referred to as end-stage renal disease (ESRD) and represents severe derangement of kidney function, with an eGFR of <15mL/min/1.73m². The incidence of ESRD in the UK is increasing at a rate of 5-8% per annum (29).

Renal replacement therapy: dialysis versus transplantation

Before considering transplantation as a treatment option, it is necessary to put transplantation in perspective by considering the alternative options available. A key concept of this thesis is whether to embark on transplantation, or whether to remain on dialysis and/or remain on the transplant waiting list. Each route involves its own subtle risks and benefits.

The definitive treatment for ESRD is renal replacement therapy. Renal replacement therapy comprises three main treatment modalities: haemodialysis, peritoneal dialysis, and renal transplantation. A patient with ESRD may undergo a period of each type of renal replacement therapy

during their lifetime. However, long-term haemodialysis and peritoneal dialysis are associated with significant excess morbidity and mortality compared to transplantation (30).

A major drawback of dialysis is the sequelae and complications of accelerated atherosclerosis (31). This is such a significant risk that cardiovascular disease accounts for approximately 45% of deaths in patients with ESRD (32). This is due to three main processes. Firstly, the presence of CKD alone results in protein loss through albuminuria (33), and carbamylation of low-density lipoprotein (LDL) cholesterol through uraemia (34); both of which are well-defined, independent and prominent risk factors for coronary artery disease. Secondly, proinflammatory mechanisms are stimulated during dialysis, through cytokine production and upregulated expression of adhesion molecules on vascular endothelium, which promote the deposition of atherosclerotic plaque within arterial vasculature. Finally, derangement of calcium metabolism in CKD and dialysis further contributes to the development of vascular calcification (35). These processes resulting in accelerated atherosclerosis are even observed in children and young people on dialysis, who would otherwise lack the typical cardiovascular risk factors of hypertension and dyslipidaemia observed in older adults (36).

Following transplantation, some cardiovascular risk factors may persist, such as hypertension. Likewise, additional risks of cardiovascular morbidity may develop, such as new onset diabetes after transplantation (NODAT), associated with calcineurin-inhibitors (i.e., tacrolimus maintenance immunosuppression). None-the-less, a functioning renal transplant is associated with reduced cardiovascular complications compared to dialysis patients.

Although a selection bias exists such that patients activated on the transplant waiting list are younger, less comorbid, of a higher socioeconomic status and in position of better access to healthcare, compared to those deemed unsuitable for transplantation, there likely remains an overall long-term patient survival benefit of transplantation over dialysis (30). Although the initial risk of death is almost three times higher in transplanted patients in the first 100 days post-operatively compared to waiting-listed patients, a clear survival benefit is seen after approximately 250 days following implantation (30). However, for an individual patient, the risks of remaining on a transplant waiting list versus accepting a

sub-optimal transplant offer remain challenging to quantify because of the potential risks associated with sub-optimal kidney donors and the difficulty in knowing when a patient might receive another offer or if a subsequent offer is likely to be of better quality or not. Quantifying the risk of each option is a major challenge in modern-day clinical practice.

An important aim of successful kidney transplantation is improvement in health-related quality of life (QOL), compared to dialysis (37). QOL also has a positive correlation with long-term patient survival in chronic kidney disease (37). Wyld et al conducted a comprehensive meta-analysis examining quality of life in patients undergoing haemodialysis, peritoneal dialysis and renal transplantation, involving 190 studies, approximately 56000 patients and 326 different QOL tools (38). With transplantation used as the reference group, QOL mean estimates were lowest in patients on long-term haemodialysis (0.69, 95% CI 0.59-0.80). Peritoneal dialysis patients had higher mean QOL estimates than haemodialysis (0.72, 95% CI 0.62-0.83), though there was no statistical difference between these two dialysis modalities (p=0.08). When combining both dialysis modalities, dialysis patients had significantly lower QOL estimates compared to renal transplantation (0.70, 95% CI 0.62-0.78). A systematic review performed by Purnell et al, involved 46 studies examining differences in participation in life activities, including travel, recreation, freedom, work, and physical activity (39). The study demonstrated higher rates of participating in these areas following renal transplantation relative to dialysis. Improved physical activity seen post-transplantation is also associated with reduced cardiovascular morbidity (40). Improved QOL following transplantation is also demonstrated in the paediatric population (41). Successful kidney transplantation is therefore considered to provide superior QOL, freedom to fully participate in recreation and work, and confers better long-term survival compared to dialysis. Kidney transplantation is also less costly after the first year of transplantation (42).

The complicating factor is that the efficacy of kidney transplantation, as a treatment option, varies between patient to patient. The renal function that a graft provides a patient, and the length of its survival, depends greatly on a number of important factors. These include, but are not limited to, donor type (living and deceased), donor age and donor comorbidities. In particular, deceased donor kidneys have greater variability in quality. In a number of situations, declining a deceased donor kidney offer may be the

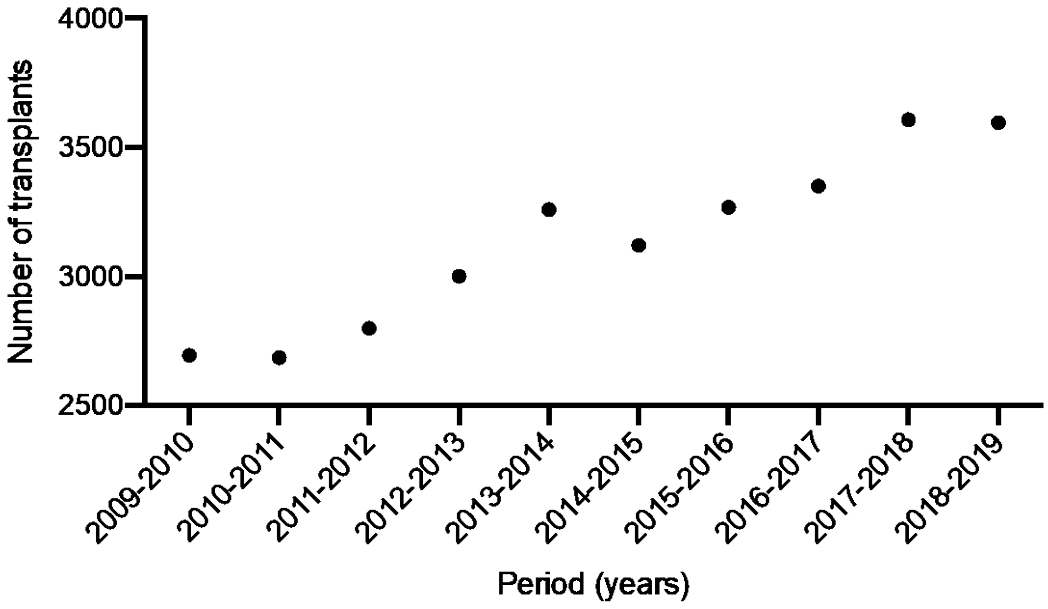
correct decision. Inappropriate organ discard rates are reportedly high across most modern transplant units, at approximately 18-20% in the US, 10-12% in the UK and 8% in Europe (43) (further evidence supporting the notion that organ discard rates are inappropriately high are considered in Section 1.4.5). The need for assessment of organ quality is therefore an important focus of this thesis.

Despite the advantages of transplantation over dialysis, it cannot currently be offered to all patients with ESRD. This is, in part, due to the finite supply of donor organs. With limited advances in artificial organs and kidney xenotransplantation, a significant discrepancy in organ supply and demand has developed (44). An understanding of the processes driving organ demand and supply is necessary to appreciate the increasing use of suboptimal kidney donors, as well as the changes in organ retrieval, preservation, and allocation.

Organ demand

Kidney transplantation began in the United Kingdom in 1960 and was soon recognised as the gold standard treatment for end stage renal disease for most patients (30, 42). The demand for kidney transplantation rose significantly as the service became more widely available. By the year 2000, 6154 patients were on the deceased donor kidney transplant waiting list (45). The merging of blood and transplant services occurred in 2005, forming NHS Blood and Transplant (NHSBT). Despite the change in name, NHSBT's responsibilities did not change significantly, and continued to include the national coordination of donor registration, oversight of national organ allocation policies, management of the deceased donor waiting list and the management of data within UK transplant registry. Data from NHSBT indicate that organ demand was rising, with the number of patients on the deceased donor waiting list peaking in 2009. Equivalent organisations internationally, such as United Network for Organ Sharing (UNOS) in the United States, also displayed mounting organ demand (46). The number of kidney transplants have continued to increase over the last decade (Figure 1) and meeting demand remains a major public health challenge.

Figure 1 - Number of kidney transplants performed in the UK over the last 10 years, prior to the SARS-Cov-2 pandemic in late 2019-2020



Adapted from: NHSBT annual report on kidney transplantation, published November 2019 (45). Years 2009-2019 considered in order to demonstrate the general trend in organ transplantation prior to the SARS-Cov-2 pandemic (discussed in Section 1.1.6)

The growing demand for kidney transplantation is partly due to the mounting realisation that kidney transplantation is the optimal treatment for the majority of patients with the ESRD (30). However, demand has been driven by resulted from the increasing prevalence of ESRD (29). Diabetes mellitus (DM) has been described as one of the largest epidemics in the world (47), and is characterised by chronic hyperglycaemia. The incidence of DM type II has doubled in the last two decades, with approximately 400 million people affected in the year 2015 (47). This figure is expected to rise to 600 million in the next 20-30 years (48). Diabetic nephropathy is a complication of DM and is a common cause of CKD and ESRD in the UK, as well as internationally (49). Hypertension is also increasing in prevalence and is expected to affect 60% of the world's population by 2025 (50). The prevalence of CKD attributable to hypertension is challenging to estimate as patients with suspected hypertensive nephropathy rarely undergo kidney biopsy. However, it is estimated to be the second most common cause of ESRD internationally (51).

With kidney transplantation in significant demand, research in recent years has justifiably focused on increasing the number of potential donors and improving organ supply. It was through this conscious process that the use of suboptimal kidney donors became more acceptable and the need for better donor assessment became a science of its own.

1.1 Organ supply

Kidneys donated for organ transplantation originate either from living donors or deceased donors. Early provision of transplant services primarily considered young living donors as the ideal source of organs. If potential recipients had no living donor options, young intensive care patients diagnosed with unrecoverable brain injury, with otherwise good renal function, were considered for those on the deceased donor waiting list. However, as the demand for kidneys increased, so did the shortfall in organ supply, resulting in increasing rates of patient death whilst on the transplant waiting list. Strategies that have been employed to improve the supply of organs for transplantation can broadly be categorised into:

- increasing the potential donor pool – considering novel groups of patients that were not originally thought to be suitable e.g., older donors with comorbid conditions. Also, increasing access to living donor transplantation, or expanding donation criteria for living donors, may also improve access to transplantation overall.
- improving the quality of organs already retrieved from donors – devising means of ameliorating graft function before and after transplantation
- reducing organ discard – devising means of better assessing organ quality, to avoid inappropriate organ offer declines

1.1.1 Living donor kidney transplantation

Living donors represent the ideal source of kidneys for recipients as they provide superior graft survival compared to deceased donor transplantation, and superior patient survival compared to

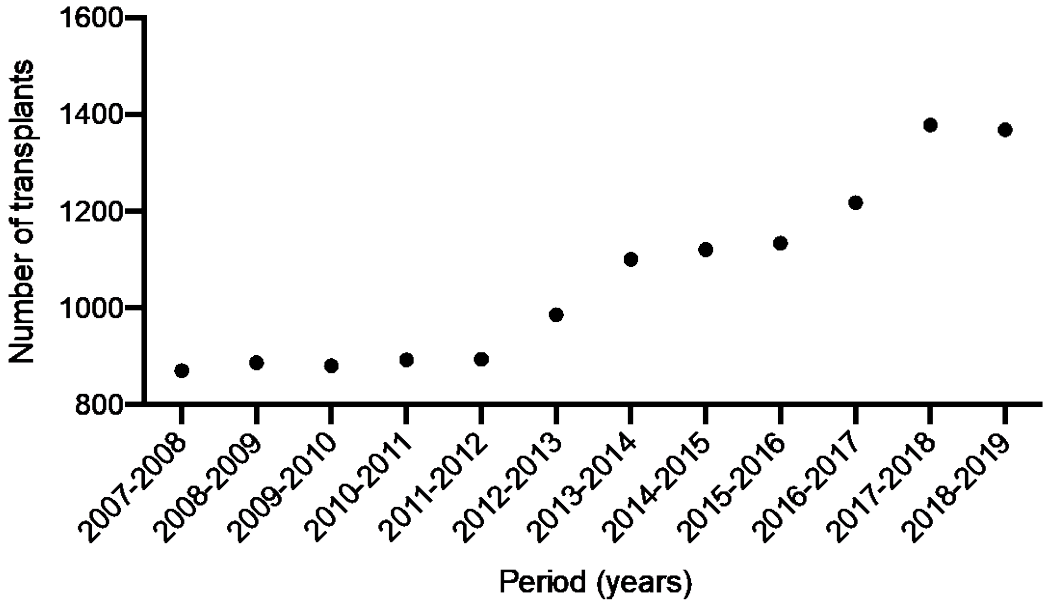
remaining on dialysis (52, 53). This is because living donors are selected on the basis of relatively fewer comorbidities and good renal function. Living donor kidney transplantation does not usually necessitate national allocation (except in non-directed altruistic donors) through a national waiting list and can overcome incompatibility issues through the living kidney donor sharing scheme, or through planned pre-transplant immunodepletion and desensitisation protocols (54, 55). Nevertheless, LDKT represents only 28% of annual transplant activity in the UK, and although the number of kidney transplants are generally increasing, LDKT numbers remain static (44). The increasing rate of kidney transplantation in the UK has not been achieved through living donor transplantation. Given the ability to thoroughly assess living donors prior to donation and the relatively low rates of early graft dysfunction or loss, this thesis does not consider living donors in detail. However, some of the strategies for predicting and ameliorating graft function in deceased donor kidneys that are considered in this thesis may be applied in living donor transplantation, especially if there is a concern about potential graft function and longevity.

1.1.2 Donation after brain death (DBD)

DBD donors are confirmed as irreversibly brain dead if they meet specific diagnostic criteria (56). However, DBD donors continue to have spontaneous cardiac activity, with perfusion of organs with oxygenated blood. This means that during organ retrieval, cessation of organ oxygenation and initiation of cold organ preservation occur almost simultaneously. The ability to begin the 'warm phase' of the organ retrieval process, carry out systemic heparinisation, cannulate the aorta, and with limited ischaemic injury prior to cold preservation, has made DBD donors the preferred deceased donors, at least from a logistical perspective. However, the number of DBD donors in the UK has not maintained its upward trajectory, perhaps due to decreasing rates of brain injuries due to improved safety regulations and a decline in road traffic accidents (Figure 2) (57).

Although young DBD donors are a valuable source of high-quality organs and the number of DBD donors began to increase again from 2013 an alternative supply of deceased donor kidneys has had to be established in order to help close the gap between organ supply and demand.

Figure 2 - Number of kidney transplants from donation after brain death (DBD) donors in the UK 2007-2019



Adapted from: NHSBT annual report on kidney transplantation, published November 2019 (45). Years 2007-2019 considered in order to demonstrate the general trend in organ transplantation prior to the SARS-Cov-2 (discussed in Section 1.1.6)

1.1.3 Expanded criteria donors

In response to the critical shortage of kidneys for transplantation, various strategies have been employed to increase the supply of suitable kidneys for transplantation. Given that mortality is raised 3-4 times in patients on the deceased donor kidney transplant waiting list relative to the general population (58), it is considered justified to consider 'higher risk' donor kidneys for transplantation. There are many different ways to define apparently 'higher risk' deceased donor kidneys.

'Expanded criteria donors (ECD)' were categorised as those aged >60 years, or >50 years with two of the following features: hypertension, raised serum creatinine ≥1.5 mg/dL (132μmol/L), or death from cerebrovascular event on the basis that risk of graft failure was 1.7 times or more higher than non-ECD donors (59). It has been found that transplantation of ECD kidneys was associated with a survival

benefit compared to patients on dialysis whilst on the US deceased donor waiting list (60). However, the use of ECD kidneys alone was not sufficient to bridge the shortfall in the supply and demand for kidneys. This oversimplistic approach of 'low' and 'high' risk donors based on three donor characteristics does not fully capture the complexity of risk management and decision-making in transplantation. Furthermore, the concept of ECD or 'marginal' kidney donors can be considered to include other donor characteristics considered high-risk, such as extremes of donor age, severe acute kidney injury (AKI), donor history of malignancy or chronic viral infection, and anatomical variation and abnormalities. The use of the ECD definition is increasingly giving way to more complex donor risk indices, providing a quantitative spectrum of risk, based on validated donor characteristics. Donor risk indices are explored in more detail in Section 1.5.1. However, in the course of this thesis, I will demonstrate how donor risk indices are still not sufficient in predicting outcomes. The need composite tools, which include a number of other clinical variables may be more promising.

1.1.4 Donation after circulatory death

Donation after circulatory death (DCD) describes retrieval of organs for the purpose of transplantation once death has been confirmed using circulatory criteria. The more frequent use of DCD donors was a critical strategy in the expansion of the donor pool. Donation after circulatory death is described as 'controlled' when donor cardiac arrest occurs within close proximity to a prepared organ retrieval team (61). Uncontrolled DCD donors have undergone an unexpected cardiac arrest, with either absent or unsuccessful cardiopulmonary resuscitation (CPR). The categories of DCD donors are described in Table 2.

Table 2 - Categories of donation after circulatory death (DCD) donors

Category	Terminology	Setting	Description
Category I	Uncontrolled	Found dead IA – out of hospital IB – in hospital	Sudden unexpected cardiac arrest without any attempt of resuscitation medical team
Category II		Witnessed cardiac arrest IIA – out of hospital IIB – in hospital	Sudden unexpected cardiac arrest with unsuccessful resuscitation medical team
Category III	Controlled	Planned withdrawal of life sustaining treatment	Expected cardiac arrest in close proximity to a prepared organ retrieval team
Category IV	Uncontrolled controlled	Cardiac arrest of a brain-dead patient	Unexpected cardiac arrest of a brain stem dead patient in hospital with unsuccessful resuscitation

Source: modified from *Thuong M et al. New classification of donation after circulatory death donors definitions and terminology. Transplant international 2016;29(7):749-759*

In the UK, uncontrolled DCD donor kidney transplantation is rare due to the logistical challenges of pre-hospital CPR and the challenges of gaining the Coroner's decision within a limited time-frame (62). Given its rarity in the UK, uncontrolled DCD donor kidney transplantation is not considered in detail in this thesis. Category III (controlled) DCD donors (cDCD) make up the overwhelming majority of DCD donor retrievals in the UK (>99%), based on the findings in Chapter 3 of this thesis. However, uncontrolled DCD donor kidney transplant is an area of interest, with evolving evidence from Spanish experiences (63, 64).

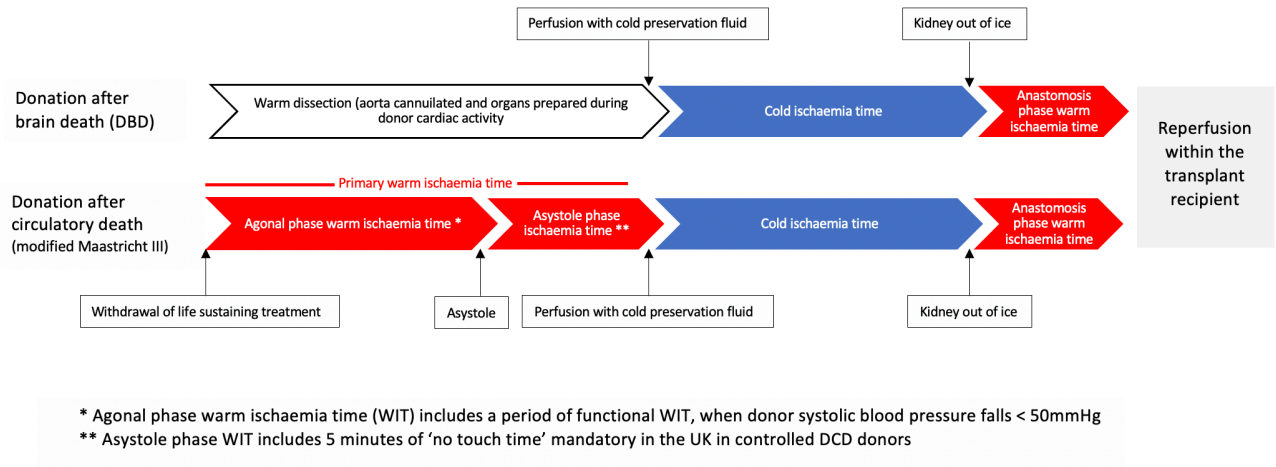
cDCD donors differ from DBD donors in several ways. cDCD donors have undergone cardiac arrest following withdrawal of life-support prior to the retrieval process. In the UK, there is a 5 minute 'stand-off' between loss of cardiac output to confirmation of death. A super-rapid retrieval approach is

commonly undertaken, whereby the infra-renal aorta or common iliac artery are cannulated, the aorta is clamped above the level of the celiac artery, the IVC is incised for blood venting and the organs are perfused with cold preservation fluid (typically Custodian HTK, or Belzer University of Wisconsin storage solution, Bridge to Life Ltd). The period of time from withdrawal of life sustaining treatment to reperfusion with cold preservation fluid in cDCD donors has been termed primary warm ischaemia (WIT) time by NHSBT, although other definitions of primary warm ischaemia exist (43). This additional ischaemic injury forms one distinguishing feature of cDCD donors from DBD donors who do not undergo significant warm ischaemia during retrieval. Primary WIT may be divided into two separate phases (65):

- Agonal phase: period of time from withdrawal of life sustaining treatment and ending when the donor becomes asystolic. Agonal phase also includes a period of 'functional WIT', when donor systolic blood pressure drops below 50mmHg, resulting in which poor organ perfusion (66)
- Asystolic phase: period of time from donor asystole (ending when the organs are perfused with cold preservation fluid)

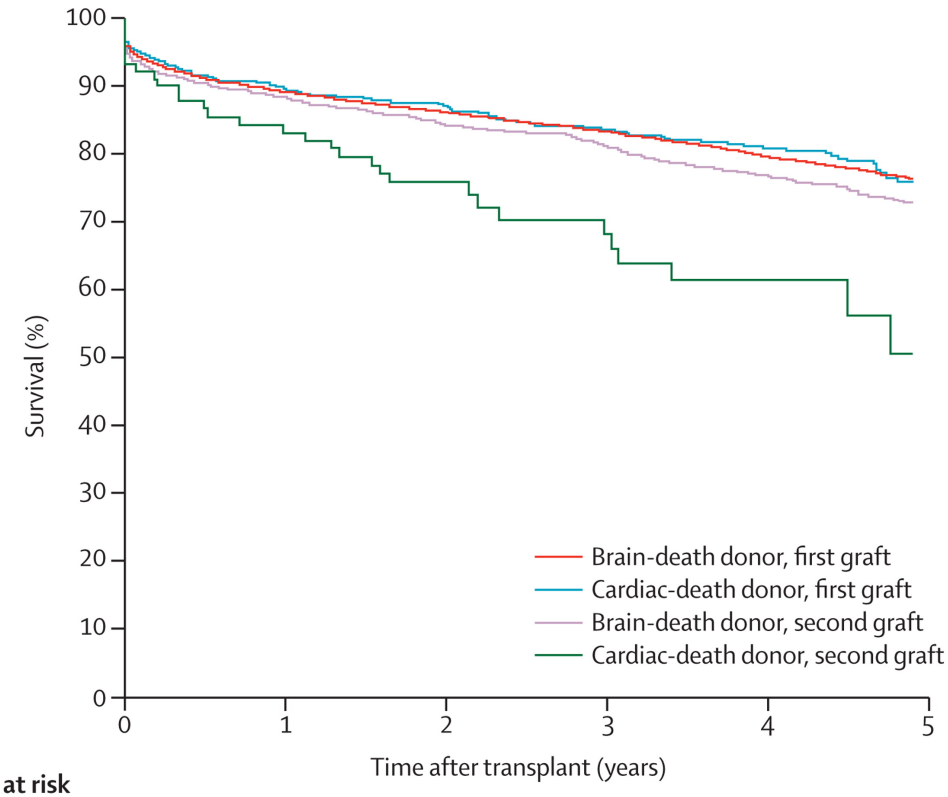
The differences in retrieval phases between DBD and cDCD donors is presented in Figure 3.

Figure 3 - The phases of organ retrieval based on deceased donor type



cDCD donors also introduce additional challenges, as donors may not reach asystole in a timeframe suitable for organ retrieval or may sustain a period of organ hypoperfusion prior to cardiac arrest rendering the organ severely damaged. The additional ischemic injuries sustained by circulatory-death kidneys has led to concerns that DCD donors are an inferior source of organs compared to DBD donors (67). However, outcomes following first graft cDCD donor kidney transplantation have proven to be acceptable, with essentially identical graft survival to DBD transplantation (Figure 4) (6).

Figure 4 - Differences in graft survival between donation after brain death (DBD) and donation after circulatory death (DCD) donor kidney transplantation in recipients of first or second graft



Number at risk						
Brain-death donor, first graft	6759	5906	5227	4440	3591	2747
Cardiac-death donor, first graft	739	638	504	345	242	156
Brain-death donor, second graft	1151	1000	880	751	599	464
Cardiac-death donor, second graft	89	73	48	33	16	7

Source: Summers DM et al. Analysis of factors that affect outcome after transplantation of kidneys donated after cardiac death in the UK: a cohort study. Lancet 2010;376(9749):1303-1311.

DCD donor kidney transplantation has increased in frequency over the last twenty years and now represent 40% of deceased donor kidney transplantation in the UK (44). The increasing utilisation of DCD donors is likely to have been the dominant factor in the rising transplantation rates (2). Furthermore, studies comparing differences in outcomes between ECD DBD and ECD DCD donors have suggested no difference in graft survival between the two groups (68, 69).

Despite the strong evidence supporting the continued use of DCD donor kidneys, they are relatively underused outside of the UK, with only 14% of deceased donor kidneys in the US coming from DCD donors compared to 38% of deceased donor kidneys implanted in the UK (43).

1.1.5 Dual adult kidney transplantation

Another strategy to improve the supply of kidneys is to consider grafts that may not provide enough function individually to be implanted together. Although this concept may be applied to small paediatric donors, when kidneys come from adult deceased donors this is known as double or dual adult kidney transplantation (DAKT). Although implanting both kidneys into a single recipient halves the potential utilisation, these grafts may have been subject to discard if only considered for implantation individually. Hence DAKT was devised to improve organ utilisation and patient outcomes, mainly in kidneys from older donors (70) or other situations in which the nephron mass may be reduced due to comorbid states or graft injury in the peri-transplant period (71).

The choice of whether a pair of grafts should be used individually or as DAKT is challenging and controversial (72). Histological assessment of the kidney can be used to determine thresholds for single versus dual transplantation, either by the proportion of glomerulosclerosis (73, 74), or using histological scoring systems such as the Karpinski or Remuzzi scores (75, 76). Alternatively, some centres use a number of donor and recipient factors to form a decision on an individual basis.

DAKT has comparable five-year graft and patient survival compared to single kidney transplantation from older donors (77), but it is yet to be determined what is the best means of guiding utilisation decisions regarding single versus dual adult kidney transplantation.

1.1.6 Novel coronavirus 2019

The global supply of organs for transplantation had an unexpected and dramatic fall due to the novel coronavirus pandemic from 2019 to present day (Severe acute respiratory syndrome novel coronavirus 2019 - SARS-CoV-2) (78). Although organ retrieval and transplantation rates fell due to competing pressures on health services to deliver care to patients with SARS-CoV-2, implantation rates also fell due to concern about SARS-CoV-2 infection in donors and recently transplanted patients (79). Indeed, mortality after testing positive for SARS-CoV-2 was 10.2% in patients with solid organ transplants in the UK (79). In kidney transplant recipients, in-hospital mortality was 21%, and rose to 50% if admitted to intensive care (80). Vaccination therefore became a key public health message, with transplant units being encouraged to engage patients in individualised risk-benefit discussions (81). Whilst donor and recipient risk assessment were important prior to 2019, SARS-Cov-2 resulted in further complexities when considering perioperative risk.

The SARS-Cov-2 pandemic also caused significant hurdles in recruiting and carrying out research in transplantation.

1.2 Transplant immunology

The assessment of organ quality is a highly complex process. Much of this complexity arises from the immune processes that take place during donor death, organ retrieval, implantation, and the resulting interactions on a cellular level between donor and recipient tissue. Due to the damage that can be sustained by donor organs during these steps, understanding these processes is therefore a necessary pre-requisite to a more complete appreciation of donor-recipient risk assessment. The amelioration of graft dysfunction also acts at an immune level.

1.2.1 History of transplantation and transplant immunology

Transplantation is the engraftment of human cells from a donor to a recipient with the aim of restoring function (82). This may involve individual cells, tissues, or an entire organ.

Although the first verified case of successful organ transplantation occurred in 1954 between monozygotic twins Ronald and Richard Herrick, historically the concept of transplantation preceded this by two thousand years. Historically, organ transplants had been referred to in numerous sources, the earliest reference to transplantation is that of a lower limb graft performed by two doctors Cosmos and Damien in ancient Arabia, circa 200 AD. However, there is no evidence that the skill necessary for transplantation had been developed at this time. Vascular reconstruction necessary for organ engraftment was developed in the relatively modern era by Alexis Carrel, first published in 1902. The ability to restore blood flow through an organ with vascular anastomoses was considered a significant breakthrough in organ transplantation and resulting in Carrel being awarded the Nobel Prize in Physiology or Medicine in 1912. Although grafts from Carrel's experiments appeared to be initially successful, all grafts were overcome by the recipient's immune response.

Recognition of various immune cells, and their functions, was fundamental in the process of immunosuppression development. The success of the first organ transplant in 1954 was primarily due to the blood group and human leukocyte antigen (HLA) matching of the donor to the recipient (discussed in greater detail in Section 1.2.3). In this case, the donor was genetically identical to the recipient, as he was the monozygotic twin of the recipient. Given that monozygotic twins only occur in 3 of 1000 deliveries, a major challenge in organ transplantation is successful matching of tissue type between organ donor and recipient. Suppression of the recipient immune system is necessary to avoid the sequelae of the tissue mismatch, namely rejection and graft loss. Other immune-mediated processes take place at the time of death, in the case of deceased donors, during organ retrieval and preservation, and in the early transplant period. An understanding of transplant immunology is therefore fundamental to successful transplantation.

1.2.2 Innate and adaptive immune system

The immune system of vertebrates may be broadly divided into two effector systems: the innate and the adaptive (also termed acquired).

The innate immune system provides the first line of defence against potential pathogens, primarily due to its rapid response to stimuli. The innate immune system is inherited and present immediately at birth. Although original thinking was that stimuli from exposure to microorganisms did not result in enhancement or adaptation of the innate immune system, it is now known that myeloid cells may undergo epigenetic changes after exposure to a pathogen that may allow them to respond more effectively if re-stimulated.

The innate immune system protects the host immediately from exposure to a potential pathogen, prior to the generation of the slower adaptive immune response. It does this by directly recognising microbes through its pattern recognition receptors, such as Toll-like receptors (TLRs) (83). Damage-associated molecular patterns (DAMPS) are endogenous molecules that are released from damaged or dying cells, and essentially act as 'danger' signals to the innate immune system. DAMPS may originate from within cells (intracellular) consisting of immunogenic molecules released from the breakdown of necrotic or apoptotic cells. Examples of intracellular DAMPS include calcium-binding protein S-100, high-mobility group box protein 1 (HMGB1), or uric acid. DAMPS may also origionate from the extracellular compartment. Extracellular DAMPS include certain glycoproteins, proteoglycans, and glycosaminoglycans. DAMPS bind to pattern recognition receptors, including TLRs. Activation of TLRs in turn result in inflammatory gene expression in attempt to fight pathogens and mediate tissue repair (84)

In contrast, immune cells from the adaptive immune system acquire structurally unique receptors during their development, enabling multiple populations of cells with differing receptors. Cells exposed to their specific antigen undergo clonal expansion. In certain cell types, notably B cells, receptor affinity also increases for their particular antigen, to enable stronger binding. Populations of cells within the adaptive immune system will therefore change over time, adjusting to differing environments, as a means of 'learning' to respond to relevant pathogens.

Examples of components of the innate immune system include a number of cells, receptors and molecules that are summarised in Table 3.

Table 3 - Components and actions of the innate immune system

Component	***Action***
Physical barriers	*Epithelial, endothelial, and membranous surfaces prevent entry of pathogens*
Pattern recognition receptors	*Inherited receptors stimulating downstream effector pathways e.g., Toll-like receptors*
Phagocytic cells (monocytes, macrophages, and neutrophils)	*Engulf cells expressing antigens that bind to pattern recognition receptors*
Antimicrobial peptides, (e.g., defensins, cathelicidins)	*Aid in the phagocytosis of pathogens*
Cytokines	*Inter-cellular signal proteins that mediate inflammation e.g., interferons, interleukins (ILs) and chemokines*
Cytotoxic enzymes	*Expressed by epithelial or phagocytic cells that destroy microorganism cell membranes e.g., lysozyme*
Complement	*Circulating cell-associated pattern recognition receptor C1q which binds to antibodies that are fixed to microbes (or damaged cells) resulting in activation of a cascade of other complement factors. C3b covers and promotes phagocytosis of the cell (opsonisation)*
Microbiome	*Populations of commensal flora that compete against pathogens for space and nutrients e.g., gut or skin commensals*

In contrast to the innate immune system, the adaptive immune system demonstrates specificity and memory for antigens. During the first exposure, the adaptive immune system provides a relatively slow effector response, due to the time taken for clonal expansion of the relevant cells in response to antigen, as well as the production of antibodies. T and B cells comprise the central components of the adaptive immune response.

Antigen-presenting cells (APCs, discussed in depth in Section 1.2.3) trigger T and B cell responses. Various T cell subtypes exist with distinct functions:

- Cytotoxic T cells (characteristically expressing CD8 cell-surface proteins)
- T helper cells (characteristically expressing CD4 cell-surface proteins)

Naïve T cells are produced and matured within the thymus, later circulating within the blood and lymphatics. T cell activation results in entry into cell cycles as well as expression of cell adhesion proteins to enable effective leukocyte migration to areas of inflammation. Activated T cells roll along vascular and lymphatic endothelium, undergo adhesion to endothelial cells, before stopping and transmigrating across the endothelial barrier to enter inflamed tissue. Activated T cells undergo clonal expansion. B cells produce antibodies, forming a key component of the adaptive immune system.

1.2.3 Organ immunogenicity

The immunogenicity of organs differs, with small bowel, heart and lung requiring relatively greater immunosuppression than the kidney. Liver transplantation requires less immunosuppression to avoid rejection, with episodes of rejection relatively more responsive to treatment compared to the kidney. This thesis focuses exclusively on kidney transplantation in humans. However, there are parallels in biological processes in other organs and species, which will be considered where relevant to this work.

One of the functions of the immune system is to differentiate host cells from non-self. This process is known as allorecognition (considered Section 1.2.4). Once recognised, a multitude of effector mechanisms attempt to destroy foreign pathogens; a process called the alloresponse. Allorecognition occurs primarily through distinguishing non-self cell surface material, known as antigens. Three main groups of antigens are considered clinically relevant in human transplantation: ABO blood groups, major and minor human leukocyte antigens (HLA).

ABO antigens are glycoproteins which are present on the cell surfaces of various tissues, but most importantly erythrocytes and vascular endothelium. Individuals may have one of four phenotypes: A, B,

AB and O. Additionally, individuals produce antibodies to the antigens that are not expressed, causing opsonisation and a subsequent immune response against non-self ABO-expressing cells. Thus, persons expressing A antigens produce anti-B antibodies and *vice versa*. Persons blood group O (expressing only antigen H) produce both anti-A and anti-B antibodies, and persons expressing both A and B antigens do not synthesise anti-A or anti-B antibodies. These ABO antibodies in the serum are formed following exposure to ABO blood group antigens in food and commensal microorganisms. Transplantation of A, B or AB blood group organs into incompatible recipients (where anti-A or anti-B antibodies are present) results in hyperacute rejection (discussed later), unless immunodepletion is undertaken prior to transplantation (55). Organs from blood group O donors may be transplanted into any recipient (once blood containing anti-A and anti-B antibodies has been flushed out of the organ with preservation fluid) given that the tissue itself does not express AB antigens.

HLAs are a set of cell surface antigens that is encoded by genes found on the short arm of chromosome 6 in humans. This area of genetic code is known as the major histocompatibility complex (MHC). The resulting HLAs are significantly more diverse than ABO antigens, with two main classes (HLA classes I and II), six main types (class I: HLA-A, HLA-B, HLA-C; class II: HLA-DP, HLA-DQ and HLA-DR) with thousands of allelic variations (85). For this reason, HLA mismatch remains a significant challenge in organ transplantation due to the extensive polymorphism between individuals and therefore the difficulty in finding an HLA-identical match.

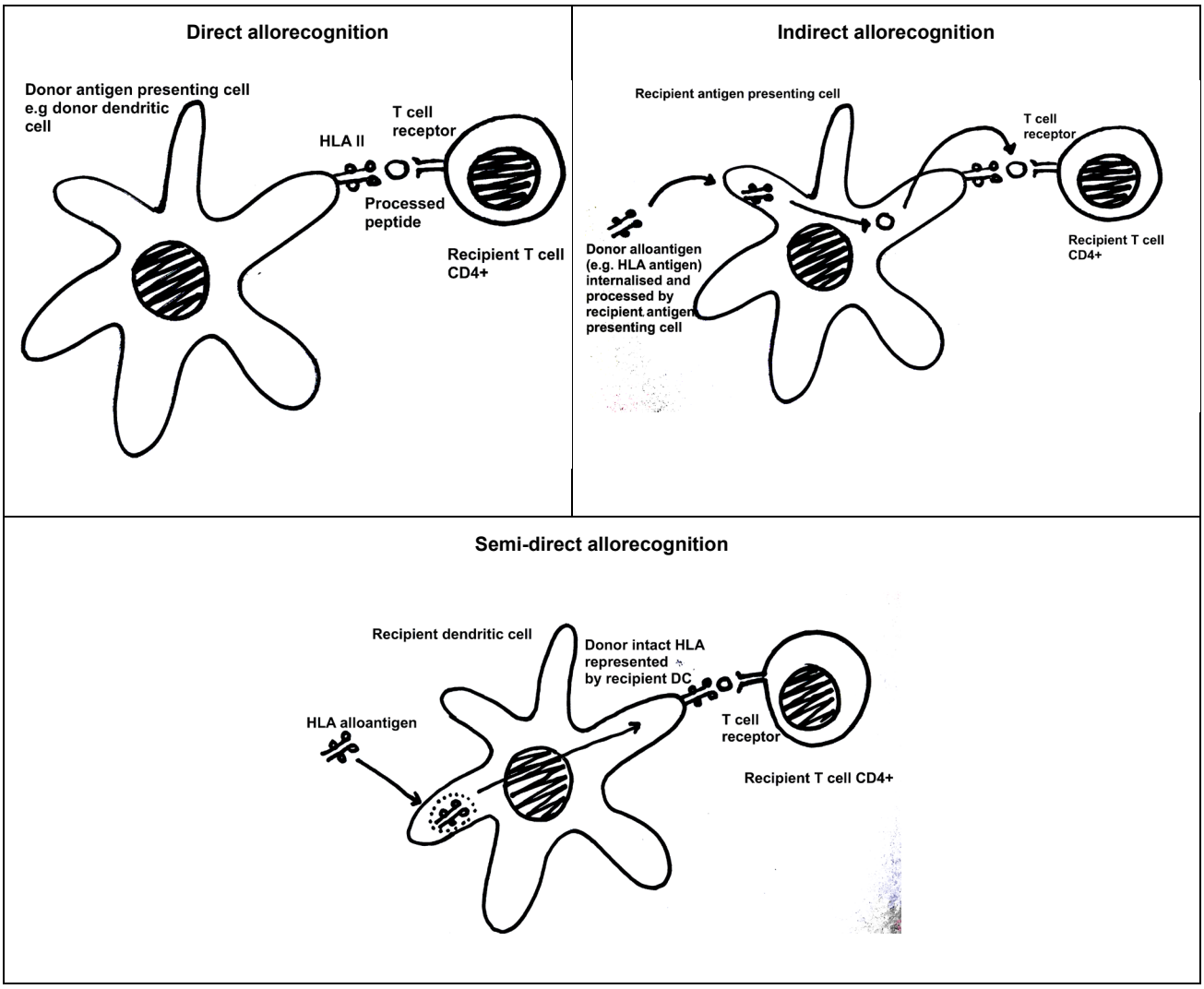
HLA class I antigens are present on almost all human nucleated cells. The function of HLA class I antigens are to present protein fragments found within the cell, such as the peptides from an invading viral particle, in order for cytotoxic T cells to destroy the infected cell. HLA class II are present on 'professional' antigen presenting cells (APCs), meaning that their function is to display extracellular foreign peptides specifically to T helper cells (86). Dendritic cells, macrophages and B cells express HLA class II antigens and are therefore considered professional APCs. T helper cells (characterised by their expression of CD4) act by binding to the complex formed by the HLA class II expressing foreign antigen presented by the APC through the process of allorecognition.

1.2.4 Allorecognition pathways

The immune system is able to detection of genetically encoded polymorphisms between disparate individuals within the same species, in a process termed allorecognition. This allows immune cells to discriminate between self and non-self cells and proteins. In vertebrates, allogeneic material is recognised by T lymphocytes through their antigen receptors. This results in activation of pro-inflammatory allospecific T cells, designed to respond to foreign or invading pathogens. However, in a non-tolerant and immunocompetent recipient, this process leads to rejection of transplanted allogeneic organs and tissue.

There are three main pathways of allorecognition: direct, indirect and semi-direct allorecognition. All three pathways are relevant in clinical transplantation, and are summarised in Figure 5.

Figure 5 - Pathways of allorecognition



Direct allorecognition

Soon after transplantation donor-derived immune cells, from within the graft, pass into the recipient circulation, and into the recipients secondary lymphoid tissue. These donor-derived cells are known as Passenger Leukocytes (PLs). Naïve T lymphocytes within the lymphatics recognise intact allogeneic HLA expressed by PLs, without the need for antigen processing by recipient antigen presenting cells – this is termed direct allorecognition. MHC class I and II alloantigens are recognised as intact proteins on the surface of *donor* APCs, by CD8+ T cells and CD4+, respectively (87). Unlike conventional T cell responses to an antigen, direct allorecognition results in a high frequency of T cell reactivity, with a polyclonal T cell response.

Indirect allorecognition

Lechler and Batchelor demonstrated that transplanted organs that that *lack* PLs may still succumb to rejection (88). This is because the indirect pathway still exists following transplantation.

Indirect allorecognition occurs when an alloantigen is internalised by the recipient APC (e.g. by a dendritic cell), which is process and presented as a peptide fragment, ready for a self-restricted recognition by its own recipient CD4+ T helper cell. Although CD8+ T cells can a,so recognise processed antigens though the indirect pathway, this is not considered clinically relevant in vascularised solid organ transplants.

Semidirect allorecognition

It is has been established that leukocytes can exchange molecules via cell-to-cell contact. Indeed MHC class I and II molecules may be transferred between recipient and donor dentritic cells following organ transplantation (sometimes termed MHC cross-dressing). This provides an opportunity for semi-direct allorecognition, where recipient APCs acquire intact HLA molecules from donor cells and present these to recipient T cells. In the semidirect pathway, HLA alloantigens are re-presented by the recipient's APC – not as a processed allopeptide, but as a conformationally intact protein.

Semi-direct and direct allorecognition are both consequences of PLs having close interaction with the recipient's immune system. The importance of PLs as a target for intervention in the prevention of immune-mediated injury is considered in Chapter 6.

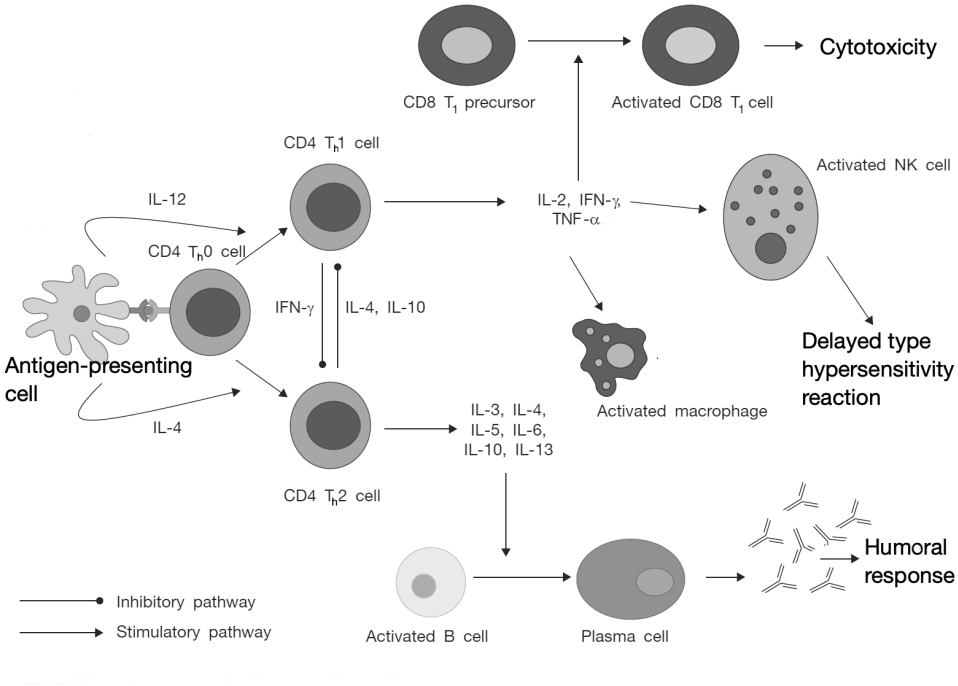
Following direct, semi-direct and indirect allorecognition, T helper cells secrete a number of cell signal peptides, called cytokines, which have stimulatory down-stream effects. T helper cells can be categorised into two sub-types based on their pattern of cytokine release. T helper 1 (T_h1) cells differ from T helper 2 (T_h2) cells in that they predominantly secrete cytokines IL-2, TNF-α, IFN-γ, whilst T_h2 cells predominantly secrete interleukins 3-6, 10 and 13.

There are three main effector pathways in the adaptive alloimmune response that result from cytokine release from T helper cells:

1. Cytotoxic T cells (expressing CD8): activation of cytotoxic T cells by IL-2, TNF-α and IFN-γ results in cell destruction through release of perforins, granzymes and Fas receptor-mediated apoptosis.
2. Delayed type hypersensitivity reaction: TNF-α and IFN-γ produced by T helper cells results in phagocytosis by macrophages.
3. Humoral response: alloantigen-specific B cell activation results in antibody production. This is initially IgM production but with sustained stimulation results in IgG antibody production with a high affinity for the specific antigen. Cells coated in antibodies promote targeted cell death through activation of natural killer (NK) cells and macrophages. Antibodies coating the antigen also activate the complement cascade culminating in production of the C5 membrane attack complex (C5-9) which perforates cell membranes, resulting in cell death.

The process of antigen presentation, T helper binding and release of cytokines and the three main effector pathways that lead to graft rejection and destruction are summarised in Figure 6.

Figure 6 - T helper cell binding to antigen presenting cells, cytokine release and activation of effector pathways



Interleukin (IL); Tumour necrosis factor (TNF); interferon (IFN). Adapted from **Phillips BL** & Callaghan C. The immunology of organ transplantation. Surgery - Oxford International Edition 2020;38(7):353-360.

Tissue resident immune cells have a homeostatic role within the kidney. This is summarised below:

- *Dendritic cells:* Found primarily within the tubulointerstitial compartment of the kidney. Interaction with antigens promotes production of chemokines to attract effector cells such as neutrophils. Dendritic cell maturation state may be regulated by interaction with tubular epithelial cells
- *NK cells:* effector cells of the innate immune system, are primarily found in the tubulointerstitial compartment of the kidney, and kill foreign cells by releasing perforin and granzyme. NK cells also produce pro-inflammatory cytokines.
- *CD4+ cells and CD8+ T cells:* these persist in peripheral tissues, such as the kidney, following infection. CD69 expression may act as a means of distinguishing tissue-resident CD4+ and CD8+ cells from those in circulation.

1.2.5 Human Leukocyte antigens

In clinical transplantation, HLA-A HLA-B and HLA-DR mismatches between donor and recipient are considered to be clinically relevant (89, 90). The presence of mismatch at each HLA loci (0-2) can be conveyed by the HLA mismatch (HLA MM). For example, an HLA MM of 000 means there is no mismatch at each HLA-A HLA-B and HLA-DR loci, although HLA-DQ mismatching has also recently shown to influence transplant outcomes (91). The degree of HLA MM was categorised into four groups by the 2006 UK National Kidney Allocation Scheme (Table 4) (92). A new UK National Kidney Offering Scheme was introduced in 2019 with updated HLA MM categories.

Table 4 - Human Leukocyte Antigen (HLA) mismatch level according to the 2006 UK National Kidney Allocation Scheme

Level	HLA mismatch summary	HLA mismatch combinations included
1	000	000
2	[0 DR and 0/1 B]	100, 010, 110, 200, 210
3	[0 DR and 2 B] or [1 DR and 0/1 B]	020, 120, 220, 001, 101, 201, 011, 111, 211
4	[1 DR and 2 B] or [2 DR]	021, 121, 221, 002, 102, 202, 012, 112, 212, 022, 122, 222

1.2.6 Rejection

Rejection is the immune-mediated destruction of an allograft through the effector pathways outlined in Section 1.2.3 (above) and is classified by tempo of onset and effector mechanism. Hyperacute rejection takes place immediately to several hours post-transplantation, acute rejection occurs weeks to months after transplantation and chronic rejection occurs months to years later. Rejection may also be characterised by the underlying mechanism, namely cell- or antibody-mediated rejection (or both). Kidney transplant rejection is confirmed histologically, following a graft biopsy, and the findings classified by internationally accepted features (Banff classification) (93). The Banff classification is regularly updated in order to provide consistent reporting, for clinical and research purposes. In the days and week following kidney transplantation, acute rejection is only distinguishable from other forms of graft dysfunction histologically. Rejection may be subclinical or could cause a detectible deterioration in graft function. However, most episodes of acute rejection are successfully treated.

1.2.7 Immunosuppression

Prevention of an alloimmune response can be achieved using immunosuppressive medication. Since the action of these drugs is not specific to the transplanted organ, immunosuppression also increases the risk of infection and cancer. Immunosuppression therefore requires a fine balance between avoidance of rejection and minimising the risk of opportunistic infections such as those caused by cytomegalovirus, Epstein Barr virus and *Candida albicans*. Immunosuppression carries the risk of de novo cancers, such as skin cancers (especially non-melanotic cancers), and risk of post-transplant lymphoproliferative disorder.

There are five main types of immunosuppressive medications, with differing modes of action:

- Corticosteroids, such as methylprednisolone and prednisolone, acting in multiple ways such as suppression of cytokine production
- Calcineurin inhibitors, such as tacrolimus and cyclosporin, inhibit interleukin (IL)-2 production and therefore preventing T cell activation. Both tacrolimus and cyclosporin require drug-level monitoring as their absorption, metabolism and excretion varies between patients
- Biological therapies, including monoclonal and polyclonal antibodies
    - Basiliximab acts as an anti-CD25 monoclonal antibody to prevent T cell activation through IL-2 blockade
    - Alemtuzumab acts as an anti-CD52 monoclonal antibody leading to T and B cell depletion
    - Eculizumab acts as a monoclonal antibody against complement component 5 (C5)
    - Antithymocyte globulin (ATG) acts as a polyclonal antibody that causes T cell depletion
- Antimetabolites, such as mycophenolate mofetil (MMF) and azathioprine, act by inhibiting DNA synthesis in order to prevent lymphocyte proliferation

- Mammalian target of rapamycin (mTOR) inhibitors, such as sirolimus and everolimus, act by inhibition of IL-2 to prevent T cell activation

Immunosuppressants can broadly be categorised according to their use. Induction immunosuppression takes place prior to transplantation, typically at the induction of anaesthesia prior to surgery. Commonly used induction protocols include methylprednisolone and a biological agent such as basiliximab or alemtuzumab. Following transplantation, maintenance immunosuppression is undertaken for the life of the graft in most patients. Historically, immunosuppression comprised oral cyclosporine, MMF or azathioprine, and prednisolone, depending on individual patient circumstance and local unit practices. Prednisolone doses are reduced over time and may stop completely in some patients. However, over the last ten years, oral tacrolimus use became increasingly prevalent in Western health systems, and was incorporated into National Institute of Health and Care Excellence (NICE) recommendation in 2017. The target serum tacrolimus differs between transplant units and patients; however, most units base this on immunological risk stratification of the recipient at the time of transplantation. This risk may depend on various factors, such as the presence of anti-HLA antibodies, recipient ethnicity, and whether or not the recipient had received a transplant previously. At our centre, recipients considered high risk may receive alemtuzumab induction instead of basiliximab.

Additional immunosuppressants is used in clinically suspected, or histologically confirmed acute rejection, depending on the type.

1.3 Organ injury during organ retrieval, preservation, and reperfusion

Much of the focus of immune therapy has been on organ rejection. However, there are other important immune-mediated processes that take place within the organ of interest even prior to transplantation. In contrast to rejection, there have been relatively few immunological interventions that

focus on reducing potentially avoidable and reversible organ injury during organ retrieval, preservation, and reperfusion.

1.3.1 Donor acute kidney injury

Deceased donors experience significant pathophysiological changes during death, as well as potentially during the acute episode that resulted in their death. This may make the organs more vulnerable to further episodes of injury during preservation and transplantation. The susceptibility to injury is likely to differ between organs, based on factors such as donor-type, donor age and cause of death.

In DBD donors, the injury caused to organs appears to derive from the inflammatory milieu caused by brain infarction (94). In brain death, ischaemia of the pons results in significant autonomic dysfunction, resulting in circulatory dysfunction with both hypertension and bradycardia (95, 96). Ischaemia of the medulla oblongata causes a catecholamine storm and hypertensive crisis, but with peripheral vasoconstriction (95, 96). These circulatory disturbances can cause organ ischaemia (97). As a result, DBD donors display up-regulation of pro-inflammatory gene expression, such as those coding for cytokines IL-1, IL-6, IL-8 when compared to living donors (98). DBD donors also express higher levels of endothelial adhesion molecules in the kidney, such as E-selectin, aiding in the migration of leukocytes into tissues (98). HLA class I and class II expression also increases, with resulting increased inflammatory cell migration into the kidneys (99). Increased migration of PLs from the circulation into the kidney could result in higher transfer of donor antigens into recipient circulation during reperfusion after transplantation, resulting in enhanced direct allorecognition (see Section 1.2.3). This means that passenger leukocytes include tissue resident cells, present before the inflammatory response, as well as newly arrived leukocytes responding to acute inflammation.

Although DCD donors do not meet the criteria for brain death, they have typically sustained significant brain injury. This state of brain injury also results in disruption to the organ homeostasis (94). However, DCD donor kidneys are exposed to additional periods of warm ischaemia (summarised in

Figure 3). Hypoxia of the kidney during this period results in mitochondrial dysfunction, depletion of adenosine-triphosphate (ATP) and failure of cell volume and electrolyte regulatory mechanisms (100). Increased caspase-3 activity suggests that these processes lead to programmed cell death (101). The resulting injury to the kidney is proportionate to the duration of warm ischaemia time and is associated with early post-transplant graft dysfunction (101, 102).

1.3.2 Static cold storage

To avoid the sequelae of ischaemia following loss an oxygenated blood supply to the organ, the gold standard method of organ preservation is static cold storage, in which the organ is cooled to 4^{o}C (103). Cellular enzymic function is slowed to approximately 12%, in order to reduce consumption of intracellular energy stores (104). The purpose of cold preservation fluids is to cool the organ down, flush out blood and microthrombi and to maintain the osmotic environment to ensure cell viability. This phase, termed cold ischaemia time (CIT), begins once the organ has been perfused with cold preservation fluid and ends once the organ has been removed from cold storage. Cold storage is a simple and inexpensive means of organ preservation during transportation, and organ and patient preparation. However, use of cold preservation solution in the absence of machine perfusion, termed static cold storage (SCS), leads to gradual accumulation of lactic acid resulting in decreasing intracellular pH. Prolonged periods of SCS eventually leads to depletion of ATP stores and failure of ATP-ase dependent anion pumps. In kidney transplantation, increasing cold ischaemia is associated with increased rates of delayed graft function (DGF; broadly referring to poor early function with eventual recovery) and primary non-function (graft never functions). Limiting CIT has therefore proven to be an important goal in clinical transplantation.

1.3.3 Ischaemia reperfusion injury

Ischaemia is the restriction of an oxygenated blood supply to an organ and reperfusion occurs when oxygenation is restored. Ischaemia reperfusion injury (IRI) defines the deleterious sequelae that results from this process. IRI may occur when the original blood supply to the tissue is restored (e.g.,

following limb ischaemia or acute coronary syndrome) but also refers to the scenario in transplantation in which reperfusion occurs in the recipient of an allogenic organ.

The processes that take place in IRI are complex, with several interlaced pathways, involving interactions between the vascular endothelium and elements of the innate immune system such as macrophages, dendritic cells, cytokines, and the complement cascade.

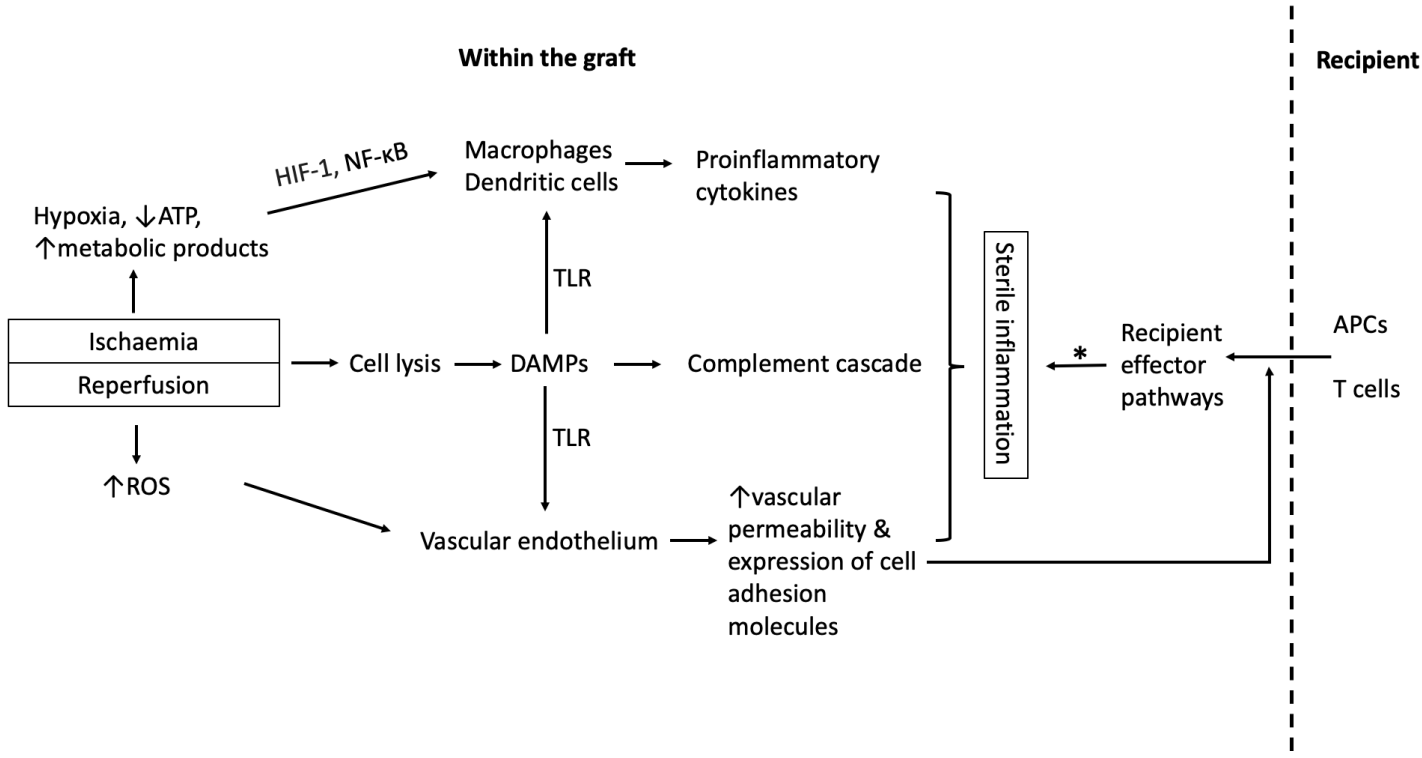
In the first instance, sudden and prolonged interruption of oxygenated blood results in hypoxia, anaerobic respiration, rapid depletion of ATP and the accumulation of metabolic products. Low concentration of cellular oxygen is a triggering factor for the activation of transcriptional regulators, such as hypoxia-inducible factor-1 (HIF-1) and nuclear factor-κB (NF-κB) (105). These signalling molecules promote acute inflammation within the graft by increasing neutrophil adhesion and endothelial permeability. Ischaemic tissue becomes the site of a local immune response. Reperfusion leads to a sudden increase in oxygen concentration, resulting in the development of reactive oxygen species (ROS). Local production of ROS results in oxidation of proteins, lipids, and nucleic acids of the vascular endothelial cells, causing further damage (106). The inflammatory response in this setting is often termed 'sterile inflammation', as it generally occurs in the absence of a pathogenic organism. Cell lysis that occurs as a result of these injuries releases various molecules that further stimulate the graft's innate immune system. DAMPS (discussed in Section 1.2.2) are an important group of such substances and are present in the transplant recipient circulation within three hours of transplantation (107). The concentration of DAMPS has been shown to directly correlate to the duration of cold ischaemia time (108). DAMPS can act directly on the graft's endothelial cells and immune cells to induce production of pro-inflammatory cytokines and chemokines. DAMPS promote inflammation by binding to pattern recognition receptors, such as TLRs (107). Kidney transplant recipients with non-functional TLR-4 have subsequent lower levels of proinflammatory cytokines and improved early transplant function (107). High mobility group box 1 is one of the most prominent intracellular DAMPs and is significantly upregulated in deceased donor kidneys (107).

The activation of complement is also fundamental in the orchestration of IRI (109). In this context, TLRs also promote activation of the complement cascade. Evidence suggests that complement activation is predominantly donor kidney-derived (110).

The heightened inflammatory milieu that results from these inter-dependent pathways within the transplant, along with increased vascular permeability, provides an opportunity for the migration of recipient immune cells into the graft, as well as leakage of donor cells into the recipient circulation. Whilst it has been well established that early phases of IRI are driven primarily by the innate immune system, evidence suggests that the adaptive immune system plays a role in IRI (111, 112). Leukocytes in general, and PLs specifically, have been widely implicated in IRI. T and B lymphocyte-deficient animals have demonstrated markedly reduced IRI responses (112). Lung transplant models have also shown that IRI is predominantly mediated by the donor's PLs, rather than the recipient's leukocytes (113).

Given the potential interaction between donor and recipient immune cells within a pro-inflammatory environment, direct and indirect allorecognition is therefore abundantly possible, meaning that IRI can lead to further injury, and possibly graft loss, through rejection. IRI has been shown to increase organ immunogenicity through the activation of antigen presenting cells (114) and promotion of alloantibody production (115), thus increasing the likelihood of acute rejection. The processes of IRI discussed are summarised in Figure 7. Limiting IRI is therefore a potential target for intervention in the amelioration of early graft function. Reducing IRI may also have the added benefit of lowering the risk of organ rejection. The use of machine perfusion technology in transplantation is introduced in Section 1.5.3, and its potential use in reducing IRI are explored in Chapter 6.

Figure 7 - Ischaemia reperfusion injury in transplantation, with opportunities for allorecognition and organ rejection



* Opportunities for allorecognition and organ rejection

ATP – Adenosine triphosphate, ROS – reactive oxygen species, HIF – hypoxia inducible factor, NF-κB - nuclear factor-κB, DAMPs – damage-associated molecular patterns, TLR – Toll-like receptors, APCs – antigen-presenting cells

1.3.4 Post-reperfusion syndrome

Graft reperfusion is a critical point during organ transplantation as it may be associated with recipient intraoperative cardiovascular instability, termed "post-reperfusion syndrome" (PRS). This is most commonly seen in orthotopic liver transplantation (OLT), reportedly occurring in 8-58% of OLT (116, 117). PRS was first described in OLT, with severe cardiovascular dysfunction following reperfusion of a newly transplanted liver, characterised by a rapid decrease in systemic mean arterial pressure (MAP), as well as bradycardia, reduced systemic vascular resistance and increased pulmonary filling pressures (118). PRS therefore poses a significant challenge to anaesthetists, intensivists, and transplant surgeons. In OLT, PRS is associated with graft injury and PNF (119, 120), increased transfusion

requirements, longer mechanical ventilation, prolonged intensive care stay (121) and early post-operative death (119).

In OLT, PRS is defined as a >30% fall in MAP from the pre-reperfusion to within five minutes of reperfusion of the graft, lasting for at least one minute (120). PRS also demonstrates an augmentation of the recipient's immune system, with a rise in activated inflammatory cells in circulation (122).

PRS following kidney transplantation has also been demonstrated in smaller observational studies (123, 124). In a case-control study of 150 consecutive kidneys, Bruhl *et al* defined PRS as a reduction in mean arterial pressure of at least 15% within five minutes of kidney reperfusion within the recipient, lasting a least one minute (123). Using these parameters, Bruhl *et al* demonstrated a PRS incidence of 4% (n=6) within their study. This was associated with expanded criteria donor kidneys, recipient age >60 years and recipient diabetes mellitus on univariate analysis. The increased incidence of PRS in OLT compared to kidney transplantation is likely to be explained by the differences in blood loss, portal hypertension and hyperdynamic circulation associated with advanced cirrhosis (125).

In deceased donor kidney transplantation, PRS was associated with increased hospital length of stay (11 vs 5 days, $p<0.0001$) and increased rate of graft failure within 6 months compared to those without (16% vs 6%, $p=0.01$).

The pathogenesis of post-reperfusion syndrome is complex and is mediated through a variety of immune pathways, including overlap with IRI, and interaction with the circulatory system. Following organ reperfusion, there is a sudden bolus of cold, acidic and potassium-rich fluid exiting the graft into systemic circulation (126). This results in ventriculo-arterial decoupling, a phenomenon in which the heart and arterial system temporarily lose their coordinated efforts to propel blood forward through circulation (127). University of Wisconsin preservation fluid is particularly rich in potassium (120mmol/L), and evidence suggests that flushing the organ with cold Ringer's lactate or human albumin solution via the inferior vena cava may improve recipient haemodynamics (128).

Donor-derived immune cells may also suddenly exit the graft, and an inflammatory response ensues in which there is a sudden rise in cytokines (129) and complement proteins (130). Within the

graft, IRI ensues, in which there is significant endothelial dysfunction resulting in vasodilatation (discussed in detail in Section 1.3.3 – Ischaemia-reperfusion injury). Ventriculo-arterial decoupling and sudden vasodilation results in a fall in systemic blood pressure. There are a number of deleterious sequelae of intra-operative hypotension including acute kidney injury (131), the need for intensive care and prolonged hospital admission (121). However, it should be noted that the study of PRS pathophysiology has been mainly in the context of OLT, and there may be differences between organ type that need to be better defined if strategies and interventions to reduce PRS are to be considered.

Currently the mainstay of preventing and treating PRS is the optimisation of intravascular volume, the correction of electrolyte and metabolic derangement and the use of inotropes and vasopressors (132). However, the use of machine perfusion technology in transplantation is introduced in Section 1.5.3, and its potential use in preventing PRS are explored in Chapter 5.

1.4 Transplant outcomes

"If you do not measure something, you cannot change it" – Peter Drucker, writer of business and management philosophy

Measuring clinically relevant outcomes is critical in improving the delivery of care (133). Interventions in transplantation not only include novel therapies, but also evolving policies related to organ retrieval, allocation, and implantation at a national and international level. Measuring the impact of these interventions is necessary to inform the transplant community as to their efficacy.

Outcomes should ideally be relevant to the patients themselves and can often be discordant to the physician-centred outcomes that have been traditionally used (134). The Medical Outcomes Study Short Form (SF-36) is the most commonly used and most relevant quality of life score used in patients with ESRD (135).

The timeframe in which outcome measures can be expected varies. In most cases, transplant patients are expected to live for several decades post-transplantation, meaning that desired outcomes, such as patient and graft survival, are censored (a statistical condition in which a value of observation is only partially known) for an indeterminate period of time. Predictors of long-term outcomes, for example early outcome measures with longer-term implications, are necessary to determine the likely long-term impact of an intervention.

Statistical methods, such as regression modelling, estimate the relationship between one or more independent variables (termed predictors or covariates) and a dependent variable (known as the outcome). Linear regression, for example, estimates the line of best fit, with the fewest sum of squared distances between the true data, and the line. This allows the investigator to estimate the conditional expectation of an outcome. Regression modelling is therefore widely used for prediction and forecasting. However, the term 'predict' can be misleading if it is interpreted as the ability to predict beyond the limits of the data.

Beyond patient and graft outcome metrics, there are also important system metrics that determine how well a service is performing. Organ decline and discard rates may not be directly relevant to an individual patient but has an important bearing on the function and effectiveness of a national transplant programme.

1.4.1 Primary non-function

Primary non-function (PNF) describes a state in which a transplanted organ fails to ever adequately function from the time of implantation, irrespective of cause. Patients requiring dialysis immediately post-transplantation may either have PNF or DGF. Therefore, grafts that fail to provide renal function three months post-transplant are usually considered to have PNF.

PNF is considered a serious and poor outcome, as the patient has been exposed to all the risks of major surgery and induction immunosuppression, without any of the benefits of a working allograft. Recipients of kidneys with PNF are approximately 12 times more likely to die within the first year of

transplantation compared to recipients with grafts that survived more than 30 days (136). PNF is indistinguishable to DGF in the first three months, because clinicians are unable to determine whether function will eventually begin. Patients with PNF also undergo invasive investigation such as transplant biopsy. Furthermore, transplantation is a sensitising event, meaning patients are likely to develop anti-HLA antibodies, leading to subsequent increased chances of positive crossmatch against future potential donors with increased chances of rejection following transplantation (137).

The rate of PNF varies according to donor type. The rate of PNF is approximately 1.9% in living donor transplantation, 3% in DBD donor kidney transplantation, and 4% in DCD donor kidney transplantation (138, 139), however these rates vary internationally (138, 140, 141). Early graft loss in the US is possibly as high as 5.5% (142). This may vary because of differences in induction immunosuppression.

Retransplantation has been shown to increase the risk of PNF in DCD donor kidneys, but not in DBD donor kidneys (supplementary data in reference (138)). Other potential risk factors for PNF include donor age, and prolonged cold and warm ischaemic times (140). Donor acute kidney injury was considered a concern for PNF according to a UK analysis, with AKI stage 3 (creatinine rise of >354μmol/L or three-fold increase from baseline) associated with a statistically higher rate of PNF (143). However, a large propensity score-matched cohort study from the USA indicates that AKI status does not predict PNF rates (144).

1.4.2 Delayed graft function

In kidney transplantation, DGF describes a state of temporary graft dysfunction in the early post-transplant period. Various definitions of DGF exist (145) and are summarised in Table 5.

Table 5 - Variations in the definition of delayed graft function in the literature

Definitions
Dialysis-based definitions of delayed graft function
• The requirement for dialysis in the first post-operative week
• The requirement of dialysis in the first post-operative week excluding the first 24 hours
• The requirement for two or more episodes of dialysis in the first post-operative week
• The requirement for dialysis in the first 10 days post operatively
Serum creatinine-based definitions delayed graft function
• No fall in serum creatinine by ≥10% on three consecutive days in the first post-operative week
• Serum creatinine >220umol/L on seventh post-operative day
• Serum creatinine >220umol/L on tenth post-operative day
• Fall in creatinine by ≤ 30% between the first and second post-operative day
Hybrid definitions delayed graft function
• Dialysis in the first post-operative week or no fall in serum creatinine in first post-operative 24 hours
• Dialysis in the first week or serum creatinine >220umol/L on seventh post-operative day

Adapted from Mallon DH et al Defining delayed graft function after renal transplantation: simplest is best. Transplantation 2013;96(10):885-889.

However, DGF is most commonly defined as the need for dialysis within the first seven days of transplantation, regardless of cause (145). This definition has been adopted by the UK transplant registry. For early graft dysfunction to be classified as DGF, dialysis must therefore have commenced within the first week of transplantation, with subsequent recovery of graft function with dialysis-independence. This distinguishes DGF from PNF, in which the patient requires dialysis within the first week of

transplantation and continues to require dialysis long-term (although there is no national definition, the requirement of dialysis after three months is a locally adopted cut-off to suggest PNF is likely).

DGF is usually a clinical manifestation of IRI during kidney transplantation (146). However, DGF may also be caused by any process impairing immediate function on the graft, such as acute rejection. DGF is often used as an early and clinically relevant outcome measure following transplantation. Without immediate graft function, patients with DGF require in-patient dialysis, have increased length and cost of hospital stay (147), increased risk of acute rejection (148, 149) and may be subjected to invasive investigations such as transplant biopsy (150). The presence of DGF is also associated longer-term outcomes, as it doubles the risk of graft loss in living donor kidney transplantation (151) and DBD donor kidney transplantation (6, 67, 152). However, its relevance in DCD donor kidney transplantation has been questioned. Although the risk of DGF in kidneys from DCD donors is approximately 49%; twice as common as in DBD donor kidneys (68, 153), previous UK registry analyses found that DGF had no long-lasting influence on outcomes in first-time recipients following DCD donor kidney transplantation(6). US data has also suggested that DGF in DCD donor kidneys did not lead to inferior graft survival (154). However, these studies were performed when DCD donor kidney transplantation was in its infancy relative to living-donor and DBD kidney transplantation and may be underpowered and represent relatively lower risk donors in other aspects (such as donor age). It is therefore unclear whether DGF impacts longer-term graft outcomes in DCD donor kidney transplantation.

1.4.3 Monitoring graft functionality

Graft function can be measured over time, either as a binary outcome (functional versus failed) or as a spectrum of function. Graft function is often described as a binary outcome in the context of clinical research within survival and regression analyses. In reality it is more complex because there is variation between nephrologists when to re-initiate dialysis. As a spectrum of function, estimated GFR is generally considered the best way to monitor long-term kidney transplant function in a clinical setting (155), and can be done so using a creatinine-based equation, such as the 4-variable MDRD (see Equation 1).

Although serum cystatin C demonstrates greater correlation with measured GFR than serum creatinine, it is currently not widely used (156). The use of the MDRD eGFR in monitoring transplant function is distinct from the original population of patients with un-transplanted chronic kidney disease in which it was developed (157). As a result, the MDRD eGFR is not as accurate in kidney transplant recipients as it is in non-transplanted CKD patients, but demonstrates greater accuracy compared to other creatinine-based eGFR equations such as the Cockcroft-Golt and Nankivell equations (27).

Evidence suggests that eGFR at one-year post-transplantation is a predictor of longer-term graft outcomes. A UNOS registry analysis involving 100,000 deceased donor kidney transplants found that eGFR at one year was strongly associated with graft survival (158). In another UNOS registry analysis, eGFR at five years demonstrated a strong correlation with the eGFR at one year (r=0.94), and eGFR at one year was the strongest predictor of five-year eGFR (159). Donor type, recipient age, DGF and acute rejection status within year 1 were no longer statistically significant predictors of five-year GFR when one-year GFR was included in the regression model (159). However, the difficulty of using eGFR as an outcome measure in transplantation is that this cannot be reliably measured in patients who return to dialysis. Since dialysis removes serum creatinine from circulation, eGFR increases temporarily. There are two commonly used methods of dealing with this issue, neither of which is satisfactory. Firstly, patients with graft failure may be excluded from the eGFR analysis. This over-estimates the remaining patients' eGFR and means that the sample size decreases over time as more grafts fail. Secondly, one may assign an eGFR to all patients that have had graft loss. As eGFR cannot be accurately measured in patients with failed grafts, this is an estimate. However, there is no agreed eGFR figure to use with a range of filtration rates of 5-15mL/min/1.73m^2 described (160).

1.4.4 Graft and patient survival

Graft survival refers to the duration of dialysis-independence with a functioning transplant. Graft survival is considered a patient-orientated outcome as the period of dialysis-independence is considered

of high importance to patients (134). All cause graft survival is the duration from transplantation to return to dialysis or retransplantation or death, whichever occurs first. Death-censored graft survival (DCGS) is defined as the number of days from transplantation to the date of graft failure (defined as the return to long-term dialysis or retransplantation, whichever happened first) censoring for patient death. Patient survival is defined as the number of days from transplantation to patient death. The most common statistical methods of demonstrating graft and patient survival are with Kaplan-Meier curves, with the log-rank test to allow comparison between different groups (univariate) or with Cox proportional hazards regression models (multivariable).

1.4.5 Organ decline and discard

Organ utilisation is defined as the action of making practical and effective use of organs from potential deceased donors (161). Maximising the potential for organ donation and transplantation has become increasingly important because mortality in patients remaining on the waiting list has been clearly demonstrated (37-39). Deceased donor kidneys are a finite and precious resource, that have been shown to significantly improve the QOL in patients living with ESRD. Organ utilisation is a key concept in this thesis, as accurate donor assessment, with mitigation of risk, is needed to avoid unnecessary organ decline and discard.

Organ decline is when a transplant centre chooses not to accept the offer of an organ (pre- or post-retrieval), either for a named-patient offer, or for any of the other potential recipients at their centre. Organs which are declined by a centre may be accepted and implanted at another accepting centre. Organ discard pertains to decline of a retrieved organ by all UK transplant centres, and the organ is either disposed of or is used for research purposes. Organ decline and discard rates are not patient or graft-related outcomes. However, organ decline/discard are important system outcome measures, given the discrepancy in organ supply and demand. Organ decline/discard rates are a measure of the effectiveness of many processes in organ transplantation, namely organ retrieval, preservation, allocation, and decision-making.

The process of considering an organ offer is a complex, typically involving a discussion between transplant surgeon, the patient's local nephrologist and the patient themselves. The organ offer is considered in the context of what is most appropriate for the recipient. Whilst one offer may be declined for one particular patient, the same offer may still be acceptable for another patient. For example, younger and less co-morbid patients may be aiming for organs with the longest estimated graft survival. Older, comorbid, highly sensitised or surgically complex patients may have lower expectations, such as a dialysis-independent period, without necessarily an expectation of improved patient survival. The new UK Organ Offering Scheme 2019, introduced in September 2019, was developed to improve the process of matching graft life expectancy with patient life expectancy (162). It attempts to achieve this by quantifying both donor and recipient risk (See Section 1.5.1 Donor risk indices) so that each donor quartile (D1-D4) may be offered first to equivalent recipients (R1-R4).

Strategies used to increase the pool of donated kidneys has led to the utilisation of donors with advanced age and comorbidities, as discussed in Section 1.1. Although this has increased transplantation rates, there is also increased perceived variability in organ quality. With limited techniques to assess organ quality prior to transplantation (163), donor kidney decline and discard rates have increased over time. There is also a concern that methods of assessing organ quality, such as donor kidney biopsy, may inadvertently increase organ discard (164, 165).

In the UK, deceased donor kidney discard rates increased, from 5% in 2002-2003 to 12% in 2011-2012 (7). In 2012, the UK Kidney Fast-Tract Scheme (KFTS) was introduced to rapidly offer kidneys at risk of discard to participating centres (166). However, since the introduction of the KFTS, it is estimated that one in five kidneys are discarded unnecessarily (i.e. unnecessary organ discard rate is estimated at 20%) (166). In the US, kidney discard rates appear to be even higher (43, 165). Although direct comparisons are difficult, overall, the utilisation rate of recovered kidneys in the US is 81%, compared to 90% in the UK (43).

Discard rates are particularly high in the US in kidneys from ECDs, reaching 41% (164). Differences in utilisation rates between the UK and US may be explained by differences in risk appetite,

but also by variations in retrieval and allocation policies, reliance on donor kidney biopsy as well as geographical differences (43).

Organ decline can be broadly categorised as donor-, organ- or recipient-related factors, or logistical. In the UK, donor-related causes of kidney decline included unsuitable virology (26%), past medical history (20%) and age (11%) (7). Other donor-related reasons accounting for <10% each are poor perfusion, long cold ischaemia time and organ damage. In the US, where there is greater reliance on donor kidney biopsy appearances for quality assessment, this is cited as one of the main reasons for organ discard (164, 165).

However, organ decline/discard rates alone are not necessarily a useful metric, but rather *appropriate* decline/discard. The latter is much harder to measure. Studies examining discard after organ retrieval have been conducted to determine whether the decision not to implant was appropriate. One UK study examined 20 consecutive discarded deceased donor kidneys by a panel of surgeons to determine whether discard was appropriate in each case. Twenty percent of discarded kidneys were thought to be usable, meaning they could feasibly have been implanted into an acceptable recipient. However, it is even more challenging determining whether declining a kidney was appropriate if it was declined before organ retrieval, due to the limited data available. Data is usually limited to studies in which at least one organ (e.g., heart, lung, liver, single kidney) was retrieved and implanted. One study found that no kidneys were retrieved from 8% of liver donors and 3% of heart and/or lung donors. Declined deceased donor kidneys were generally from older, obese donors with comorbidities such as hypertension and diabetes (167).

Maximising utilisation of DCD and older donors has been identified as a key challenge set out by NHSBT, as is improving consistency in decision-making between clinicians and units (see 'Taking Organ Utilisation to 2020') (161). Also recently published by NHSBT was a national strategy aimed at meeting the needs over the next ten years, where it was agreed that new perfusion technologies and techniques were necessary to achieve higher levels of organ utilisation.

1.5 Predicting and ameliorating graft function

Approximately, 6% of deceased donor kidney transplants no longer function after one year (168). By five-years post-transplant 13% of deceased donor kidney transplants have failed, and 13% of patients have died (168). Identifying risk factors for graft loss is fundamentally important in our endeavour to reduce graft loss and identify appropriate organs for transplantation and their recipients. Despite the importance of organ assessment, there are no national or international guidelines available to clinicians that comprehensively cover deceased donor organ assessment. This is primarily due to the paucity of high-level evidence in the field of deceased-donor organ assessment.

Improved knowledge about factors affecting short- and long-term transplant outcomes expand the possibilities for optimal organ utilisation and the avoidance of unnecessary organ discard. A small number of tools have been devised to aid transplant physicians and surgeons during the decision-making process of organ acceptance and implantation, especially for kidney offers from donors that may be suboptimal. However, the evidence supporting these tools is often questionable or has originated in populations distinct from the target group of patients to which it is applied.

The ability to predict poor graft outcomes helps clinicians in at least two ways. Firstly, it enables clinicians to longevity match kidneys with recipients, whereby the highest quality organs are offered to patients expected to live the longest and the lower quality organs are offered to patients with shorter predicted survival. This essentially optimises organ use. Secondly, it allows clinicians to identify specific groups of donor kidneys or recipients that would benefit from a specific intervention aimed at improving graft function. Improving graft function is intended to cross two important early thresholds: from untransplantable to transplantable, and from delayed graft function to immediate function.

1.5.1 Donor risk indices

In order to broadly estimate donor risk at the time of organ offering or transplantation, donors can be categorised into standard criteria donors (SCD) or ECDs based on their age, comorbidities and serum creatinine (59), as described in Section 1.1.3. The relative risk of graft loss in ECDs is >1.7 when

compared to kidneys from donors aged 10-39 years, with normal serum creatinine, no history of hypertension and death from causes other than stroke. This approach essentially divides donors into low and high risk. This provides a basic framework for discussing and documenting risk categories. It may also help to focus organ offers to recipients that are most likely to accept the organ, to avoid prolonged organ storage during offering (169). However, the SCD/ECD generalisation has been criticised for simplifying a complex problem. The relative risk of graft loss within the ECD category is wide (ranging from 1.74 to 2.69).

Considering the relative risk of graft failure as a spectrum led to the development of more granular donor risk indices. The most commonly used donor risk indices are summarised in

Table 6. The Kidney Donor Risk Index (KDRI) was developed from UNOS national data between 1995-2005 using approximately 70,000 first-time deceased donor kidney-only transplants (169). The KDRI uses 14 donor and transplant factors that were found to be independent predictors of graft loss or patient death, relative to a healthy 40-year-old donor.

Since the KDRI was developed and validated using a US population, the UK Kidney Donor Risk Index (UKKDRI) was developed using a locally representative population of 4570 donors and validated against 3050 donors (5). The UKKDRI uses five donor characteristics found to be independently predictive of graft survival over a three-year period, namely donor age and weight, history of hypertension, days in hospital and the use of adrenaline or epinephrine support. The KDRI and UKKDRI are only moderately predictive of graft failure when validated against their indexed populations (C-statistic 0.63 and 0.62 respectively). The predictive value of these indices is similar in non-UK/US populations (170). Never-the-less, an adapted version of the UKKDRI forms part of the new 2019 UK deceased donor National Kidney Offering Scheme (162). However, it is important that the original UKKDRI tool was developed based on data from donors between 2000-2007, when considering its applicability to donors more recently (5).

Table 6 - Commonly used donor risk indices in deceased donor kidney transplantation

Donor risk index	Donor variables considered
Original 2012 UK Donor Risk Index (UKKDRI) (5)	Donor age, history of hypertension, donor weight, days in hospital, use of adrenaline
UK Kidney offering scheme 2019, modified UKKDRI (162)	Donor age, donor height, history of hypertension, donor sex, donor cytomegalovirus (CMV) seropositivity, donor eGFR, days in hospital
Kidney Donor Risk Index (KDRI) (169) *	Donor age, height, weight, ethnicity, history of hypertension, history of diabetes, cause of death, serum creatinine, hepatitis C status, donation after circulatory death (DCD) donor status
Kidney Donor Profile Index (KDPI) (171)	Cumulative percentage scale of the KDRI (above)

* Two versions of the KDRI exist: the original non-normalised KDRI used a single reference donor. Subsequently, the reference donor figure was chosen to be the median of the data to produce a normalised version of the KDRI.

1.5.2 Donor kidney histology

A pre-implantation kidney biopsy (PIKB) of deceased donor kidneys can be taken in order to estimate the quality of the organ, in an attempt to aid utilisation decisions. Pre-existing chronic donor renal disease may result in altered appearances of the glomeruli as a result of scarring. Glomerulosclerosis is the presence of glomerular scaring and was one of the first histological markers of donor kidney quality. Glomerulosclerosis is thought to signify nephron loss, and is thought to be a surrogate marker for advancing donor age (74). Other components of the renal parenchyma, namely the

interstitium, tubules and blood vessels have subsequently been considered relevant, resulting in the formulation of various chronic histological scores; the most commonly used are summarised in Table 7.

Table 7 - Summary of the main pre-implantation kidney biopsy scoring systems

Pre-implantation kidney biopsy scoring system	Explanation
Karpinski score (75)	Score 0-3 based on severity of chronic glomerular, interstitial, tubular, and vascular appearances, for a total score of 12. The most severe lesion is used to provide the vascular score. More than 20 glomeruli needed for adequacy
Remuzzi (or Pirani) score (70, 76)	Score 0-3 based on severity of glomerular, interstitial, tubular, and vascular appearances for a total score of 12. If vascular changes are focal, the most severe lesion is used. More than 25 glomeruli needed for adequacy. An adaptation of the Remuzzi score also exists for the purpose of the PITHIA trial (see (172))
Maryland Aggregate Pathology Index (173)	Scores determined by periglomerular fibrosis, arteriolar hyalinosis, glomerulosclerosis and artery thickness
Banff chronic interstitial fibrosis (CIF) score (174)	Score based on percentage of chronic interstitial fibrosis with<6% cortical area scoring 0, 6–25% scoring 1 (mild), 26–50% scoring 2 (moderate) and >50% scoring 3 (severe)
Histologic fibrosis score (175)	Score based on the presence of cortical fibrosis on at least four microscopic fields at 100x magnification: no evidence of fibrosis scoring 0, one field of fibrosis scoring 1, less than half of fields with fibrosis scoring 2 and more than half of the fields demonstrating fibrosis scoring 4

Remuzzi and Karpinski scores are very similar, both providing scores 0-3 for appearances in the glomerular, interstitial, tubular, and vascular components of the biopsy. The only difference between Remuzzi and Karpinski scores are how they define vasculopathy and the number of glomeruli needed for adequacy (76, 176).

PIKBs may be obtained through a number of biopsy techniques. These include wedge, core, and punch biopsy, which each have their own advantages and disadvantages. Wedge biopsies are performed

by cutting a triangular-shaped specimen using a scalpel blade. Core biopsies are performed using a biopsy core needle ranging from 14-16 gauge in diameter and 100-160mm deep. Punch biopsies are performed using a circular blade to cut a cylindrical specimen ranging from 4-6mm in diameter and 5mm deep. The shape of each biopsy affects tissue representation. For instance, wedge biopsy may over represent superficial tissue and under-represent deeper tissue because of its shape. Core biopsies may better represent vascular tissue which are more substantial in size deeper in the kidney. Biopsies of all kinds carry with them a risk of bleeding.

PIKB staining and preparation techniques may also vary. Donor kidney biopsy may be examined as a frozen section or may be immediately fixed in formalin. Frozen section biopsies are challenging as they need to be reported shortly after procurement and evaluated by an on-call pathologist, potentially outside working hours and not necessarily by a renal histopathologist (177). Frozen sections have also shown poor reliability for the assessment of mesangial cellularity, capillary wall thickness, the presence of small thrombi and acute tubular necrosis (178). There are a number of staining techniques for examining donor kidney biopsies and these are considered in Section 2.3.3, Table 11.

The predictive ability of PIKB in the assessment of older, deceased donors has been a focus of interest in the last 20 years. In a landmark study, Remuzzi et al compared the outcomes of 62 kidneys transplanted after PIKB assessment from donors aged ≥60 years versus a matched comparator group of kidneys transplanted without histological assessment (70). Recipients of kidneys from donors aged ≥60 years that underwent PIKB demonstrated superior graft survival relative to single grafts without histological assessment. PIKB was also found to be an independent predictor of graft survival on multivariable analysis. However, since then there have been a number of other studies examining the association between Remuzzi/Karpinski scores and graft survival, with conflicting findings.

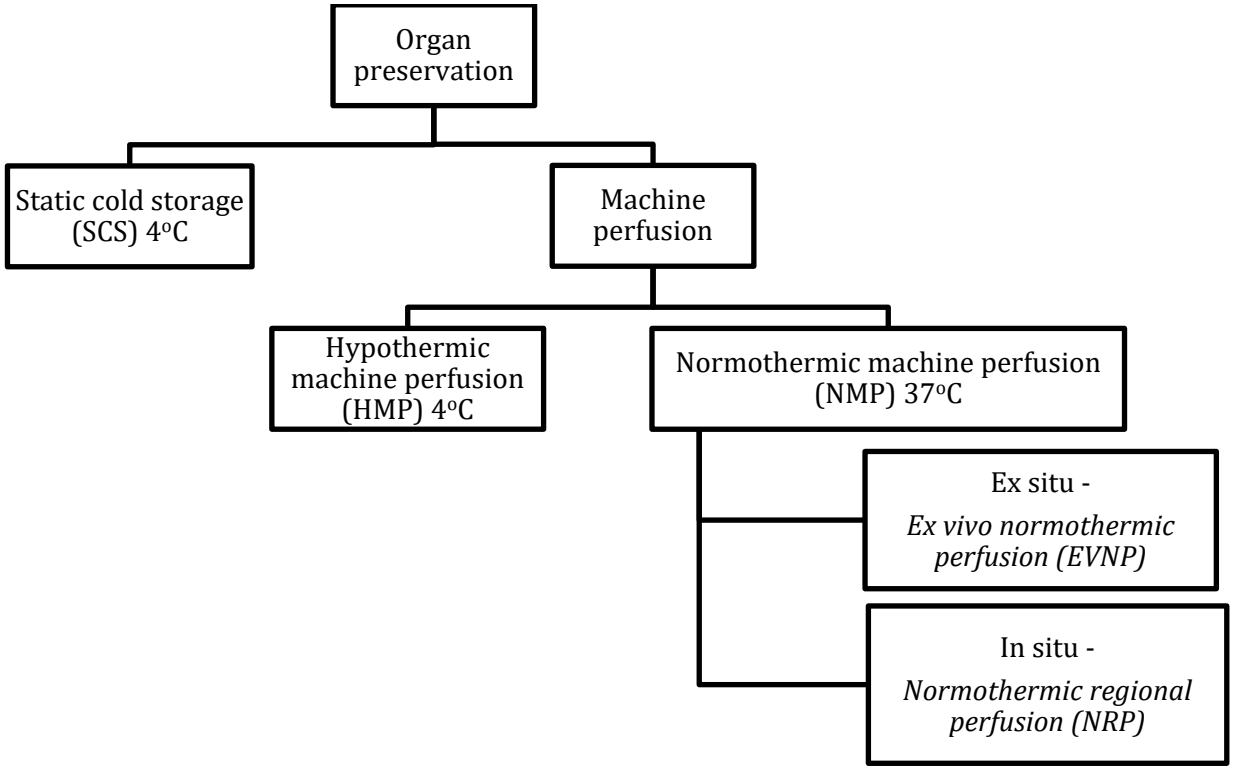
A systematic review of the literature found 47 studies examining the utility of PIKBs between 1994-2014 (179). This found that glomerulosclerosis was not associated with graft survival in seven of fourteen studies. PIKB was associated with graft failure in four out of eight studies. A large UK single-centre study specifically examining Remuzzi scores in a contemporaneous cohort of donor kidneys found

that kidneys with Remuzzi scores of ≥5 were associated with inferior graft survival (73% at one year) with high rates of primary non-function (12.5%) (180). However, it is yet to be determined whether systematic use of PIKB optimises organ utilisation (172), and is therefore considered as a focus of interest in this thesis.

1.5.3 Machine perfusion

The period of cold organ preservation (introduced in Section 1.3.2) also provides a possible opportunity for organ assessment. Static cold storage provides a number of limited means of assessing organ quality, most notably the surgeon's own subjective appraisal of the kidney (e.g., adequacy of cold flush, organ damage, organ size, presence of concerning lesions, vascular quality). More sophisticated means of organ preservation have allowed the development of objective measures of organ quality and function to be investigated and for the potential to actively improve organ quality. Whilst SCS involves placing the organ in fluid that is not circulated through the organ (discussed in Section 1.3.2), machine perfusion allows preservation fluid to be actively circulated through the organ at a chosen temperature, either within the donor at the time of retrieval, or following retrieval (Figure 8).

Figure 8 - Nomenclature of organ preservation method



In situ refers to perfusion of organs within the donor, ex situ refers to perfusion of the organ in isolation following retrieval. In addition to hypothermic and normothermic perfusion, there also includes subnormothermic perfusion, with a target temperature of 32°C

Hypothermic machine perfusion

Hypothermic machine perfusion (HMP) allows cold preservation fluid to be circulated through the organ at a chosen pressure. Various devices can provide continuous or pulsatile, oxygenated or non-oxygenated cold preservation fluid flow through the renal artery and out through renal vein. Aside from digital monitoring of the perfusion temperature, the intended benefits of HMP are potentially the quantitative reading of organ quality through renal resistance measurements, the reduction of DGF and improvements in graft survival.

When considering the ability of HMP to predict graft outcomes, a cohort study performed in the Netherlands examined the performance of 336 deceased donor kidneys to determine whether kidney

vascular resistance over a certain threshold was associated with DGF (181). Although renal resistance appeared to be an independent predictor factor for DGF on multivariable analysis, analysis of received operator characteristic (ROC) curves demonstrated low predictive accuracy for DGF (C-statistic of 0.58). This would indicate that HMP would not be an accurate method of predicting DGF.

When considering the ability to ameliorate graft function, a landmark randomised controlled trial (RCT) performed in Holland, including 336 pairs of deceased donor kidneys (672 kidneys in total) were randomly allocated to static cold storage or HMP prior to transplantation, in order to compare rates of DGF (as well as PNF and 1-year graft and patient survival) (182). HMP during this Dutch study involved non-oxygenated pulsatile flow of cold preservation fluid at a fixed systolic perfusion pressure of 30mmHg. Donor characteristics were the same between the two groups, and included 42 (12.5%) DCD donors. Although the rate of DGF was not statistically different between the two groups, logistic-regression analysis showed that when adjusting for clinically important variables, the risk of DGF was significantly reduced following HMP (OR 0.57, p=0.01). No difference in PNF, or patient and graft survival, were observed. However, these results were not replicated in a UK-based RCT, in which the study was stopped after 45 pairs of deceased donor kidneys because no advantage in DGF rate was observed (183). However, there were fundamental differences between the UK study compared to the Dutch study. UK study only involved DCD donor kidneys whilst only a small proportion were DCD donors in the Dutch study, and HMP was performed at the recipient hospital in the UK study, whilst HMP was started at the donor hospital in the Netherlands.

Further studies have amassed body of evidence enabling a Cochrane review and meta-analysis to be performed in order to determine whether HMP was superior to SCS in the reduction of DGF (184). Sixteen studies, including 2266 kidney transplant recipients, were found, comparing HMP and SCS (fifteen studies were included in the meta-analysis). In this meta-analysis, HMP was associated with reduced relative risk of DGF in DCD donor kidneys (RR 0.75, 95% CI 0.64-0.87) and in DBD donor kidneys (RR 0.78, 95% CI 0.65-0.93). Seven perfusions were needed to treat to prevent DGF in DCD donor kidneys, compared to SCS, and 14 perfusions needed to prevent an episode of DGF in DBD donor kidneys. However, the meta-analysis was limited by the small number of studies included in each subgroup

analysis, and a number of studies were performed more than 10 years ago when the donor risk profile was considerably different. It was also not possible determine the optimal perfusion solution. Finally, the data in the meta-analysis was also not granular enough to determine whether oxygenated/non-oxygenated or pulsatile/non-pulsatile perfusion was superior. However, a large multi-centre RCT performed after publication of this meta-analysis examined the use of oxygenation during HMP (185). Two hundred and sixty-two deceased donor kidneys were transplanted across 5 countries after being randomised to either oxygenated HMP or static cold storage. No difference in one-year graft survival was observed between the groups.

Since hypothermic and normothermic machine technologies have been developed in parallel eras, there is considerable debate whether cold preservation methods are the future of organ preservation, or whether perfusion at body temperature has greater potential (186). In contrast to normothermic machine perfusion, HMP keeps organs in relative hibernation, meaning that functional outputs, like urine output, are not reliable measures of organ quality. However, HMP has several advantages over normothermic machine perfusion, such as the relative cost, technological simplicity, and that pump malfunction would not necessarily result in warm ischaemia since it would be comparable to SCS.

Normothermic machine perfusion

Technology enabling cardio-pulmonary bypass surgery and extracorporeal membrane oxygenation can be used to perfuse organs with warm oxygenated blood. Normothermic machine perfusion may be performed whilst the organs remain within the donor, termed normothermic regional perfusion (NRP), or following retrieval, typically termed ex vivo normothermic perfusion (EVNP) (Figure 8)

NRP is only necessary in DCD donors, since DBD donors continue to have spontaneous cardiac activity, until asystole is induced by the retrieving surgeons. NRP achieved by cannulation of the donor femoral artery and vein, with restoration of regional perfusion to the abdominal organs, following donor

asystole. Cannulation of the femoral vessels may be performed pre-mortem, although this is not currently permissible in the UK. Intra-arterial balloons or arterial clamps in the proximal abdominal aorta and the contralateral femoral artery prevent recirculation beyond the abdominal viscera. NRP was developed to avoid donor warm ischaemia following DCD donor retrieval, primarily to assess and improve the function of liver allografts. However, there is evidence from a large European study that NRP may improve early kidney graft function (187). Padilla et al compared 770 standard retrievals versus 770 NRP retrievals in DCD donors in a propensity score analysis. They found that although PNF rates were comparable, standard retrieval was associated with higher rates of DGF (odds ratio 1.97, 95% CI 1.43-2.72).

Although the concept of ex situ perfusion of organs was first pioneered by Alexis Carrel more than 100 years ago (188), this was reintroduced more recently in a canine model of kidney transplantation by Lauren Brasile, in which cellular function could be restored using an oxygenated acellular perfusate (189-192). When translated to human kidneys, Brasile et al demonstrated molecular signs of organ repair following oxygenated perfusion at sub-physiological temperatures (32^{o}C) and pressure (35mmHg) (193).

The implementation of EVNP using blood as a perfusate, at body temperature and physiological pressures, was pioneered by Hosgood and Nicholson (194). Following retrieval and subsequent cold preservation, normothermic machine perfusion was able to restore intracellular adenosine triphosphate (ATP) levels, as well as reversing some of the deleterious effects of prolonged cold storage in porcine kidneys (194) Normothermic perfusion also resulted in improved renal blood flow and creatinine clearance compared to static cold storage alone (195). The safe use of ex vivo normothermic perfusion (EVNP) in humans prior to transplantation was first demonstrated by Hosgood and Nicholson in 2011 (196), in which the recipient of a kidney treated with EVNP demonstrated non-inferiority to a paired kidney undergoing cold storage alone. Since its introduction in clinical research, early observational studies have demonstrated two potential benefits of EVNP prior to transplantation: organ assessment and amelioration of graft dysfunction.

In a series of 74 discarded human kidneys, Hosgood and Nicholson examined characteristics associated with superior organ function. Using ROC curves, they were able to determine thresholds of renal blood flow, urine output and macroscopic appearance during EVNP to formulate an organ quality score (197) (Table 8). Kidneys scoring 1 were considered the highest quality, with organs scoring 1-3 considered to be transplantable. Organs scoring 4-5 were considered to be untransplantable due to the likely chance of primary non-function. This newly formulated EVNP score was then applied to 36 kidneys transplanted into humans, in which kidneys scores ranged from 1-3. A second multicentre study has supported the assertion that kidneys declined by UK centres scoring 1-3 can be transplanted with satisfactory early patient and graft outcomes (198). This suggests the potential for EVNP to help reduce unnecessary organ discard by acting as an adjunct to organ acceptance decisions. Biomarkers in urine produced during EVNP also showed potential for organ viability assessment (199).

Table 8 – Ex vivo normothermic perfusion kidney assessment score

Perfusion parameter	**Score**
Macroscopic appearance	
Excellent perfusion: global pink appearance	1
Moderate perfusion: patchy appearance	2
Poor perfusion: globally mottled, purple, or black appearance	3
Renal blood flow (adjusted to organ weight)	
≥ 50 mL/min/100g	0
< 50 mL/min/100g	1
Urine output	
≥ 43 mL/min/hour	0
<43 mL/min/hour	1
Total score 1 – 5	

Adapted from Hosgood SA, et al. Ex vivo normothermic perfusion for quality assessment of marginal donor kidney transplants. The British Journal of Surgery 2015;102(11):1433-1440.

It has also been reported that EVNP may ameliorate graft function prior to transplantation. Eighteen ECD kidneys underwent EVNP for 60 minutes and demonstrated a reduced rate of DGF (5.6%) compared to a group of historic control kidneys which underwent SCS alone (36.2%, p=0.01) (200). Although the two groups were matched for donor and recipient age, a greater proportion of recipients of EVNP were not established on dialysis at the time of transplantation, acting as a significant confounding factor. The role of EVNP in the reduction of DGF in deceased donor kidney transplantation has yet be demonstrated in a RCT (201).

Hosgood and Nicholson identified poorly perfused kidneys with potentially the greatest to gain from EVNP. At the time of retrieval, kidneys that appear to be patchy or mottled due to incomplete flushing of blood after in situ and/or backbench perfusion with cold preservation may specifically benefit from EVNP, according to reports from Hosgood and Nicholson (202). The Hosgood/Nicholson method of normothermic perfusion also allowed them to test an array of experimental agents in the amelioration of IRI, such as erythropoietin (203), nitric oxide (204) and carbon monoxide (205), hydrogen sulphide (206), small interfering RNA (207), sildenafil (208), 1400Wa (nitric oxidase inhibitor) (209) and cyclic helix B peptide (210). Furthermore, temperature-dependent therapies such as mesenchymal stromal cells and multipotent adult progenitor cells could potentially be delivered directly to organs during to ameliorate IRI and improve graft function (211-214). These avenues of development in normothermic machine perfusion are discussed further in Section 7.3.

Ex vivo normothermic perfusion randomised controlled trial (EVNP RCT)

The EVNP trial is a multicentre open-label RCT investigating the effect of EVNP on initial graft function in DCD donor kidney transplantation, compared to kidneys undergoing static cold storage alone (201). The trial began in 2016 and completed recruitment in early 2020 but has not yet presented or published the results. The EVNP trial is being specifically discussed in this introductory chapter for several reasons. Firstly, the EVNP trial represents the largest and most robust clinical trial considering the use of EVNP in kidney transplantation. Secondly, the technical aspects of performing EVNP in

Chapters 4, 5 and 6 are drawn directly from the methods used in the EVNP trial. Finally, Chapter 5 represents a subgroup analysis of the wider EVNP RCT. An understanding of the EVNP trial is therefore necessary in detailing the background of the thesis. Specifically, it is important to appreciate what the EVNP RCT aims to demonstrate, and the questions that it is not designed to answer.

The inclusion and exclusion criteria of the EVNP RCT are shown in Table 9. Patients are randomised in a 1:1 ratio to either SCS followed by one hour of EVNP prior to transplantation or static cold storage alone. The recruitment target was 200 patients in each group (376 patients needed to detect a 30% relative reduction in DGF (from 50% to 35%) with a power of 80% at a statistical significance of 0.05).

Table 9 - Inclusion and exclusion criteria for the EVNP RCT

Inclusion criteria	Exclusion criteria
• Patients undergoing controlled DCD donor kidney transplantation • DCD donor aged ≥18 years • Prospective kidney transplant recipients aged ≥18 years • Patients undergoing their first or second kidney transplant • Patients who have given written informed consent	• DCD donor aged <18 years • Uncontrolled DCD donor kidney transplantation • Patients undergoing their third or subsequent kidney transplant • Patients receiving multi-organ transplants • Patients receiving dual kidney transplants • Donor kidneys that have undergone hypothermic machine perfusion

Adapted from Hosgood et al. Protocol of a randomised controlled, open-label trial of ex vivo normothermic perfusion versus static cold storage in donation after circulatory death renal transplantation. BMJ open 2017; 7(1): e012237

The primary outcome measure of the EVNP RCT is the rate of DGF, defined as the need for dialysis in the first seven days of transplantation, regardless of cause. The secondary outcome measures include PNF rates, DGF duration, functional DGF rates (defined as a <10% fall in serum creatinine for three consecutive days in the first week of transplantation), rates of biopsy-proven acute rejection, serum creatinine and eGFR at 1, 3, 6 and 12 months after transplantation, one-year graft and patient survival.

The inclusion criteria of the EVNP are remarkably wide. However, the results EVNP trial will not necessarily apply to other donor organ types, such as DBD donor kidneys or living-donor kidneys.

Notably, the EVNP RCT does not consider hypothermic machine perfusion as a comparator group. The question of how warm and cold machine perfusion compare will remain outstanding. Likewise, differences in perfusate composition, concentration of oxygen delivery and the application of additional hardware will not be considered. The EVNP RCT does not consider the use of white cell filters – however the use of white cell filters is examined by me in a basic science experiment in Chapter 6 (not linked to the main EVNP RCT). The EVNP RCT seeks to deliver a short period of warm perfusion (60 minutes). The benefits and challenges of prolonged EVNP will remain an area of interest. Finally, the EVNP RCT is not specifically designed to investigate the role of organ viability assessment, or to validate the existing EVNP organ quality score.

2 Chapter 2: Predictive ability of donor kidney histology on kidney transplant outcomes

Work from this chapter has been published in the peer-reviewed journal *Transplant International*:

Phillips BL, Kassimatis T, Atalar, K, Wilkinson H, Kessaris N, Simmonds N, Hilton R, Horsfield C, Callaghan CJ. Chronic histological changes in deceased donor kidneys at implantation do not predict graft survival: a single-centre retrospective analysis. *Transplant International*. 2019 May;32(5):523-534. doi: 10.1111/tri.13398. Epub 2019 Feb 8. PMID: 30636065

This chapter has also been presented orally at the British Transplantation Society Annual Congress in March 2018, and I received the clinical Medawar Medal 2018 for this work.

Within this Chapter, I performed the overwhelming majority of the data collection, and all data analyses and interpretation. Histological specimens were analysed by consultant renal histopathologists at Guy's and St Thomas' NHS Foundation Trust.

There was no direct funding for this research

2.1 Abstract

The examination of donor kidney histology has been used as a tool to predict kidney transplant outcomes. However, the evidence-base for this approach is considered to be weak and may have contributed to high rates of kidney discard in the US relative to the UK. The aim of this work was to determine the predictive ability of donor histological findings at implantation on short- and long-term transplant outcomes. A retrospective observational study was performed of adult single kidney-only transplants from deceased donors between 2006 and 2015. Karpinski (K) scores, which quantify chronic donor histological damage, were collected, and graft and patient outcomes were compared using univariate and multivariable statistical methods. In the second part of the analysis, K scores of single and dual kidney transplants from donors aged 60 years and over were examined to determine whether knowledge of the score prior to transplantation would have influenced the decision to proceed. During the study period 408 eligible kidney transplants were performed. Kidneys with K scores 5-12 had lower one-year eGFRs (median (IQR) 51 (37-66) vs 35 (26-52) mL/min/1.73m^2, p<0.001) and three-year eGFRs (median (IQR) 52 (34-64) vs 35 (24-52) mL/min/1.73m^2, p<0.001) compared to kidneys scoring 0- 4. However, there was no significant association between K score and death-censored graft survival (DCGS) on univariate and multivariable analyses. Subgroup analyses considering individual glomerular, interstitial, tubular and vascular components of the score, or K scores from donors aged >50 and >60 years did not demonstrate an association with DCGS. Sensitivity analyses altering the adequacy threshold for the number of glomeruli on biopsy did not reveal an association with DCGS. The utilisation analysis suggested that systematic use of pre-implantation kidney biopsies would have resulted in 26.4% fewer patients receiving kidney transplants. This analysis suggests that the current method of quantifying chronic donor histology is not a helpful tool for clinicians making kidney utilisation decisions. This work suggests that use of PIKB may paradoxically increase organ decline rates. Further work is required to improve the predictive ability of other histological tools in the prediction of graft function and survival. Other methods of assessing donor risk are needed, either in isolation or in combination with histological analysis of donor kidneys, such as from large registry analyses or organ perfusion technologies.

2.2 Introduction

Methods of predicting kidney transplant outcomes prior to implantation, using donor, organ and recipient characteristics, have been widely investigated (163). Large retrospective registry analyses have identified deceased donor clinical characteristics, such as age and hypertension, to be associated with graft failure (5, 169). It has therefore been hypothesised that these causes of chronic injury may be accurately reflected at a microscopic level within the kidney. Donor kidney biopsies have therefore been used to identify chronic injury, with the suggestion that these histological features may be associated with poor graft outcomes (75, 76). Living donors are selected on the basis that they are in good health, with few or no comorbidities, in order to reduce the risk of chronic kidney disease to donors in the future. For this reason, graft survival in living donor kidney transplantation is higher than deceased donor kidney transplantation (median graft survival 19.2 years versus 11.7) (215). Since living donor kidneys are relatively high in quality, the use of pre-implantation kidney biopsies in living donors is uncommon, but can be performed to detect subclinical CKD (216).

Kidney biopsies to detect chronic changes can be performed at two possible opportunities. Firstly, *before* the decision has been made to implant the organ (and before reperfusion). This approach has the intention of waiting for urgent histological analysis to aid in the decision as to whether the organ should be implanted or not. This approach is widely termed a pre-implantation kidney biopsy (PIKB) and is extensively used in the United States, with approximately 75% of kidneys from extended criteria donors being biopsied (164). PIKBs are intended to provide the transplant team with an estimation of organ quality in order to inform and direct organ utilisation. For example, PIKBs suggesting poor quality may be discarded or implanted as dual grafts. The second opportunity to perform a kidney biopsy is *after* the decision to implant the organ has been made. These biopsies can be performed either before or after reperfusion of the organ by the surgeon and are commonly known as time-zero biopsies. Time-zero biopsies therefore do not influence the decision-making process prior to transplantation but provide information on 'baseline' chronic changes to compare with subsequent post-transplant biopsies. The

timing of the biopsy and the terminology in the literature can be confusing. It is important that both the timing and intent of the biopsy are made clear.

Despite their widespread use in the US, the evidence supporting the use of PIKBs is weak (176, 179). Previous studies examining the association between chronic donor histological changes at implantation and graft failure have often been small in number, used inconsistent techniques, included both living and deceased donors, included a high proportion of younger and lower risk deceased donors, or originated from transplant units that commonly performed PIKBs (thus biasing utilisation decisions and therefore the outcomes) (reviewed in (179)). Studies examining time-zero biopsies are better equipped to investigate the predictive ability of donor histological assessment on graft outcomes, as these have, by definition, *not* influenced the decision to use the organ or not. The widespread use of PIKBs has been proposed as one of the underlying causes for the perceived high rate of kidney discard in the US (46, 217). In the latter reference, Reese et al demonstrated that biopsy findings were the most common primary reason for kidney discard in both low risk donors (28%; KDPI <85) and higher risk donors (39%; KDPI >85) (217).

The first aim of this study was to determine whether chronic donor histological changes at the time of implantation were predictive of graft outcomes. To reach this end, a single centre retrospective cohort study was performed examining the outcomes of deceased donor kidney transplants that had undergone time-zero core biopsies. The population of donors and recipients transplanted in our centre are representative of the current UK transplant programme, with a high proportion of older donors with co-morbidities, and a high proportion of donation after circulatory death (DCD) donors (2, 8, 68). Our centre also performs relatively few PIKBs, but instead frequently performs time-zero biopsies. This means that our centre is well-placed to perform this type of study, as the results of these biopsies did not influence the final decision to transplant the organ, thus reducing selection bias. The second aim of the study was to determine whether the systematic use of PIKBs impacts organ utilisation. To do this, a second analysis was performed to determine whether utilisations decisions based on PIKB results altered the use of single and dual adult kidney transplants (DAKT) and whether there was a difference in organ discard rates.

2.3 Methods

2.3.1 Study design

A retrospective observational cohort study of patients transplanted between July 2006 to January 2015 at a single transplant unit in the UK was performed. Inclusion and exclusion criteria are detailed in Table 10. Time-zero biopsies are seldom performed in living donor kidneys at our centre, and this donor type was therefore excluded. Small paediatric donors were excluded for the same reason. Multi-organ transplantation (such as simultaneous pancreas and kidney or simultaneous liver and kidney) was excluded as these types of procedure are much less common than kidney only transplantation and are not representative of typical kidney-only graft survival.

Table 10 - Inclusion and exclusion criteria

Inclusion criteria
Deceased donor kidney transplants (DCD and DBD donors)
Donor age ≥10 years
Recipient age ≥18 years
Kidney-only transplant
Exclusion criteria
Living donor kidney transplantation
Double kidney transplantation
Multiorgan transplantation (in combination with another organ type, e.g., pancreas or liver)

2.3.2 Immunosuppression therapy

This cohort study spanned across two eras of immunosuppression therapy. Between July 2006 and January 2012, immunosuppression entailed induction with intravenous basiliximab, and maintenance with oral cyclosporine, mycophenolate mofetil and prednisolone. From January 2012,

cyclosporine was replaced with oral tacrolimus. Induction therapy and the target serum tacrolimus level was determined by the immunological risk stratification (according to the presence of anti-HLA antibodies, recipient ethnicity, and previous organ transplantation).

2.3.3 Kidney biopsies

On the whole, biopsies of the donor kidney were taken after the decision had been made to implant the organ (i.e., without the intention of the biopsy guiding organ utilisation). Only one biopsy was taken prior to organ transplantation, with the intention of guiding organ utilisation.

Biopsies were obtained using a 16-gauge core needle by the operating surgeon under direct vision. Kidney biopsies obtained in this way provided specimens which are approximately 1.29mm by 25mm. Specimens were then fixed in 10% buffer-aqueous formaldehyde (formalin) and embedded in paraffin wax (FFPE). Specimens were then cut into 29 sections and prepared onto nine glass slides. Tissue sections were stained with haematoxylin and eosin (12 sections on four slides), periodic acid-Schiff (nine sections on three slides), periodic acid silver methenamine and Masson's Trichrome (four sections on one slide for both stains) prior to light microscopy. The purpose of each of these stains is summarised in Table 11. Preparation of renal biopsies were consistent with Renal Pathology Society guidance (218).

Table 11 - Staining of donor kidney biopsies

Stain used	Purpose
Haematoxylin and eosin	Localisation of the cell nuclei and extracellular proteins allowing the composition of the tissue to be seen
Periodic acid-Schiff	Demonstration of the basement membrane of glomeruli (in red), and to assess its thickness. Arteriolar hyalinosis and tubular atrophy are also demonstrated if present
Periodic acid silver methenamine (Jones)	Accentuation of argyrophilic substances (in black), including the glomeruli and glomerular basement membrane. Periodic acid-Schiff and periodic acid silver methenamine used together allow a double contour to be seen in various pathological processes including ischaemia
Masson's Trichrome	Demonstrates fibrin, including glomerular and interstitial fibrosis

Kidney biopsies were analysed by one of five renal histopathologists and reported within a week of transplantation. However, it was not possible to determine the inter-observer variability based on the data available. Each kidney was given a Karpinski (K) score based on the histological appearances. The total K score is made up of the individual scores of the interstitial, glomerular, tubular and vascular components (Table 12) (75). Biopsies were considered inadequate if the sample contained <20 glomeruli and were excluded from the main outcome analyses. Sensitivity analyses were undertaken to determine whether altering the definition of biopsy 'adequacy' by decreasing or increasing the number of necessary glomeruli influenced the predictive ability of the Karpinski scoring system. However, where the number of glomeruli was lowered to less than 20, analysis was limited to samples where a score had been given regardless of being identified as adequate (i.e., analysis could not include inadequate samples where the histopathologist did not, or could not, provide a K score).

Table 12 - Components of the Karpinski score

Glomerular score	
0	No global sclerosed glomeruli
1	<20% global glomerulosclerosis
2	20-50% glomerulosclerosis
3	>50% glomerulosclerosis
Interstitial score	
0	No cortical parenchyma replaced by fibrous connective tissue
1	<20% of cortical parenchyma replaced by fibrous connective tissue
2	20-50% of cortical parenchyma replaced by fibrous connective tissue
3	>50% of cortical parenchyma replaced by fibrous connective tissue
Tubular score	
0	No tubules affected
1	<20% of tubules affected
2	20-50% of tubules affected
3	>50% of tubules affected
Vascular score	
Both arterioles and arteries are assessed, and the most severe lesion determines the final vascular score	
Arteriolar narrowing or hyaline arteriolosclerosis	
0	No changes
1	Increased wall thickness less than the diameter of the lumen
2	Wall thickness equal to the diameter of the lumen
3	Wall thickness greater than the diameter of the lumen (extreme luminal narrowing or total occlusion)
Arterial sclerosis or intimal fibrous thickening	
0	No changes
1	Increased wall thickness less than the diameter of the lumen
2	Wall thickness equal to the diameter of the lumen
3	Wall thickness greater than the diameter of the lumen (extreme luminal narrowing or total occlusion)
Total score out of 12	
Adapted from: Karpinski J, et al. Outcome of kidney transplantation from high-risk donors is determined by both structure and function. *Transplantation* 1999;67(8):1162-1167.	

The K score is almost identical to the Remuzzi score (76, 176). Both scores are composed of glomerular, interstitial, tubular, and vascular components, each gaining a scored 0-3. The only differences are in their definition of vasculopathy (76, 176) and the number of glomeruli needed to define an inadequate biopsy (<20 glomeruli for the K score, <25 glomeruli for the Remuzzi score).

2.3.4 Clinical outcomes

Transplanted recipients were followed-up for five years post-transplantation or until January 2018, whichever occurred first. Cold ischaemia time (CIT) was defined as the time between organ perfusion with cold preservation fluid in the donor to re-perfusion with warm recipient blood after implantation. Donor risk was quantified using the UK Kidney Donor Risk Index (UKKDRI), which is a validated score that takes into account the donor age, weight, presence of hypertension, length of stay in hospital prior to death, and adrenaline inotropic support (5). Kidney transplant function was measured using the four-variable Modification of Diet in Renal Disease estimated glomerular filtration rate (eGFR) equation, which standardises kidney according to age, sex and ethnicity (157) (see Equation 1).

Recipients with a failed graft were assigned an eGFR of 5 mL/min/1.73m^2. Delayed graft function (DGF) was defined as the need for dialysis within seven days post-transplant, regardless of cause, and excludes kidneys with primary non-function (145). Primary non-function was defined as failure of the transplanted kidney to ever function (i.e., freedom from dialysis) within three months of transplantation, regardless of cause. Death-censored graft survival (DCGS) was defined as the time from transplantation to the date of graft failure (i.e., return to long-term dialysis, or re-transplantation, whichever occurred first), censored for patient death. Patient survival was defined as the time from transplantation to the date of death.

2.3.5 Statistical analysis

Transplant recipients were placed into two groups based on the K score from by time-zero biopsies. Kidney transplant recipients with low K scores (0-4) were compared to those with high K scores (5-12). This grouping reflects the current utilisation scoring thresholds for single versus dual adult kidney transplants in the United Kingdom (172, 180, 219). Sensitivity analyses were employed, acknowledging previous groupings of K scores 0-3 versus 4-12 (70, 75, 76).

All variable data were tested for normality using the Shapiro-Wilk test. Differences in demographic and clinical characteristics between the two groups were examined using the Chi-squared test for categorical data and the Kruskal-Wallis test for continuous non-parametric data. Wilcoxon rank test was used for non-parametric paired data. Spearman's rho was used to test the correlation between non-parametric continuous data. The number and proportion of data is detailed in the appropriate tables and were tested for randomness between the DGF duration groups. Imputational techniques would be considered for variables with >5% missing data and missing completely at random. However, this was not the case for any of the variables, therefore complete case analysis was employed throughout. Unfortunately, data on calculated reactive frequency in recipients was not collected or included in this study. Kaplan-Meier survival estimates were employed to demonstrate DCGS and patient survival between the K score groups, with patients censored at the end of the study or if lost to follow-up; univariate differences between groups were examined using the log-rank test.

Multivariable analyses were performed to identify independent predictors of glomerular filtration rate, DCGS, and patient survival. Clinically relevant candidate donor and recipient variables available at the time of transplantation, as well as K score, were included in the multivariable analyses if an association on univariate analysis with $p<0.10$ was reached. In order to identify and avoid multicollinearity, variance inflation factor (VIF) was calculated for each covariate in the multivariable regression model. Covariates were removed if the VIF exceeded 5 (220). Linear regression was used to examine factors predictive of one-, three-, and five-year eGFR. Cox proportional hazard was used to find factors predictive of DCGS and patient survival, with the results expressed as hazard ratios (HR) with

95% confidence intervals (CI) and p values derived from likelihood ratio tests. Two-sided tests were conducted and $p<0.05$ was considered statistically significant. Inter-observer variability analysis was not performed. All data in this chapter were analysed using IBM SPSS Statistics for Macintosh versions 24-25 (IBM, Armonk, NY, USA).

2.3.6 Organ utilisation analysis

In order to ascertain whether routine and systematic usage of PIKBs might have altered organ utilisation of kidneys from older donors in our unit, all kidney-only transplants from DBD or controlled DCD donors aged 60 years and over between 1 January 2012 and 31 December 2015 were examined retrospectively. This age group was used in this analysis for three main reasons:

- This age group forms part of the definition of expanded criteria donors (221)
- This was the age group originally used to describe the use of PIKB in the determination of dual versus single kidney transplantation by Remuzzi et al (70, 76)
- This is the indicated age group for the PITHIA trial, an open-label clinical trial assessing whether the provision of a national PIKB service improves kidney utilisation (172)

During this period kidneys were implanted as single or dual adult kidney transplants (DAKT) (76). The DAKT programme at Guy's Hospital began in 2012 and the decision to implant two kidneys together as DAKTs was based on locally agreed criteria (Table 13).

Table 13 - Donor and recipient selection criteria for dual adult kidney transplantation

Donor selection criteria (one or more may apply) *
Donor aged >70 years with multiple comorbidities that are likely to negatively impact graft survival (such as hypertension requiring more than one medication, ischaemic heart disease, peripheral vascular disease, diabetes mellitus type I or II)
Donor aged >60 years (or >50 years with a history of hypertension, diabetes mellitus, or significant cardiovascular disease) with 'baseline' eGFR 40-60 ml/min/1.73m^2
Donation after circulatory death donor aged >60 years with estimated cold ischaemia time of a potential second single kidney transplant of >20 hours
Kidneys with poor macroscopic appearance suggesting that adequate graft function would not be achieved by using both kidneys singly (such as small or poorly perfused kidneys)
Recipient selection criteria
Recipients aged >55 years who are dialysis-dependent
Recipients aged <55 years with clinical urgency (e.g., poor dialysis access) or likely prolonged wait for transplantation (e.g., calculated reaction frequency >85%)
Recipients who are medically fit enough to withstand a longer procedure with additional blood loss

*When a pre-implantation kidney biopsy is performed, Karpinski scoring has also been used as a guide for stratification

K scores were examined retrospectively to determine how knowledge of these scores prior to transplantation might have impacted organ utilisation decisions, using scoring thresholds initially defined by Remuzzi et al and then modified by others (180, 219). Kidneys that had received a PIKB were excluded from this part of the analysis, as utilisation decisions had already been influenced by the result of the biopsy score. In those kidneys with adequate time-zero biopsies, a utilisation algorithm was retrospectively applied to determine how organ utilisation might have been affected by current practices in UK transplant centres (172):

- if only one kidney was accepted at our centre

  - and the K score was 0 to 4, then utilisation would have been as a *single kidney transplant*

  - and the K score was 5 or above, then the organ would have been *declined*

- if both kidneys were accepted by and available to our centre

  - and both K scores were 0 to 4, then utilisation would have been as two *single kidneys transplants*

  - and the highest K score of the pair was 5 or 6, then utilisation would have been as a *dual adult kidney transplant (DAKT)*

  - and the highest K score of the pair was 7 to 12, then both organs would have been *declined*

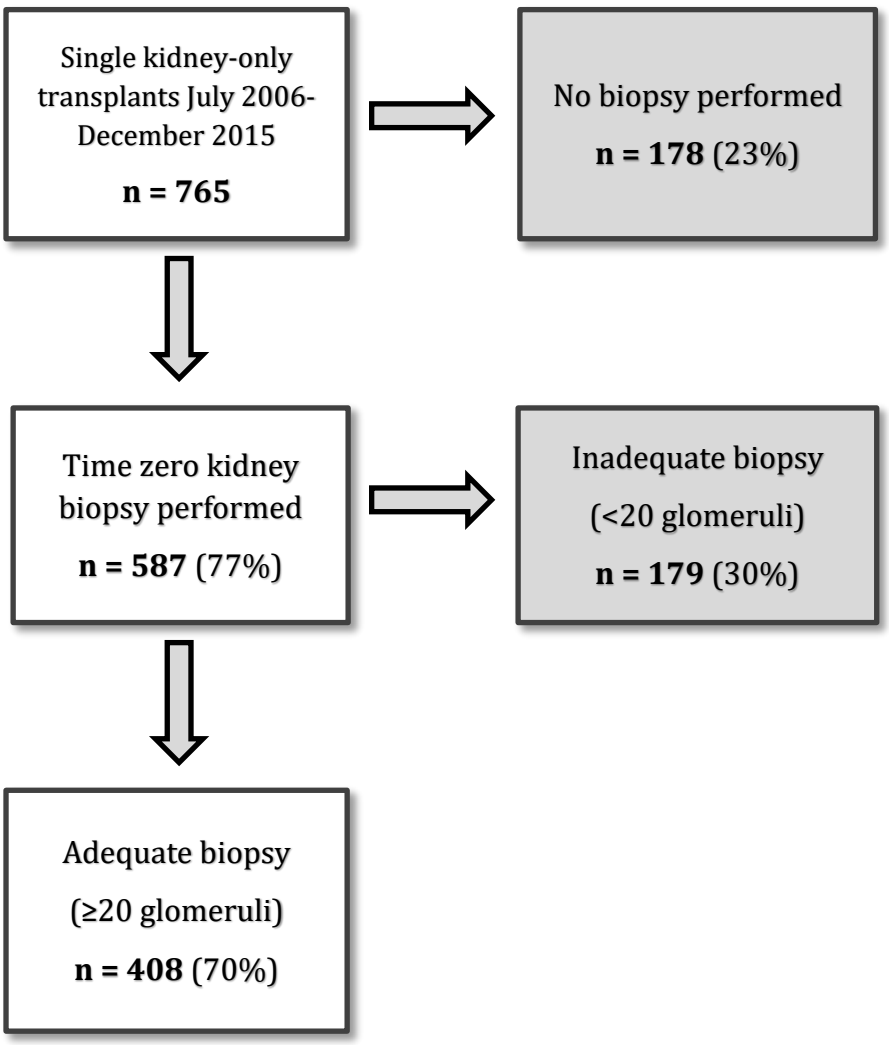
DCGS and eGFRs for single kidney transplants and DAKTs were analysed.

2.4 Results

2.4.1 Donor, recipient, operative, and biopsy characteristics

During the study period, 765 deceased donor kidneys meeting the inclusion criteria were transplanted. Recipients where no time-zero biopsy had been performed (n=178), or where the biopsy was found to be inadequate (glomerular count<20; n=179), were excluded (Figure 9). Of the 408 kidneys left for analysis, only one had had a PIKB with the intent to wait for scoring prior to transplantation. Median follow-up for transplant recipients in the study was 4.1 years (IQR 2.8-5.4 years).

Figure 9 - Flow diagram of kidney biopsies and study numbers.



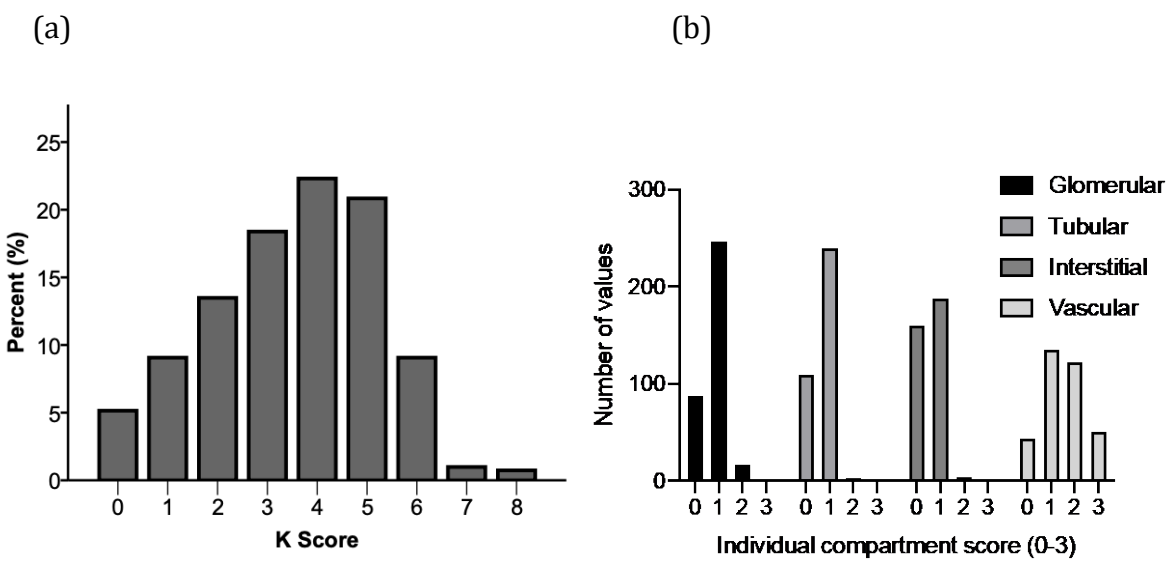
Donor, recipient, and operative demographic data of kidney transplants with adequate and inadequate biopsies are shown in Table 14.

Importantly, characteristics of kidneys with adequate biopsies were comparable to those with inadequate biopsies suggesting that data was 'missing' at random. Donors with no biopsy were generally younger with lower UKKDRI. This reflects the low biopsy rate in donors who were not expected to have chronic changes on biopsy. Of those with adequate biopsies, median (IQR) donor age was 51 (41-60) years with a high proportion of kidneys from DCD donors (n=134; 32.8%). A high proportion of donors died from a cerebrovascular event (n=241; 59.1%) and more than a third of donors fell into the 'high risk'

UKKDRI quartile (5). This suggests that the study was representative of transplantation in the modern era and therefore representative of UK transplant current practices.

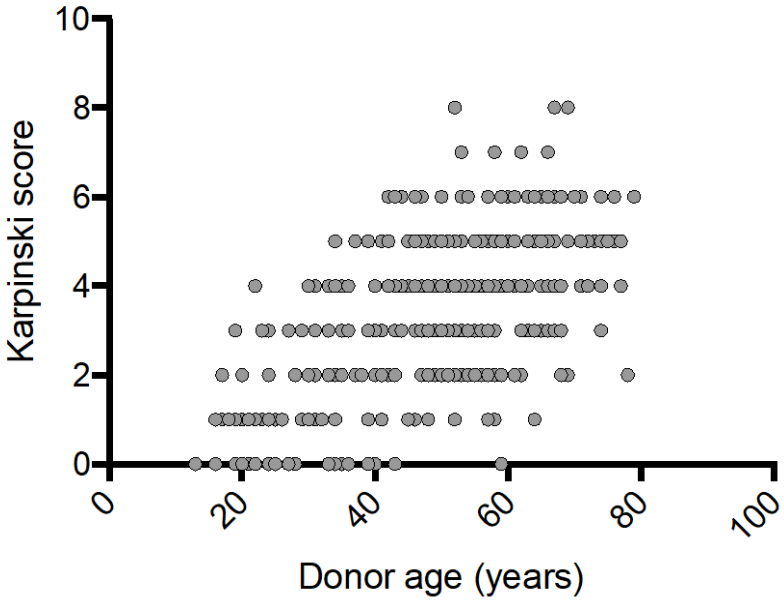
Median (IQR) K score in the entire cohort was 4 (2-5), ranging from 0 to 8 (Figure 10). Almost one-third of kidneys had scores >4 (n=129; 32%) and were implanted as single grafts. There was a moderate positive correlation between donor age and K score (Spearman's rho r=0.53, p <0.001; *Figure 11*).

Figure 10 – Histogram demonstrating the distribution of (a) total Karpinski (K) scores and (b) individual compartment scores in single kidney-only transplants from deceased donors (n=408).



** No kidneys scored 9 to 12 during the study period*

Figure 11 - Deceased donor age versus Karpinski score (n=408).



Transplants were stratified into low (0-4) and high (5-12) K score groups. Kidneys in the higher K score group were more likely to be from older donors, DCD donors, have higher UKKDRI, be transplanted into older recipients (Table 15). The higher K score group was also associated with inferior HLA mismatch levels than those in the low K score group, possibly because older recipients are offered and accept less favourable HLA mismatches.

Table 14 - Donor, recipient, and operative characteristics of kidneys with an adequate biopsy, inadequate biopsy, and those kidneys not biopsied.

Variable	Adequate biopsy (n=408)	Inadequate biopsy (n=179)	No biopsy (n=178)	Adequate versus no biopsy p value	Across all three groups p value
Donor age (years)	51 (41-60)	56 (47-66)	51 (42-59)	0.31	<0.001
Donor sex					
Male	210 (51.5%)	87 (48.6%)	96 (53.9%)	0.58	0.60
Female	198 (48.5%)	92 (51.4%)	82 (46.1%)		
Donor type					
DBD	274 (67.2%)	111 (62.0%)	114 (64.0%)	0.46	0.45
DCD	134 (32.8%)	68 (38.0%)	64 (36.0%)		
Cause of death					
Stroke	241 (59.1%)	102 (57.0%)	87 (48.9%)		
Trauma	40 (9.8%)	14 (7.8%)	17 (9.6%)	0.04	0.15
Other	127 (31.1%)	63 (35.2%)	74 (41.6)		
UKKDRI*	1.04 (0.97-1.46)	1.30 (1.00-1.53)	1.04 (0.94-1.47)	0.84	0.01
≤1.35	262 (65.8%)	96 (55.2%)	111 (68.1%)		0.02
>1.35	136 (34.2%)	78 (44.8%)	52 (31.9%)	0.57	
Recipient age (years)	51 (42-59)	52 (43-61)	59 (46-67)	0.06	0.04
Recipient sex					
Male	258 (63.2%)	118 (65.9%)	108 (60.75)		
Female	150 (36.8%)	61 (34.1%)	70 (39.3%)	0.56	0.59
Recipient ethnicity					
White	232 (56.9%)	114 (63.7%)	96 (53.9%)	0.52	0.33
Black	124 (30.4%)	43 (24.0%)	52 (29.2%)		
Other	52 (12.7%)	22 (12.2%)	30 (16.9%)		
Primary renal disease					
Diabetes mellitus	41 (10.0%)	12 (6.7%)	23 (12.9%)	0.36	0.26
Hypertension	73 (17.9%)	28 (15.6%)	25 (14.0%)		
Other	294 (72.1%)	139 (77.7%)	130 (73.0%)		
Graft number					
1	344 (84.3%)	152 (84.9%)	148 (83.1%)	0.73	0.90

>1	64 (15.7%)	27 (15.1%)	30 (16.9%)		
HLA mismatch level*†					
1	55 (14%)	24 (13.8%)	14 (8.5%)		
2	130 (33%)	56 (32.2%)	48 (29.3%)	0.21	0.54
3	188 (47%)	81 (46.6%)	89 (54.3%)		
4	26 (6%)	13 (7.5%)	13 (7.9%)		
CIT (mins)	835 (660-1027)	794 (657-1000)	849 (709-1071)	0.15	0.71

Data are expressed as median (IQR) or number (%). Chi-squared test for categorical data and the Kruskal-Wallis test for continuous non-parametric data. Wilcoxon rank test was used for non-parametric paired data.

*Missing data (UKKDRI n = 30, HLA mismatch level n = 28).

†Defined according to the UK allocation policy 2017 for deceased donor kidneys and was based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was a mismatch of 000; level 2 was a 0 HLA-DR and a 0/1 HLA-B mismatch; level 3 was a 0 HLA-DR and a 2 HLA-B mismatch, or a 1 HLA-DR and a 0/1 HLA-B mismatch; and level 4 was a 2 HLA-DR or a 1 HLA-DR and 2 HLA-B mismatch.

Table 15 - Donor, recipient, and operative characteristics of the low K score group (K score 0-4) versus high K score (K score 5-8).

Variable	K score 0-4 (n=279)	K score 5-8 (n=129)	p value
Donor age (years)	49 (35-57)	58 (50-66)	<0.001
Donor male sex	143 (51%)	67 (52%)	0.92
Donor type			
DBD	196 (70.3%)	78 (60.5%)	0.05
DCD	83 (29.7%)	51 (39.5%)	
Donor cause of death			
Stroke	115 (41%)	86 (67%)	
Trauma	32 (12%)	8 (6%)	0.73
Other	132 (47%)	35 (27%)	
UKKDRI*	1.02 (0.83 – 1.28)	1.39 (1.01 – 1.85)	<0.001
Recipient age (years)	49 (40 – 58)	54 (46 – 63)	0.005
Recipient male sex	116 (42%)	92 (71%)	0.02
Recipient ethnicity			
White	157 (56.3%)	75 (58.1%)	
Black	90 (32.3%)	34 (36.4%)	0.46
Other	32 (11.4%)	20 (5.5%)	
Primary renal disease			
Diabetes mellitus	20 (10.8%)	11 (8.5%)	
Hypertension	55 (19.7%)	18 (14.0%)	0.37
Other	205 (69.5%)	100 (77.5%)	
Graft number			
1	230 (84.6%)	114 (89.8%)	0.16
>1	42 (15.4%)	13 (10.2%)	
HLA mismatch level*†			
1	44 (16.2%)	11 (8.7%)	
2	89 (32.7%)	41 (32.3%)	0.02
3	127 (46.7%)	61 (48.0%)	
4	12 (4.4%)	14 (11.0%)	

CIT (mins)	810 (641 – 1020)	866 (643 – 1011)	0.48

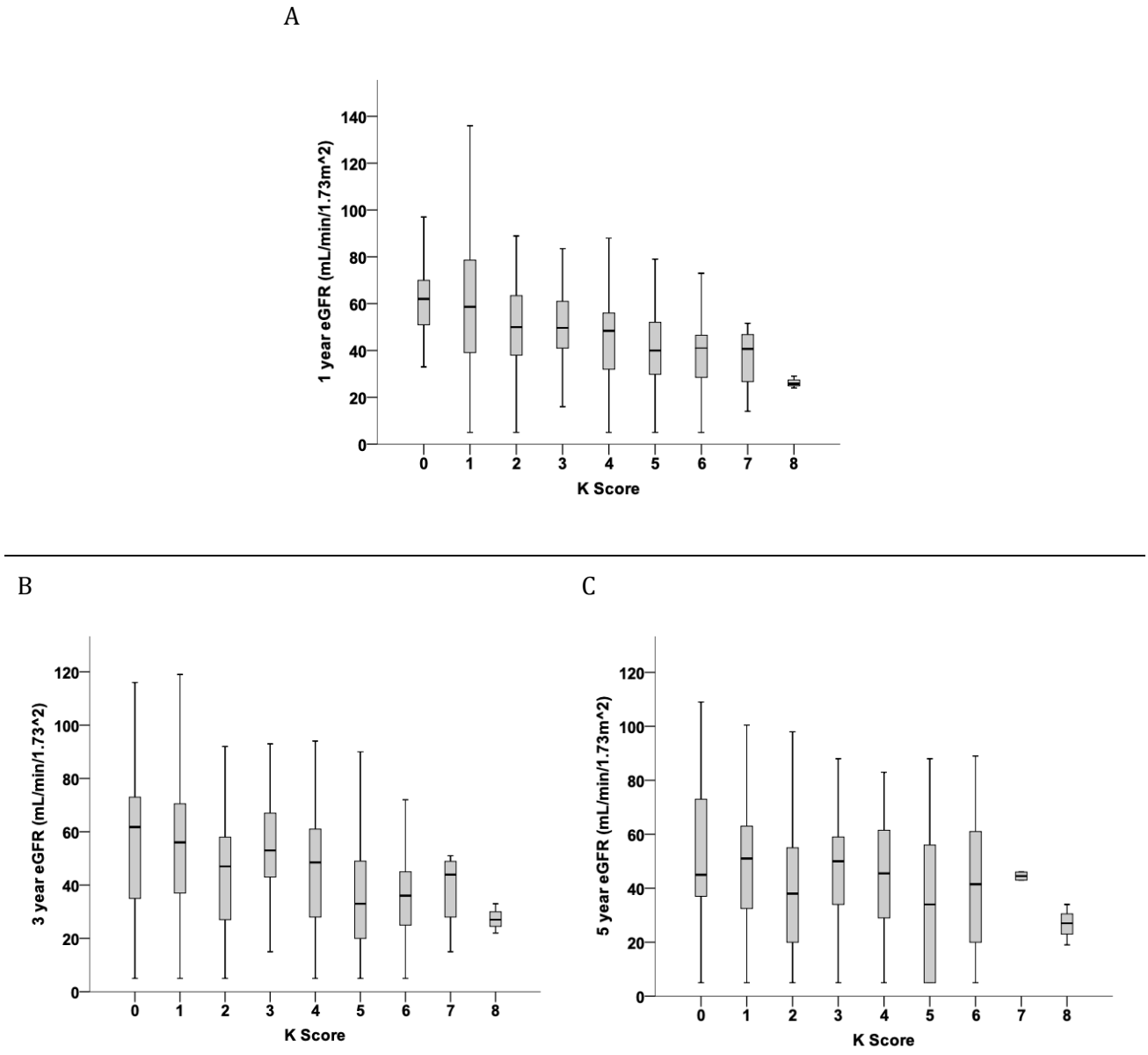
Data are expressed as median (IQR) or number (%)

*****Missing data (UKKDRI n = 10, HLA mismatch level n = 9). †Defined according to the UK allocation policy 2017 for deceased donor kidneys and was based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was a mismatch of 000; level 2 was a 0 HLA-DR and a 0/1 HLA-B mismatch; level 3 was a 0 HLA-DR and a 2 HLA-B mismatch, or a 1 HLA-DR and a 0/1 HLA-B mismatch; and level 4 was a 2 HLA-DR or a 1 HLA-DR and 2 HLA-B mismatch.

2.4.2 Graft function

There was a wide variation in post-transplant graft function when stratified by K score (Figure 12)

Figure 12 - Box and whisker plot of Karpinski (K) score and graft function at (A) one- (n=399), (B) three- (n=310), and (C) five-years (n=193) post-transplantation.



Boxes presented as median with interquartile range, whiskers as minimum and maximum figures. *Death before 1 year (n = 5), missing data (n = 4). 3-year follow-up not yet reached (n = 62). Death before 3 years (n = 16), missing data (n = 20). 5-year follow-up not yet reached (n = 179), death before 5 years (n = 24), missing data (n = 12).*

There was no correlation between K score and eGFR at one- and three-years post-transplant on an unadjusted analysis (Spearman's rho r = -0.3). When put into the grouping strategy of K scores 0-4 versus 5-12, kidneys with high K scores had significantly poorer eGFR at one- and three-years post-transplantation when compared to kidneys with low K scores. However, this did not reach statistical significance at five years (Table 16).

Table 16 - Karpinski score and graft function

Outcome	**K score 0-4**	**K score 5-8**	**p value**
1-year eGFR (n=399)*	51 (37-66)	35 (26-52)	<0.001
3-year eGFR (n=310)†	52 (34-64)	35 (24-52)	<0.001
5-year eGFR (n=193)§	46 (29-61)	36 (5-50)	0.06

Data expressed as median (IQR). Estimated glomerular filtration rate (eGFR) expressed as mL/min/1.73m^2

*Death before 1 year (n = 5), missing data (n = 4)

†3-year follow-up not yet reached (n = 62), death before 3 years (n = 16), missing data (n = 20)

§5-year follow-up not yet reached (n = 179), death before 5 years (n = 24), missing data (n = 12)

Given the intrinsic difference in DGF rates between DCD and DBD donor kidneys, the incidence of DGF between the K score groups was analysed by donor-type (DBD n= 134, DCD n=274). The DGF rates were not significantly different between the low and high K score groups in recipients of DCD donor kidneys (43.6% vs 40.8%; p=0.76) and DBD donor kidneys (32.8% vs 35.6%; p=0.66).

Linear regression analysis was used to find independent predictors of eGFR at one-year post-transplantation. Candidate variables were first tested individually in univariate analyses (Table 17) before selection for multivariable analysis (Table 18). For every increment in K score, the analysis

predicted a drop in eGFR by 2.8 mL/min/1.73m^2 in the first year of transplantation (p<0.001). In this analysis, the strongest predictor of inferior eGFR was re-transplantation, associated with a reduction in eGFR by 10mL/min/1.73m^2. This may have been due to higher sensitisation in these patients, complex anatomy and possibly inferior organ offering if more advanced in recipient age. Re-transplantation, and CIT were all independently predictive of lower eGFRs at one year.

Table 17 - Univariate linear regression analyses of eGFR at one-year post-transplant

Variable	One-year post-transplant eGFR (n=399)		
	Coefficient	95% CI	p value
K score (0-12)	-4.06	-5.23 – -2.88	<0.001
Donor age	-0.65	-0.78 – -0.52	<0.001
Donor sex			
Male	Reference	–	–
Female	-5.54	-9.74 – -1.34	0.01
Donor type			
DBD	Reference	–	–
DCD	0.35	-4.18– 4.88	0.88
Donor cause of death			
Stroke	Reference	–	–
Other	4.24	1.48 – 7.01	0.003
UKKDRI	-18.28	-23.59 – -12.95	<0.001
Recipient age (years)	-0.07	-0.29 – 0.15	0.04
Recipient sex			
Male	Reference	–	–
Female	-1.96	-6.35 – 2.42	0.38
Recipient ethnicity			
Non-black	Reference	–	–
Black	-0.76	-3.06 – 1.55	0.52
Recipient diabetes			
Non-diabetic	Reference	–	–

Diabetic	-0.32	-7.53 – 7.90	0.93
Graft number			
1	Reference	–	–
>1	-7.72	-13.88 – -1.57	0.01
HLA mismatch level†			
1	Reference	–	–
>1	-1.66	-7.86 – 4.54	0.60
Cold ischaemia time (minutes)	0.01	-0.02 – 0.0	0.06

†Defined according to the UK allocation policy 2017 for deceased donor kidneys and was based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was a mismatch of 000; level 2 was a 0 HLA-DR and a 0/1 HLA-B mismatch; level 3 was a 0 HLA-DR and a 2 HLA-B mismatch, or a 1 HLA-DR and a 0/1 HLA-B mismatch; and level 4 was a 2 HLA-DR or a 1 HLA-DR and 2 HLA-B mismatch.

Table 18 - Multivariable linear regression analyses of eGFR at one-year post-transplant

Variable*	One-year post-transplant eGFR (n=399)		
	Coefficient	**95% CI**	**p value**
K score (0-12)	-2.80	-4.17 – -1.43	<0.001
Donor sex			
Male	Reference	–	–
Female	-3.19	-7.32 – 0.94	0.13
Donor cause of death			
Stroke	Reference	–	–
Other	0.41	-2.42 – 3.24	0.77
UKKDRI	-13.02	-19.28 – -6.74	<0.001
Recipient age (years)	-0.05	-0.22 – 0.12	0.61
Cold ischaemia time (minutes)	-0.01	-0.02 – 0.00	0.03
Graft number			
1	Reference	–	–
>1	-10.19	-16.08 – -4.31	0.001

*Donor age was removed from these analyses due to multicollinearity with UKKDRI

2.4.3 Graft and patient survival

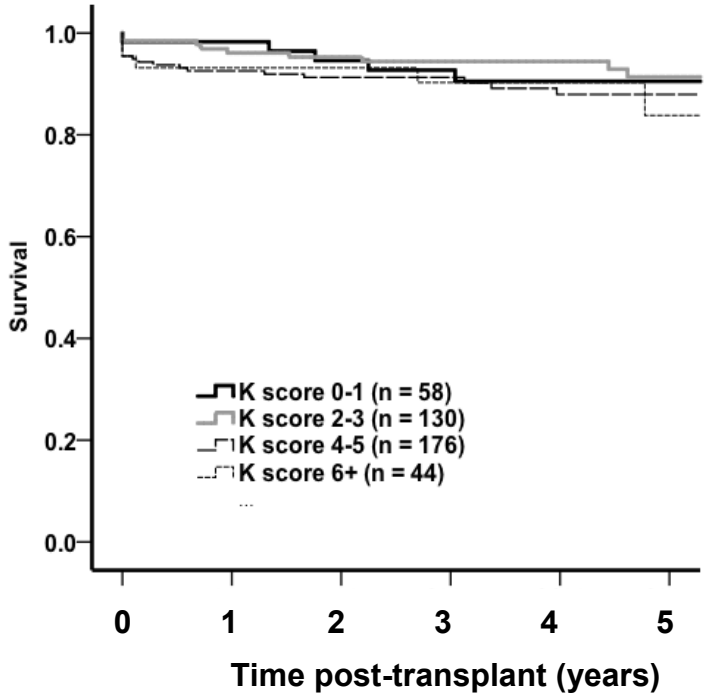
DCGS for kidney transplant recipients were high throughout the study cohort (Table 19).

Table 19 - Percentage of kidney transplant recipients with functioning grafts

	Death-censored graft survival	**Number in cohort**
Year 1 post-transplantation	94%	n=385
Year 3 post-transplantation	92%	n=377
Year 5 post-transplantation	89%	n=89

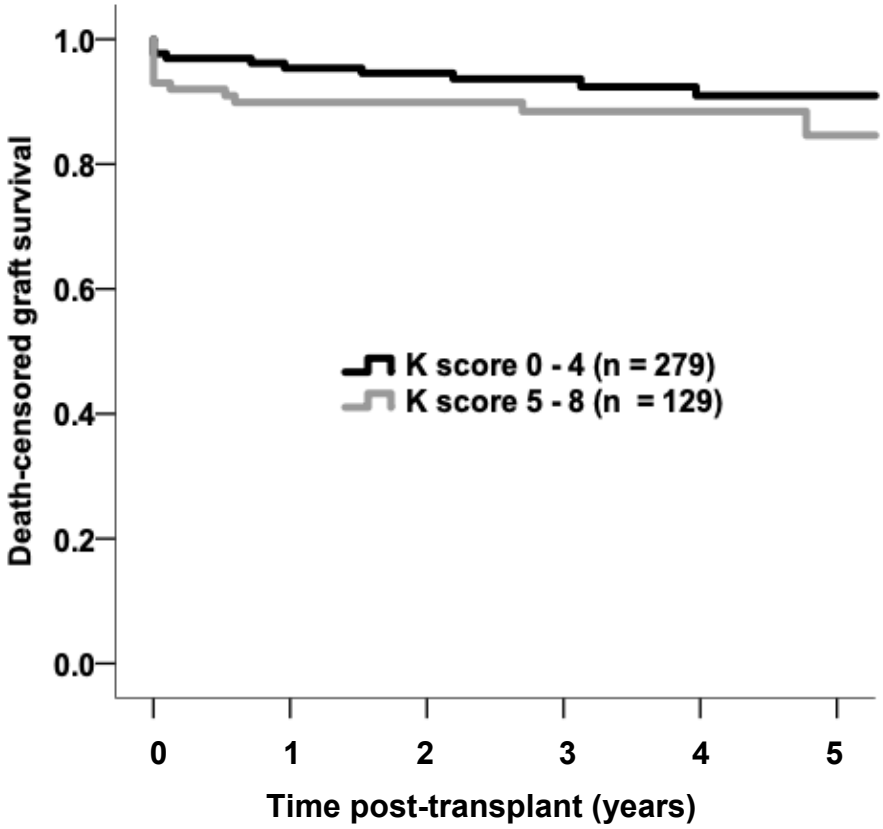
After stratifying by overall K score, no difference in DCGS was found (p=0.72; Figure 13). Interestingly, even organs with apparently severe chronic histological changes at implantation (K score 6 and above) had DCGS of more than 80% at five years, despite being implanted as single grafts (n=44).

Figure 13 - Karpinski (K) score and death-censored graft survival in single kidney-only transplants from deceased donors (n=408).



When comparing low and high K score groups there was no statistically significant difference in DCGS (p=0.26;Figure 14).

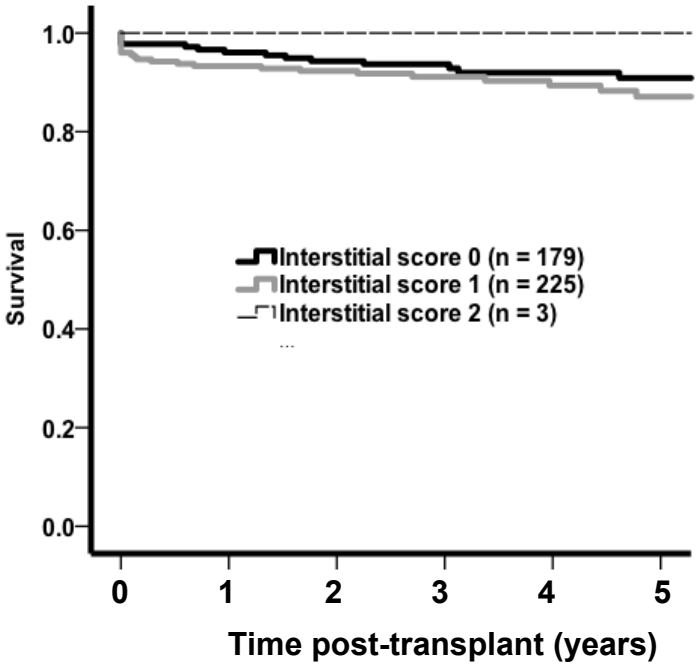
Figure 14 - Karpinski (K) score and death-censored graft survival, K score 0-4 (n=279) versus K score 5-8 (n=129)



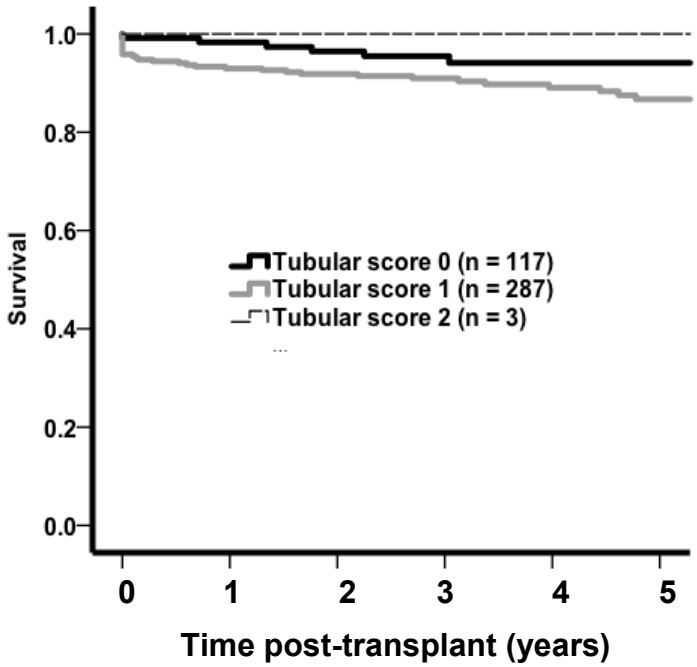
The individual components that make up the K score were also examined to see if any one element was associated with graft loss. However, no association between the interstitial (p=0.64), tubular (p=0.34), glomerular (p=0.78) and vascular component scores (p=0.30) and DCGS was found (Figure 15).

Figure 15 -Karpinski score components and death-censored graft survival (DCGS) in single kidney-only transplants from deceased donors (n=408). (A) Interstitial (p=0.64), (B) Tubular (p=0.34), (C) Glomerular (p=0.78) and (D) Vascular compartment scores (p=0.30)

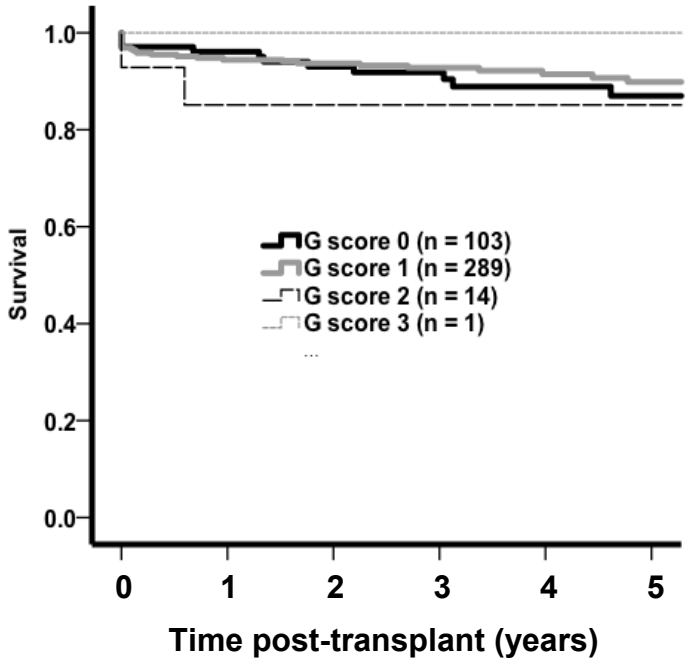
(A)



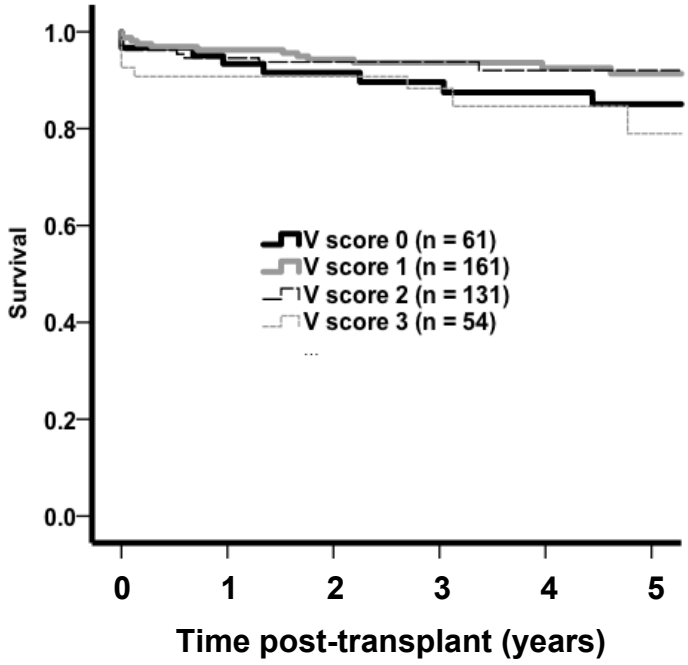
(B)



(C)


1.0
0.8
0.6
0.4
0.2
0.0
Survival
G score 0 (n = 103)
G score 1 (n = 289)
G score 2 (n = 14)
G score 3 (n = 1)
0
1
2
3
4
5
Time post-transplant (years)

(D)

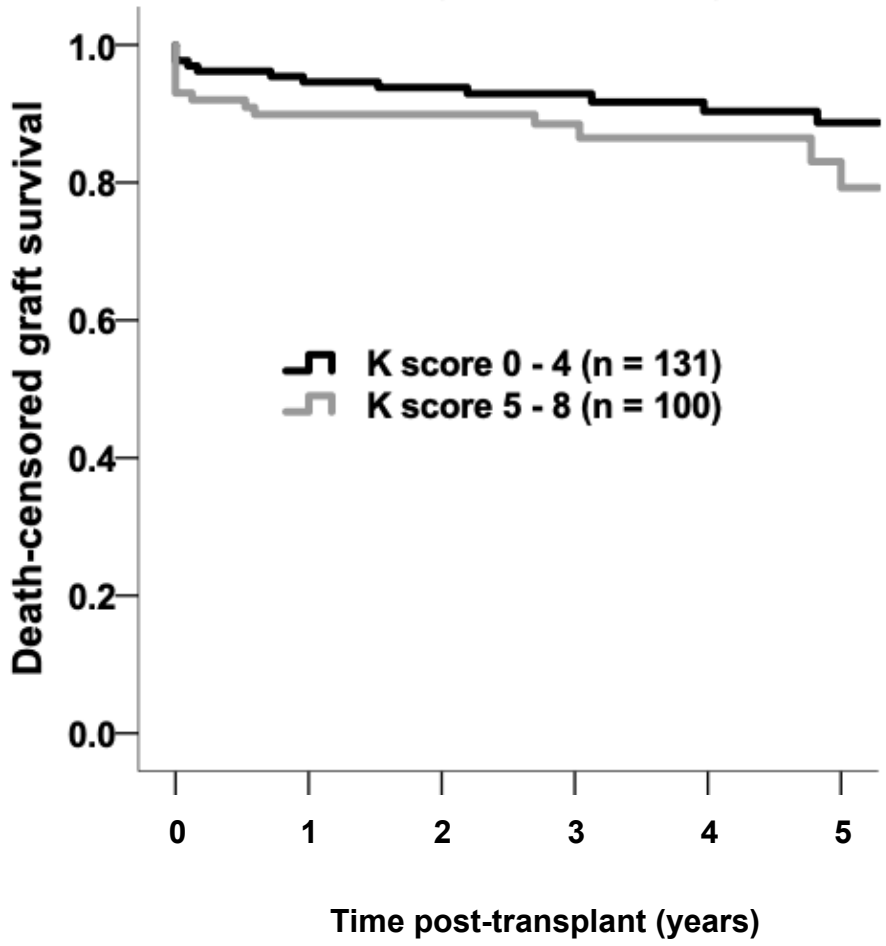

1.0
0.8
0.6
0.4
0.2
0.0
Survival
V score 0 (n = 61)
V score 1 (n = 161)
V score 2 (n = 131)
V score 3 (n = 54)
0
1
2
3
4
5
Time post-transplant (years)

Overall, the rate of PNF was 3.2% (n=13). A lower rate of PNF in kidneys with a K score of 0-4 was observed, compared to those that scored 5-8 (1.8% versus 6.2%, p=0.02). In a univariate logistic regression analysis, comparing the PNF rates in kidneys scoring 0-4 vs 5+, the higher K score was not associated with PNF (OR 0.3 95% CI 0.09-1.25, p=0.12). This may have reflected to low rates of pNF in both groups, making analysis of PNF in multivariable analyses challenging with this sample size.

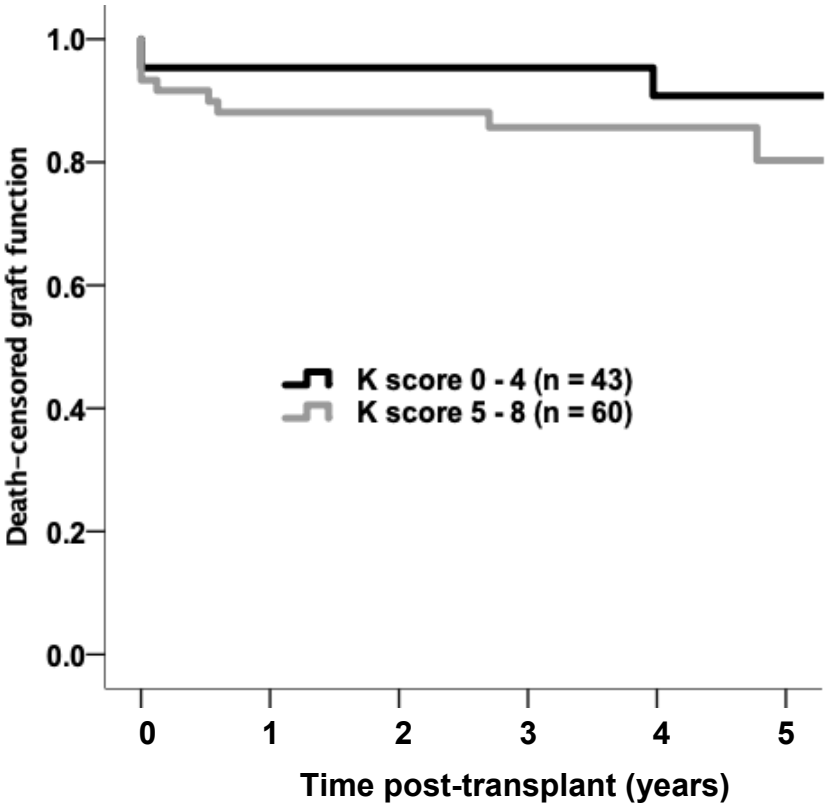
Subgroup analyses were undertaken to determine if higher K scores were associated with worse DCGS in increased risk organs, such as those from donors with more advanced ages or higher donor risk indices. No statistically significant differences in DCGS between kidneys with overall K scores 0-4 versus 5-8 were observed, when donors aged ≥50 years (n=231), ≥60 years (n=103), or UKKDRI >1.35 (n=137) were examined (Figure 16A-C). Of note, seven kidneys implanted as single grafts had K scores of ≥7. At the end of the follow-up period, all of these grafts were still functioning (recipients were dialysis independent).

Figure 16 - Sub-group analyses of Karpinski (K) score and DCGS in higher risk deceased donor groups. (A) K score 0-4 versus 5-8 in donors aged ≥ 50 years old (p=0.19); (B) K score 0-4 versus 5-8 in donors aged ≥ 60 years old (p=0.24); (C) K score 0-4 versus 5-8 in donors aged ≥ 50 years old (p=0.19); (B) K score 0-4 versus 5-8 in donors aged ≥ 60 years old (p=0.24); (C) K score 0-4 versus 5-8 in donors with UKKDRI >1.35 (p=0.44).

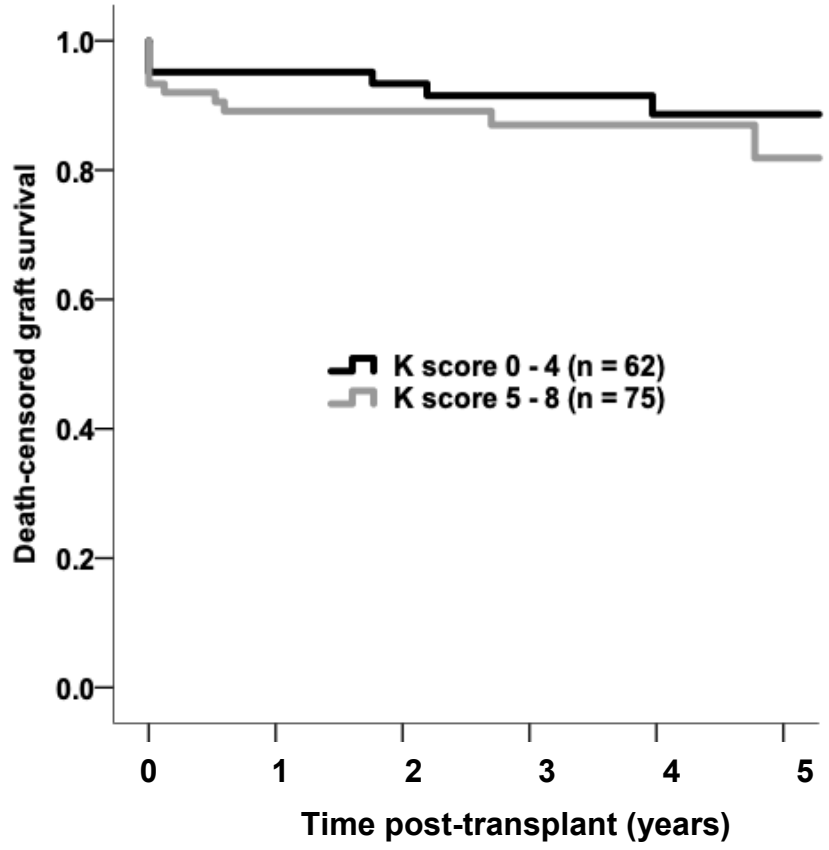
(A)



(B)


Death-censored graft function
1.0
0.8
0.6
0.4
0.2
0.0
K score 0 - 4 (n = 43)
K score 5 - 8 (n = 60)
0
1
2
3
4
5
Time post-transplant (years)

(C)


Death-censored graft survival
1.0
0.8
0.6
0.4
0.2
0.0
K score 0 - 4 (n = 62)
K score 5 - 8 (n = 75)
0
1
2
3
4
5
Time post-transplant (years)

Univariate Cox regression analysis of DCGS showed that only re-transplantation and UKKDRI had p<0.10 (Table 20), however K score did not. Re-transplantation and UKKDRI were therefore the only variables included in the multivariable analysis. Both re-transplantation and UKKDRI were independently associated with DCGS (HR (95% CI) 3.20 (1.61-6.37), p=0.001; and 2.15 (1.03-4.51), p=0.04, respectively).

Table 20 - Univariate variable Cox regression analysis of death-censored graft survival (DCGS) (n=408)

Variable	Hazard ratio	95% CI	p value
Karpinski score	1.1	0.94 – 1.33	0.20
Donor age	1.04	1.00 – 1.04	0.12
Donor sex			
Male	Reference	-	-
Female	1.12	0.60 – 2.10	0.71
Donor cause of death			
Stroke	Reference	-	-
Trauma	1.19	0.57 – 2.49	0.65
Other	1.58	0.57 – 4.39	0.38
UKKDRI	0.57	0.30 – 1.01	0.09
Recipient age	1.00	0.97 – 1.02	0.72
Recipient sex			
Male	Reference	-	-
Female	1.03	0.53 – 1.96	0.93
Recipient ethnicity			
Non-black	Reference	-	-
Black	1.34	0.70 – 2.60	0.38
Recipient diabetes status			
Non-diabetic	Reference	-	-
Diabetic	1.10	0.39 – 2.05	0.88
Graft number			
1	Reference	-	-
>1	3.00	1.50 – 5.95	0.002
Donor type			
DBD	Reference	–	–
DCD	1.01	0.51 – 1.99	0.99
Cold ischaemia time	1.00	1.00 – 1.00	0.17
HLA mismatch level†			
1	Reference	–	–
>1	0.94	0.39 – 2.25	0.89

†Defined according to the UK allocation policy 2017 for deceased donor kidneys and was based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was a mismatch of 000; level 2 was a 0 HLA-DR and a 0/1 HLA-B mismatch; level 3 was a 0 HLA-DR and a 2 HLA-B mismatch, or a 1 HLA-DR and a 0/1 HLA-B mismatch; and level 4 was a 2 HLA-DR or a 1 HLA-DR and 2 HLA-B mismatch.

Patient survival was 97% at one year (n=398), 95% at three years (n=386), and 93% at five years post-transplantation (n=379). There was no association between K score and patient death (low versus high K score groups; p=0.29). Patient survival was 99% (n=275) in the low K score group and 95% (n=122) in the high K score group at one year. At three years, patient survival was 95% (n=265) in the low K score group and 94% (n=121) in the high K score group. No candidate variables had p<0.10 on univariate Cox regression analysis, including K score.

2.4.4 Sensitivity analysis: altering the threshold for biopsy adequacy

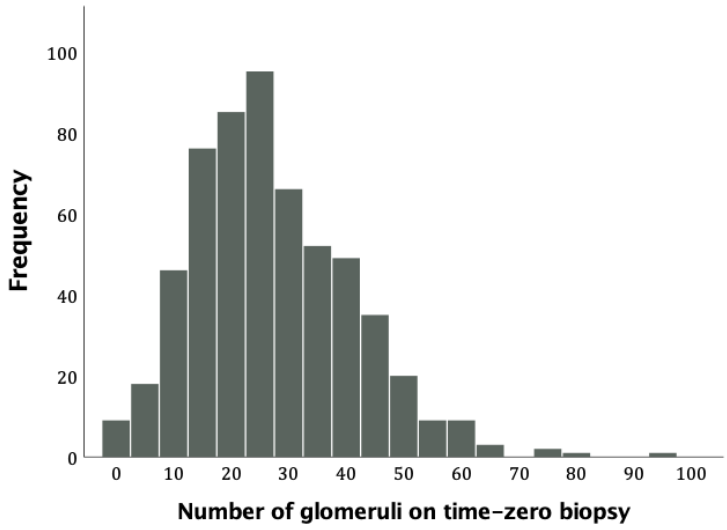
Karpinski et al defined a biopsy as inadequate if the sample contained ≥20 glomeruli, as the sample may not a representative of the population of glomeruli in the organ. Karpinski et al did not report why this threshold was chosen, and why this is different to Remuzzi's threshold of >25 glomeruli. However, the Banff reference guide to the Banff classification of renal allograft pathology suggests that adequacy may be defined as >10 glomeruli (222). Changing the definition of adequacy by reducing the number of necessary glomeruli to >10 would increase the number of available time-zero biopsies for analysis and increase the power of the study by adding an additional 53 patients to the dataset. In contrast, increasing the number of glomeruli needed for biopsy adequacy would reduce the number of biopsies for analysis but may provide better representation of the organ as a whole and improve the K score's predictive ability.

Lowering the threshold for biopsy adequacy to 10 glomeruli did not demonstrate an association between the K score and DCGS between kidneys with low K scores (0-4) and high K scores (5-12) (Figure 17). However, not all biopsies with <20 glomeruli were given a K score (missing data due to

biopsy inadequacy n=49, 9.5%). Likewise, increasing the threshold for biopsy adequacy to 30 or 40 glomeruli did not enable a difference in DCGS to be detected between low and high K scores.

Figure 17 – (A) Histogram showing the frequency of glomerular yield from kidney biopsies (B) Table demonstrating how altering the threshold for biopsy adequacy may change the death-censored survival difference between kidneys with high K scores versus low K scores

(A)



(B)

Threshold for biopsy adequacy	Sample size in each group *	Log Rank test p value
≥ 10 glomeruli	318 vs 143	0.05
≥ 30 glomeruli	293 vs 131	0.83
≥ 40 glomeruli	69 vs 37	0.43

* Groups: Karpinski score 0-4 versus 5-12

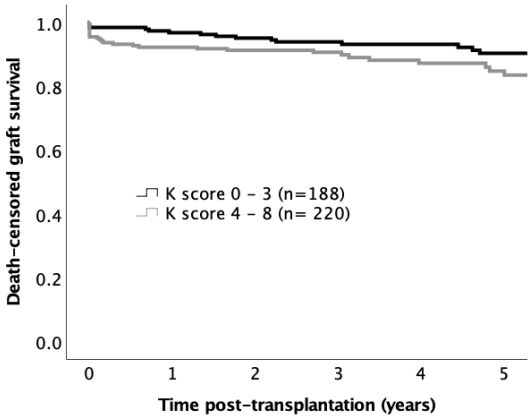
2.4.5 Sensitivity analysis: altering K score grouping

As discussed previously, recent utilisation guidance suggested kidneys with K scores 0-4 to be implanted as single grafts, and kidneys with K scores of 5 and above to be implanted as dual grafts or discarded. However, in original formulation of the Karpinski and Remuzzi scores, this threshold was defined as 0-3 implanted as single grafts, and scores of 4 and above to be implanted as dual grafts or discarded (75, 76). Therefore, the analysis was repeated to determine whether the original threshold was predictive of longer-term graft and patient survival, with the original threshold of ≥20 glomeruli.

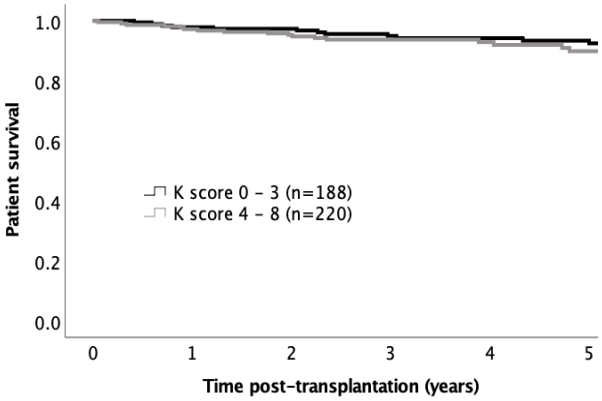
Univariate survival analyses found that comparing K scores 0-3 versus 4-12 was not associated with inferior graft survival (p=0.11) and patient survival (p=0.33) (Figure 18).

Figure 18 - Karpinski (K) score and (A) death-censored graft survival and (B) Patient survival between patients with K score 0-3 (n=188) versus K score 4-8 (n=220) (biopsy adequacy defined as ≥20 glomeruli)

(A)



(B)



2.4.6 Organ utilisation analysis

The use of PIKBs in deceased donor kidney transplantation have been advocated by some studies, typically in donors aged 60 years and over, to direct organ utilisation (e.g., grafts to be used as single transplants, as DAKT, or to be discarded) (70, 76, 180). Therefore, an analysis of older donors was undertaken to determine whether knowledge of K scores *prior* to implantation might have changed the eventual organ usage (either remaining as a single graft, or being implanted as a DAKT, or discarded).

Between 1 January 2012 and 31 December 2015, 75 single kidney transplants and 25 DAKT from deceased donors aged ≥60 years were performed, with adequate time-zero biopsies, at our centre. The donor, recipient and operative characteristics of these transplants are presented in in Table 21.

Table 21 - Donor and recipient characteristics included in the organ utilisation analysis.

	Single transplants			Dual adult kidney transplants		
	Actual (n = 75)	**Algorithm (n = 30)**	**p value**	**Actual (n = 25)**	**Algorithm (n = 12)**	**p value**
Donor age (years)	67 (64-71)	65 (63-69)	0.98	75 (70-76)	73.5 (68.8-76.3)	0.99
Donor sex						
Male	32 (64)	9 (30)	0.23	13 (52)	6 (50)	0.90
Female	43 (36)	21 (70)		12 (48)	6 (50)	
Donor type						
DBD	41(55)	18 (60)	0.62	10 (40)	5 (42)	1.00
DCD	34 (45)	12 (40)		15 (60)	7 (58)	
Donor cause of death						
Stroke	53 (71)	22 (73)		12 (48)	8 (67)	
Trauma	4 (5)	0 (0)	0.43	4 (16)	1 (8)	0.56
Other	18 (24)	8 (27)		9 (36)	3 (25)	
UKKDRI	1.80 (1.53-1.95)	1.57 (1.53 – 1.91)	0.44	1.91 (1.59-2.02)	1.98 (1.76-2.02)	0.99
Recipient age (years)	55 (45-61)	56 (47-64)	0.56			
Recipient sex						
Male	48 (64)	14 (47)	0.10	12 (48)	6 (50)	0.90
Female	27 (36)	16 (53)		13 (52)	6 (50)	
Recipient ethnicity	47 (63)	15 (50)	0.49	14 (56)	8 (67)	0.72
White	4 (5)	2 (7)		0 (0)	0 (0)	

Black Other	24 (32)	13 (43)		11 (44)	4 (33)	
HLA level†						
1	4 (5)	1 (3)		1 (4)	1 (8.5)	
2	20 (27)	11 (37)	0.40	6 (24)	3 (25)	0.68
3	41 (55)	17 (57)		12 (48)	7 (58)	
4	10 (13)	1 (3)		6 (24)	1 (8.5)	
Cold ischaemia time* (min)	740 (507-1108)	840 (594-1059)	0.44	750 (675-1020)	803 (668-1095)	0.71

Data expressed as median (IQR) or n (%)

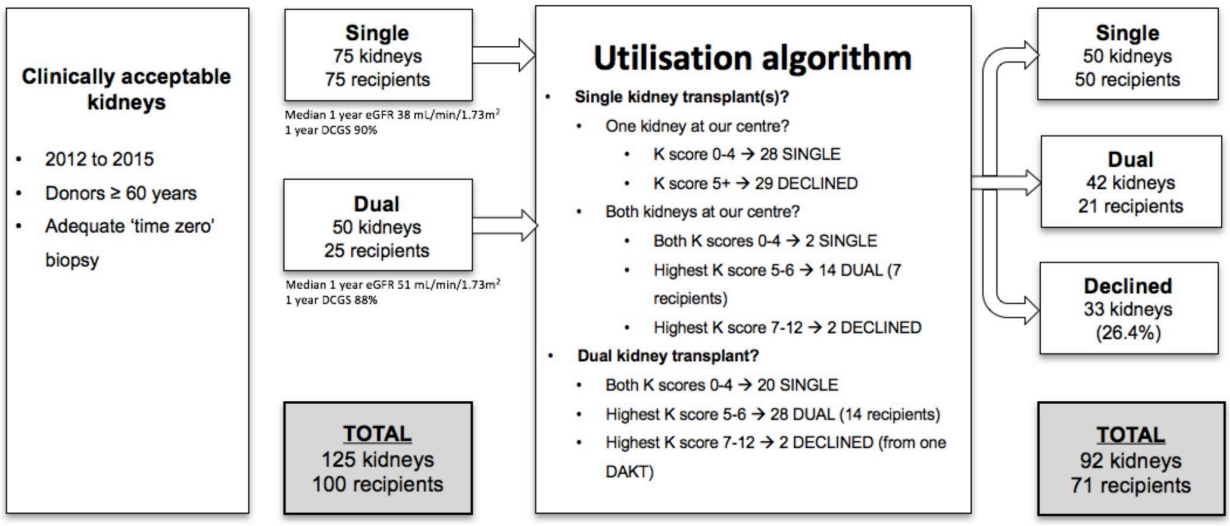
†Defined according to the UK allocation policy 2017 for deceased donor kidneys and was based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was a mismatch of 000; level 2 was a 0 HLA-DR and a 0/1 HLA-B mismatch; level 3 was a 0 HLA-DR and a 2 HLA-B mismatch, or a 1 HLA-DR and a 0/1 HLA-B mismatch; and level 4 was a 2 HLA-DR or a 1 HLA-DR and 2 HLA-B mismatch.

*For dual transplants, where there are individual cold ischaemia times for each kidney, the average of the two values is used

Of the entire cohort, 73 single and 6 DAKT were excluded due to no or inadequate time-zero biopsy. Four singles and seven DAKTs were excluded as these had PIKBs, and therefore the implant as single or DAKT had already been taken and would have biased the analysis.

Using the algorithm described in Section 2.3.6, organ utilisation decisions were redefined as if all K score results had been available pre-operatively and acted upon (Figure 19).

Figure 19 - Organ utilisation analysis considering whether following the utilisation algorithm, based on the Karpinski (K) score, would have altered the eventual use of the organ



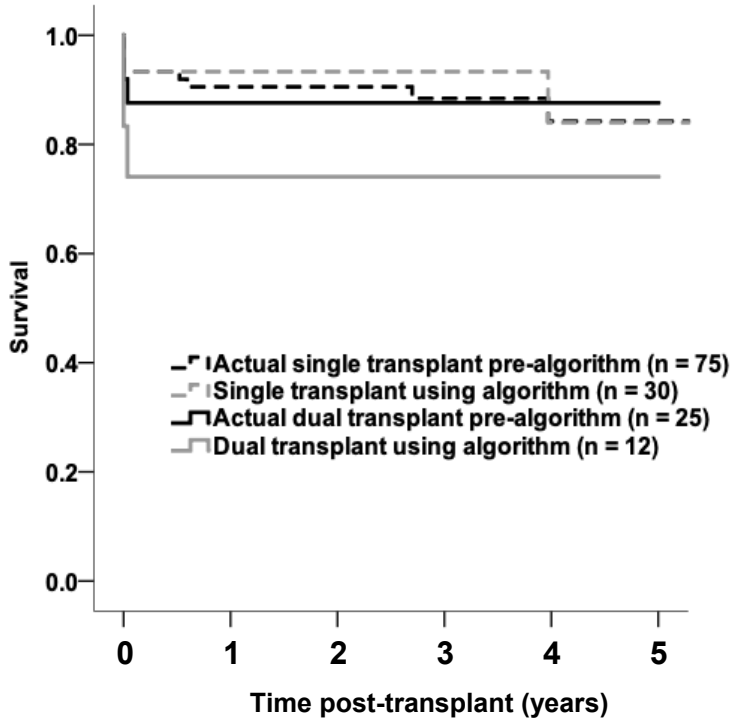
Using of PIKB to direct organ use through a commonly used kidney utilisation algorithm would have been resulted in a fall in single kidney transplants (50 versus 75 recipients) and fewer DAKT (21 versus 25 recipients). This analysis suggests that an estimated 26.4% fewer patients would have been transplanted if PIKBs had been used to direct utilisation decisions. For single kidney transplants and DAKT, where application of the algorithm did not lead to a change of utilisation group (i.e. knowledge of the K score pre-operatively would not have changed the eventual utilisation outcome), graft function and survival were analysed (Table 22 and Figure 20).

Table 22 - Actual one-year eGFR in single and dual adult kidney transplants (DAKT) versus outcomes with retrospective application of an organ utilisation algorithm based on Karpinski scores

	One-year post-transplant eGFR, mL/min/1.73m²		
	Actual	**Algorithm**	**p value**
Single kidney transplant	34 (20-48)*	42 (21-51)†	0.11
DAKT	49 (17-59)§	31 (5-55)‡	1.00

Data expressed as median (IQR)
*n = 62, †n = 26, §n = 23, ‡n = 10

Figure 20 - Actual death-censored graft survival of single and double adult kidney transplants (DAKT) from deceased donors aged ≥ 60 years, versus application of a retrospective organ utilisation algorithm based on Karpinski scores. Application of the algorithm did not significantly change death censored graft survival in single transplants (p=0.73) or dual transplants (p=0.31).



Use of the PIKB algorithm, with a reduction in organ utilisation, would not have resulted in better quality kidneys being implanted. This analysis indicates that no difference in graft function or survival would have been achieved with this strategy. It was not possible to know with certainty the effect of additional cold ischaemia time needed to wait for PIKB results. However, using the linear regression model in Table 18, it is estimated that one-year eGFR would be 0.6 mL/min/1.73m^2 lower for every extra hour of CIT. A five hour wait for PIKB results could therefore incur an estimated 3 mL/min/1.73m^2 drop in eGFR at one year.

2.5 Discussion

2.5.1 Study findings and their significance in transplantation

This chapter of my thesis examined the ability of a histological scoring system to predict outcomes in deceased donor kidney transplants. The findings from this study suggest that chronic histological changes at implantation, quantified by K scores, are not associated with long-term graft and patient survival. Sub-group analyses of older donors or those with high UKKDRI, and using different scoring thresholds, indicated that there was no association between K score and DCGS. Individual components making up the K score, comprising glomerular, vascular, interstitial, and tubular elements had no association with death-censored graft loss. Sensitivity analyses showed that altering the K score groupings to 0-3 versus 4-12 did not reveal an association with inferior outcomes. Likewise, lowering or raising the threshold for the necessary glomeruli for biopsy adequacy did not improve the predictive ability of the K score. However, in contrast preimplantation biopsies may have a predictive ability in early graft function – with a predicted a drop in eGFR by 2.8 mL/min/1.73m^2 in the first year.

The second part of the analysis suggested that routine use of PIKBs in donors aged 60 years and over, with adherence to recommended scoring thresholds (180, 219), would have resulted in a fall in the number of transplanted recipients by 26.4%, with no apparent improvement in graft quality.

Deceased donor discard rates in the UK are between 5-12%, of which a proportion were found likely to have been unnecessary (7)(8). The US has higher kidney discard rates (18-20%) compared to the UK (164). Although there are many reasons to explain the differing discard rates, including geographical, logistical, financial and policy differences (43), PIKB results are considered to be an influential factor (164, 165). Girolami et al looked at consistency between Remuzzi scores on PIKBs and the repeat Remuzzi score if the kidney was subsequently discarded. Remuzzi score derived from the whole discarded organ was lower than the initial PIKB score. This suggested that PIKBs may not be representative of the whole organ and may result in inappropriate organ discard (223).

Although PIKBs may provide further understanding of chronic donor changes at the time of transplantation, the their use in predicting long-term outcomes is put into question by the result of this study. More reliable strategies at predicting graft function and survival are needed to help clinicians in their endeavour to provide their patients with the best possible chance of dialysis independence, from a limited supply of organs, whilst minimising organ waste. Exploring potential methods are examined in more detail in Section 7.3.1 of the Concluding chapter.

2.5.2 Study findings in perspective

This is of particular interest given the a large national stepped-wedge cluster randomised trial in the UK, evaluating the impact of a national emergency PIKB service on the organ utilisation of kidneys offered from deceased donors aged over 60 years (Pre-Implantation Trial of Histopathology In renal Allografts – PITHIA; ISRCTN11708741). The trial provided UK transplant centres the option of obtaining a PIKB in order to access a Remuzzi score prior to transplantation. All UK transplant centres agreed to participate, and all centres had access to the PIKB service. The PITHIA study aimed to determine whether the provision of an urgent 24-hour donor kidney biopsy service, prior to transplantation, would increase the number and function of kidneys transplanted from donors aged over 60 years. However, the PITHIA trial was not designed to determine whether chronic changes at the time of implantation influence graft survival. On the contrary, the study assumes that PIKBs are predictive of graft survival and recommends utilisation of organs via the algorithm tested in this analysis (demonstrated by Figure 21). The results of the PITHIA trial are expected in 2023.

Figure 21 - Tea cup provided by the PITHIA trial to UK transplant units

The PITHIA trial investigators acknowledge that cold ischaemia time may be prolonged during which the biopsy is analysed (communication Emma Laing, clinical trial manager, NHSBT; Figure 22).

Figure 22 – Internal communication from the PITHIA trial to participating centres regarding estimated PITHIA report time (dated 31 May 2019). Estimated travel time between donor centre and histology centre.

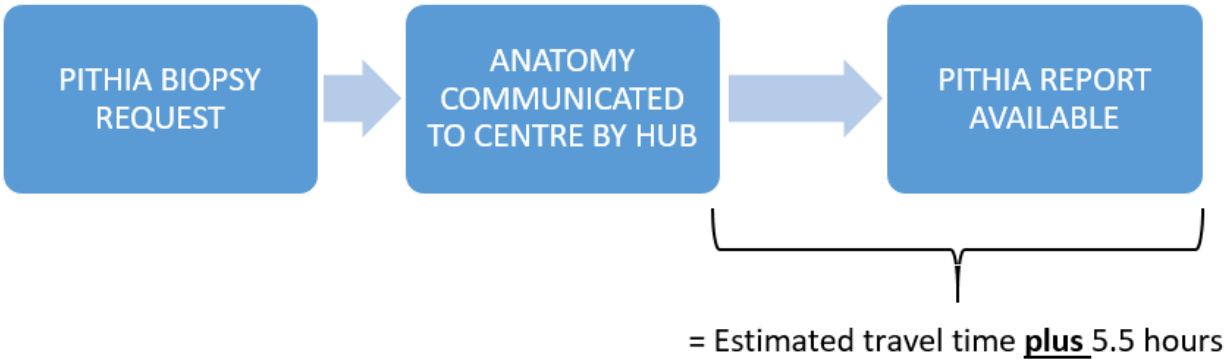


Figure reproduced with permission from Emma Lang, PITHIA trial manager

Using the linear regression model in Table 18 the additional waiting time of 5.5hrs would equate to an estimated reduction of graft eGFR of 3.3 mL/min/1.73m^2 at one-year post transplantation. However, this assumes a linear relationship between CIT and eGFR, and does not account for the difference in donor type (i.e., DCD versus DBD).

My results differ from other retrospective analyses in the literature, including a recent study performed by the University of Cambridge group (180). These discordant results may be explained by differences in donor selection, local implanting guidance and biopsy techniques. Although both studies had similar donor age profiles, the Cambridge group more frequently requested PIKBs, with 16% of single kidneys transplanted after a Remuzzi score was provided (180). This approach may have introduced a selection bias that could have influenced graft outcomes, especially since organ discard as a result of the PIKB cannot be fully accounted for. Additionally, many previous studies, including from the Cambridge group, have used wedge biopsies (9, 179) as these are thought to confer a lower risk of post-operative bleeding and provide high yield of glomeruli for analysis. However, wedge biopsies over-represent sclerosed sub-capsular glomeruli (224-226). This relates to the triangular shape of a wedge biopsy. In contrast, my analysis was based on core biopsies, which do not over-represent the sub-capsular glomerular population. Although core biopsies are thought to better sample deep blood vessels, my analysis did not find the vascular component of the K score to be associated with graft survival. Differences between my study results were unlikely to have derived from biopsy preparation, as sectioning and staining techniques were comparable between my centre and Cambridge (personal communication between Mr C. Callaghan (primary supervisor) and consultant renal histopathologist Dr V. Bardsley, Cambridge University Hospitals NHS Foundation Trust, May 2018). However, my study did not collect data on inter- or intra-observer variability of pathologists in scoring T0 biopsies and regional variation of biopsies.

Interestingly, there was a significant association between K score and eGFR in my study. K scores were also associated with a small drop in eGFR (2.8ml/min) at one year post transplant. Given that lower eGFR at one-year post-transplant is correlated with inferior long-term graft survival (227-229), and that my study found a higher rate of PNF in kidneys with K scores of 5-8, it is possible that a larger sample size might have shown that K score predicted graft survival. Our analysis would suggest that the use of empirically designed histological scoring systems and rigid scoring thresholds to determine organ utilisation seems overly simplistic (230). If chronic donor histological changes are associated with graft survival, histological scoring systems need to be developed using appropriate statistical techniques,

similar to those used to develop donor risk indices based on clinical factors (5, 169). This will require a significant dataset from multiple centres and may lead to combined clinico-pathological scoring systems (231, 232).

2.5.3 Study limitations

There are a number of study limitations worth considering. Firstly, 23% of the otherwise eligible kidneys were not biopsied and a further 30% were biopsied but did not contain sufficient glomeruli for scoring using Karpinski's criteria. Biopsies may not have been performed due to surgeon's preference, risk of bleeding in anticoagulated patients or other reasons based on perceived donor risk. The concern in this instance is the potential for selection bias, whereby biopsy data is not 'missing' at random. However, there was no statistical difference in donor, recipient, and operative characteristics between those with an adequate biopsy and those with no/inadequate biopsy, indicating that there was no demonstrable bias introduced at this stage. Adequacy rates could have been improved by using a punch biopsy technique over core biopsy techniques, as has been reported elsewhere (224, 233). As a result of the high biopsy inadequacy rates from core biopsies, my centre has begun using punch biopsies preferentially, with encouraging early results (see Appendix 1). Likewise a selection bias exists whereby only kidneys that were implanted had K score examined. This selection bias occurs because time-zero biopsies were taken post-reperfusion (and therefore only undertaken once a kidney was transplanted). Not knowing the K score of kidneys offered, but not implanted, is a weakness in the analysis.

Secondly, the retrospective utilisation analysis could only take account of those organs accepted for implantation at my unit; it is possible that access to an out-of-hours urgent histopathology service could have encouraged nephrologists and transplant surgeons to accept more marginal offers, knowing that the donor histological risk could be quantified prior to transplantation. The utilisation model described in Section 2.3.6 attempts to estimate graft usage, but may not take into account other influencing factors at the time of transplantation. It is not possible to determine its predictive ability, and therefore this limitation should be noted when considering its output.

Thirdly, this study only considered one of many histological scoring systems. It is possible that using different scoring systems may have led to positive results. Fourthly, pathology reports were not re-examined by a single histopathologist to ensure consistency between pathologists. It has been acknowledged that inter-pathologist variation exists (234), even amongst specialist renal pathologists (226, 235), which mask the association between chronic changes and graft outcome. This limitation should be considered when interpreting results from this study, and future studies designed to ensure consistence between histology reports is maintained. The fact that the lacked biopsy results from non-utilised kidneys must also caveat the findings.

The size of the study and/or the length of follow-up may have been insufficient to detect an association that might be present. Likewise, whilst study period of 2006-2015 was designed to gather enough patients int eh study, whilst leaving enough time after 2015 to gather outcome data for the latter patients, it may still not be completely representative of the donors today in 2023. This is a drawback of many retrospective studies in transplantation, where the donor population is constantly evolving.

Finally, this study considered biopsies taken post-reperfusion. It has not been extensively investigated whether post-reperfusion appearances of the kidney are the same as time-zero biopsies (typically taken during static cold storage, unperfused) when considering chronic histological appearances. Additional work is needed to determine whether it is valid to compare outcomes of studies which use different time points for the kidney biopsy.

2.5.4 Summary

In order for clinicians to make deceased donor kidney utilisation decisions on the basis of chronic donor histological changes, a tool with high predictive value for graft survival will be needed in order for it to be confidently applied to individual organs. My study suggests that, at present, there is insufficient evidence to support the systematic use of the Karpinski score to decide whether or not to implant a deceased donor kidney. Further work is needed to test other scoring systems, as well as those used in combination with other methods of assessing donor quality and risk, such as machine perfusion and large demographic registry analyses.

3 Chapter 3: Effect of delayed graft function on outcomes after deceased donor kidney transplantation

Work from this chapter has been presented at the European Society of Transplantation (ESOT) Congress 2019 in Copenhagen, Denmark, and published as an original article in a peer-reviewed journal:

Phillips BL, Ibrahim M, Greenhall GHB, Mumford L, Dorling A, Callaghan CJ. Effect of delayed graft function on longer-term outcomes after kidney transplantation from donation after circulatory death donors in the United Kingdom: A national cohort study. *American Journal of Transplantation*. 2021 Oct;21(10):3346-3355. doi: 10.1111/ajt.16574. Epub 2021 May 6. PMID: 33756062

In this chapter, I requested registry data from NHS blood and transplant (NHSBT). Additional data needed was extracted by colleagues at NHSBT (Maria Ibrahim and Lisa Mumford). I performed all statistical analyses. Prior to submission of the manuscript to peer-reviewed journals, the statistical methods were cross-checked by statistician Lisa Mumford. Coding necessary for novel mediation analysis was kindly provided by Professor Tyler J. VanderWeele, Harvard University.

There was no direct funding for this research

3.1 Abstract

A significant proportion of deceased donor kidney transplants do not function immediately post-operatively. This delayed graft function (DGF) is commonly defined as the need for dialysis within a week of kidney transplantation and is much more frequent following transplantation with a kidney from a DCD donor than after implantation of a kidney from DBD donor. While DGF after DBD donor kidney transplantation is known to be a poor prognostic factor for graft survival, seminal work published in *The Lancet* suggested that DGF after DCD donor kidney transplantation had no impact on longer-term graft survival. Since then, and partly due to this landmark work, DCD donors have become increasingly common in the UK and many other countries. DCD donors now make up approximately half of kidney deceased donors in the UK. Given increasing numbers of DCD donor kidney transplants, this study re-examined whether DGF is associated with worse long-term graft and patient survival, and if DGF duration was associated with poorer graft outcomes.

This study was a UK registry analysis of first single kidney-only transplants from controlled DCD donors aged ≥10 years to adult recipients (aged ≥18 years) between 2006-2016. Recipients were stratified according to the presence, and duration, of DGF. Kidneys with immediate post-transplant function were used as a reference group. Recipients not yet established on dialysis or who had already had a kidney transplant in the past were excluded. During the study period, 4714 kidney-only transplants from controlled DCD donors were transplanted into adult recipients. 2832 recipients (60.1%) had immediate graft function and 1882 (39.9%) had DGF. Of the 1847 recipients with DGF duration recorded, 926 (50.1%) had DGF <7 days, 576 (31.2%) had DGF 7-14 days, and 345 (18.7%) had DGF lasting longer than 14 days. After adjusting for risk factors in a multivariable analysis, the presence of DGF alone was not found to be a predictor of inferior long-term graft or patient survival. However, DGF duration of >14 days was found to be an independent predictor of death-censored graft failure (hazard ratio 1.7, p=0.001) and recipient death (hazard ratio 1.8, p<0.001) compared to kidneys with immediate graft function. This large national cohort study demonstrates that prolonged DGF after DCD donor kidney transplantation is

independently associated with inferior outcomes. DCD donor kidneys that take longer than 14 days to recover function have almost double the risk of both graft failure and patient death.

3.2 Introduction

In the United Kingdom (UK), deceased donor kidney transplantation in adult recipients is increasing, whilst numbers of living donor kidney transplants remains static (45). Up until UK transplant services were affected by the SARS-CoV-2 pandemic, a steady increase in transplantation of patients on the UK kidney transplant waiting list had been observed. This had been attributed to multiple factors, including a strong donation after circulatory death (DCD) donor kidney programme (45). The UK controlled DCD donor programme now makes up approximately 40% of deceased donor kidney transplantation. Although donation after circulatory death involves an additional hypoxic injury to the organ, these kidneys have demonstrated equivalent long-term outcomes compared to brain-death donor kidneys in UK analyses (6, 68, 138). Globally, DCD donor kidneys are still underused, representing only 11% of deceased donor kidneys in the United States. One of the reasons for this may be related to concerns regarding the high rate of delayed graft function (DGF) observed in DCD donor kidneys.

The perception that kidneys from DCD donors may be sub-optimal has been reinforced by the significant difference in early graft dysfunction rates after DCD versus DBD donor kidney transplantation. DGF is a clinical manifestation of acute kidney injury after kidney transplantation (146) and is most commonly defined as the need for dialysis within the first seven days of transplantation, regardless of cause (145). DGF may also be caused by any process impairing immediate function on the graft, such as acute rejection. The risk of DGF in kidneys from DCD donors is approximately 49%; twice as common as in DBD donor kidneys (68, 153). The duration of DGF likely reflects the organ's ability to recover from this injury. The incidence of DGF has risen over time, due to the growing proportion of DCD donors (236), as well as older donors with more comorbidities (3). Without immediate graft function, patients with DGF require in-patient dialysis, have increased risk of acute rejection(148, 149) and may be subjected to

invasive investigations such as graft core biopsy (150). This naturally leads to increased length of hospital stay and higher cost of care (147).

The presence of DGF is associated with double the risk of graft loss in living donor kidney transplantation (151) and 40% higher risk of graft loss in DBD donor kidney transplantation (6, 67, 152). The relationship between DGF and transplant survival in DCD donor kidneys has not been well-characterised due to the long delay between the start of national DCD donor programmes and eventual graft loss or patient death. Previous UK registry analyses found that DGF had *no* long-lasting influence on outcomes in first-time recipients following DCD donor kidney transplantation (6), albeit in a relatively small cohort. Likewise, US data has shown suggested that DGF in DCD donor kidneys did not lead to inferior graft survival (154). The long-term benefit of interventions aimed at reducing DGF in circulatory-death donor kidneys is questionable if DGF does not impact long-term graft survival.

These studies have provided reassurance to the transplant community, and have, at least in part, helped the expansion of controlled DCD donor kidney transplant programmes around the world. Since then, many more DCD donor kidneys have been transplanted, providing additional statistical power to investigate these issues. Increased statistical power also allows us to stratify according to DGF duration, allowing the degree of injury to be represented. The need for dialysis within the first week of transplantation may be subjective, and not necessarily reflect true acute kidney injury. More prolonged dialysis following transplantation may therefore be a better measure of acute kidney injury, with more significant implications on longer-term graft outcomes. Small single-centre studies have suggested that the duration of DGF may be correlated to inferior long-term graft outcomes in primarily in DBD kidney transplantation (237-241). Only two studies have considered duration of DGF in DCD donor kidneys, with contradictory findings (237, 242). Given the scepticism to utilise circulatory-death donors, examination of the effect of DGF duration on graft outcomes in this potential source of kidneys is of particular interest.

This risk-adjusted UK registry study assesses the influence of DGF presence, and its duration, on longer-term graft and patient survival after kidney transplantation from controlled DCD donors. These issues are particularly relevant given the on-going disparities in the utilisation of kidneys from DCD

donors between countries (43), and the growing number of interventions that seek to improve patient outcomes by reducing rates of DGF (182, 201, 243, 244).

3.3 Methods

3.3.1 Study population

Deceased donor kidney transplants from controlled DCD donors aged ≥10 years to adult recipients (aged ≥18 years) in the UK between January 2006 to December 2016 were identified through the UK National Transplant Registry, held by National Health Service Blood and Transplant (NHSBT). Follow-up of the study cohort included all data submitted to NHSBT by 1ST October 2018, with graft and patient survival censored at ten years of follow-up. All 23 adult kidney transplant centres in the UK submit mandatory data to the registry. Inclusion and exclusion criteria are summarised in Table 23.

Table 23 - Inclusion and exclusion criteria

Inclusion criteria
Controlled circulatory death donors [a,*]
Donor age ≥10 years
Recipient age ≥18 years
First-time transplant recipient
Kidney-only transplant
Exclusion criteria
Uncontrolled circulatory death donors [b]
Recipients not on regular dialysis prior to transplantation
Primary non-function
Second or subsequent kidney transplant
Double kidney transplantation
Multi-organ transplantation

[a] Expected donor cardiac arrest in intensive care or the operating room

[b] Donor out-of-hospital death, unsuccessful resuscitation, or unexpected cardiac arrest in a brain-dead donor in intensive care

* In an additional analysis, donation after brain death (DBD) donors were examined, to allow an overall comparison of the impact of DGF in cDCD versus DBD.

Kidneys undergoing hypothermic machine perfusion or normothermic regional perfusion were included in all analyses since there is evidence suggesting they influence the rate of DGF. Uncontrolled DCD donors, defined as donor out-of-hospital death, unsuccessful resuscitation, or unexpected cardiac arrest in a brain-dead donor in intensive care are not a commonly used source of deceased donor organs in the UK and were excluded. Analyses were limited to recipients of first-time transplants to avoid bias associated with retransplantation, namely higher recipient age and comorbidity, and increased HLA sensitisation. Recipients not on dialysis prior to transplantation were excluded from all analyses as these patients often do not require dialysis in the first week of transplantation due to residual native kidney

function (i.e., graft dysfunction may not manifest as the need for dialysis). Recipients of dual kidney transplant or multi-organ transplants were excluded. Recipients were followed for ten years post-transplant or until October 2018, whichever occurred first. Recipients with primary non-function of the transplant were also excluded.

3.3.2 Graft and patient outcomes and definitions

DGF was defined as the need for dialysis within seven days of kidney transplantation, regardless of cause, excluding primary non-function (145). The duration of DGF was defined as the number of days between transplantation and the last session of post-transplant dialysis. Recipients with DGF were grouped according to DGF duration:

- *Reference group:* *Immediate graft function*
- Group 1: DGF <7 days
- Group 2: DGF 7-14 days
- Group 3: DGF >14 days

These thresholds were considered to be clinically relevant since transplant biopsy is usually considered after the first or second week of DGF. Our group has also used these thresholds in previous work (237).

Graft and patient outcome measures included death-censored graft survival (DCGS), and patient survival. DCGS was defined as the number of days from transplantation to the date of graft failure (defined as the return to long-term dialysis or retransplantation, whichever happened first) censored for patient death. Patient survival was defined as the number of days from transplantation to patient death. PNF was defined as failure of the transplanted kidney to ever function within three months of transplantation (i.e., freedom from dialysis), regardless of cause. Acute rejection was defined as treatment for rejection within three months of transplantation. Donor risk was quantified by the

internationally used Kidney Donor Risk Index (KDRI) (169), as well as by the nationally validated UK Kidney Donor Risk Index (UKKDRI) (5). Cold ischaemic time (CIT) was defined as the time between donor cold perfusion and re-perfusion with the recipient's blood. Warm ischaemia time in DCD donors has two distinct phases: donor asystole phase (defined by this study as the time from circulatory arrest to perfusion with cold fluid) and recipient anastomosis phase (defined as the time from kidney removal from ice to perfusion with recipient blood). Donor asystole times in these donors were not analysed, as these data are not adequately captured by the registry. Donor risk was quantified by the Kidney Donor Risk Index (KDRI), consisting of donor age, ethnicity, history of diabetes or hypertension, serum creatinine, death from stroke, height, weight, donation after circulatory death, hepatitis C virus status, human leukocyte antigen (HLA) B and DR mismatch, cold ischaemia time, and double or en-bloc transplant (169). Obesity was defined as a body mass index of ≥30 kg/m^2. HLA mismatch level was defined according to the UK allocation policy for deceased donor kidneys and was based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci.

3.3.3 Statistical analysis

Median (IQR/range) patient follow-up was 4·0 (1.9-6.9/0-12.3) years, with graft survival censored after ten years post-transplantation. All data were tested for normality using the Shapiro-Wilk test. Differences in donor, recipient, operative or immunological characteristics between groups were examined using the Kruskal-Wallis test or the Chi-squared test. Number and percentage of missing variable data was detailed in the appropriate tables and complete case analysis was employed. Kaplan-Meier survival curves were used to demonstrate DCGS and patient survival; differences between groups were examined using the log-rank test.

Multivariable graft and patient survival analyses were performed in recipients with complete data on donor and recipient age, sex, and ethnicity, donor cause of death, recipient diabetes and hypertension, dialysis modality, HLA mismatch, HLA sensitisation, preservation method, and cold ischaemia and anastomosis times. Two-sided tests were conducted and $p<0.05$ was considered

statistically significant. Data were analysed using IBM SPSS Statistics for Macintosh version 25 (IBM, Armonk, NY, USA).

3.4 Results

3.4.1 Baseline characteristics

During the study period, 4714 kidneys from controlled DCD donors were transplanted that met inclusion criteria (see study flow diagram, Figure 21). Immediate graft function occurred in 2832 (60.1%) recipients with DGF in 1882 patients (39.9%). Median (interquartile range) patient follow-up was 4·0 (1.9-6.9) years. Donor, recipient and operative of study participants are shown in Table 24, categorised according to their DGF status (immediate graft function versus DGF).

Figure 23 - Flow diagram of eligible, excluded, and analysed transplant recipients

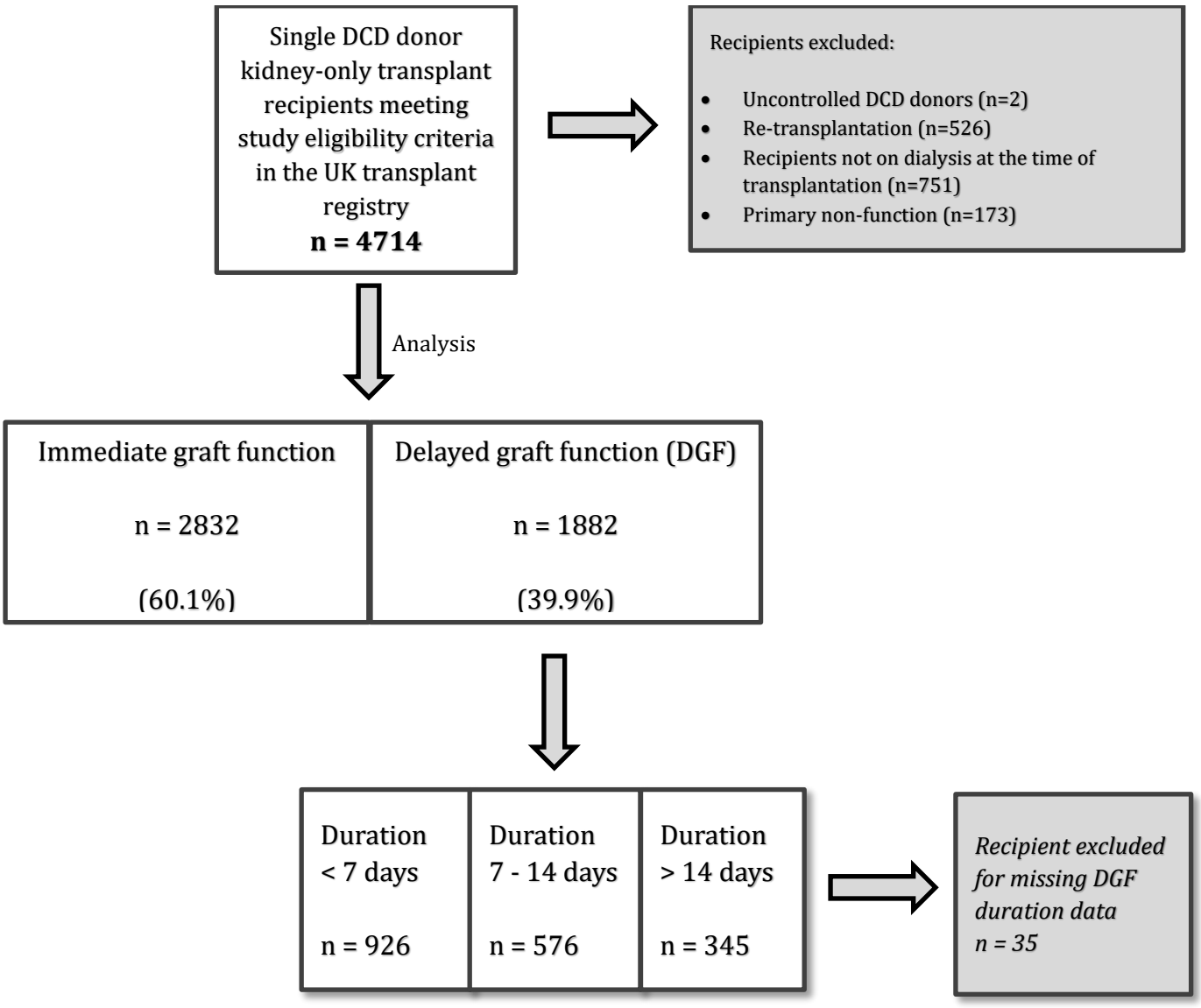


Table 24 - Baseline donor, recipient and operative characteristics of kidney transplants following donation after circulatory death with immediate graft function and delayed graft function

Variable	**Immediate function (n=2832)**		**Delayed graft function (n=1882)**		**p value**
Donor age (years)	52	(39-62)	54	(44-64)	<0.001
<40	708	(25.0%)	326	(17.3%)	<0.001
40-49	532	(18.8%)	364	(19.3%)	
50-59	745	(26.3%)	520	(27.6%)	
60-69	651	(23.0%)	496	(26.4%)	
>70	196	(6.9%)	176	(9.4%)	
Donor sex					<0.001
Male	1636	(57.8)	1199	(63.7)	
Female	1196	(42.2)	683	(36.3)	
Cause of death					0.001
Stroke	2169	(76.6)	1414	(75.1)	
Trauma	279	(9.9)	148	(7.9)	
Other	384	(13.6)	320	(17.0)	
Donor ethnicity					0.26
White	2741	(96.8)	1813	(96.3)	
Black	12	(0.4)	13	(0.7)	
Asian	40	(1.4)	36	(1.9)	
Other	38	(1.3)	20	(1.1)	
Donor diabetes mellitus	164	(6.1)	129	(7.2)	0.13
Donor hypertension	645	(24)	522	(29.5)	<0.001
Donor body mass index (kg/m^2)	26	(23-29)	27	(24-30)	<0.001
Kidney Donor Risk Index	1.45	(1.13-1.86)	1.55	(1.22-1.92)	<0.001

UK Kidney Donor Risk Index	1.07	(0.97-1.49)	1.16	(1.01-1.54)	<0.001
Terminal creatinine (micromol/L)	67	(53-88)	70	(55-96)	<0.001
Preservation method					<0.001
Static cold storage alone	2140	(79.1)	1503	(85.0)	
Hypothermic machine perfusion	538	(19.9)	261	(14.8)	
Normothermic regional perfusion	26	(1.0)	5	(0.3)	
Recipient age (years)	55	(46-63)	57	(47-64)	<0.001
<40	424	(15.0%)	233	(12.4%)	0.01
40-49	571	(20.2%)	336	(17.9%)	
50-59	782	(27.6%)	561	(29.8%)	
60-69	817	(28.8%)	583	(31.0%)	
>70	238	8.4%)	169	(9.0%)	
Recipient sex					<0.001
Male	1830	(64.7)	1317	(70.0)	
Female	999	(25.3)	564	(30.0)	
Recipient ethnicity					<0.001
White	2223	(78.8)	1381	(73.8)	
Black	160	(5.7)	174	(9.3)	
Asian	365	(12.9)	267	(14.3)	
Other	72	(2.6)	50	(2.7)	
Primary renal disease					0.001
Diabetes mellitus	252	(8.9)	226	(12.0)	
Hypertension	190	(6.7)	157	(8.3)	
Glomerulonephritis	633	(22.4)	385	(20.5)	
Polycystic kidney disease	522	(18.4)	345	(18.3)	
Other	1235	(43.6)	769	(40.9)	

Calculated reaction frequency (HLA sensitisation) >85%	56	(2.0)	30	(1.6)	0.34
Induction immunosuppression agent					0.001
ATG/ALG/OKT3	32	(1.3%)	6	(0.4%)	
Other antibody	2374	(98.7%)	1675	(99.6%)	
Recipient body mass index (kg/m²)	26.5	(23.5-30.0)	26.8	(24.0-30.3)	0.02
Dialysis modality					>0.001
Hemodialysis	1837	(64.9)	1475	(78.4)	
Peritoneal dialysis	994	(35.1)	407	(21.6)	
HLA mismatch level*					0.02
Level 1	76	(2.7)	41	(2.2)	
Level 2	691	(24.4)	416	(22.1)	
Level 3	1706	(60.2)	1134	(60.3)	
Level 4	359	(12.7)	291	(15.5)	
Warm ischemia times (mins)					
Donor asystole time	13	(11-15)	13	(11-15)	0.67
Recipient anastomosis time	36	(30-45)	40	(32-48)	<0.001
≤40	1837	(66.0%)	1026	(56.2%)	<0.001
>40	947	(34.0%)	799	(43.8%)	
Cold ischemia time (hours)	13.8	(10.7-17.3)	14.4	(11.3-17.8)	<0.001
<12	972	34.6%	539	29.0%	0.001
≥12 and <18	1226	43.6%	867	46.6%	
≥18 and <24	517	18.4%	371	19.9%	
≥24	96	3.4%	83	4.5%	

Data presented as median (interquartile range) and/or number (%).

Missing data: donor diabetes mellitus 236 (4.8%), donor hypertension 274 (5.6%), donor body mass index 132 (2.7%), KDRI 439 (9.3%), UKKDRI 230 (4.7%), donor terminal creatinine 652 (13.3%), preservation method 272 (5.8%), induction immunosuppression agent 657 (13.4%), recipient body mass index 1181 (24.2%), donor asystole time 2096 (42.9%), recipient anastomosis time 111 (2.3%), cold ischemia time 48 (1.0%).

*Defined according to the UK allocation policy 2017 for deceased donor kidneys and based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was 000 mismatch; level 2 0 HLA-DR and 0/1 HLA-B mismatch; level 3 0 HLA-DR and 2 HLA-B mismatch, or 1 HLA-DR and 0/1 HLA-B mismatch; and level 4 was 2 HLA-DR or 1 HLA-DR and 2 HLA-B mismatch.

The presence of DGF was associated with advancing donor age, male sex, hypertension, higher body mass index (BMI) and with higher terminal serum creatinine. As a result, the median kidney donor risk index (KDRI) and UK kidney donor risk index (UKKDRI) values are highest in the DGF population. DGF was also associated with preservation by static cold storage alone. Recipients with DGF were more likely to be older, male, to have higher BMI, originate from non-white ethnic backgrounds, on hemodialysis and with higher human leukocyte antigen (HLA) mismatch levels. DGF was also associated with longer cold ischemia and anastomosis times.

3.4.2 Presence of DGF and longer-term outcomes

On univariate analyses, the presence of DGF was associated with poorer graft and patient outcomes. Patients with DGF had lower median eGFR at one-, three-, and five-years post-transplant ($p<0.001$ throughout; Table 25).

Table 25- Estimated glomerular filtration rate (eGFR) of kidney transplant recipients with functioning grafts at one to five years, and presence and duration of delayed graft function (DGF)

	Immediate graft function	**DGF of any duration**	**p value [a]**	**DGF <7 days**	**DGF 7-14 days**	**DGF >14 days**	**p value [b]**
1-year eGFR (n=4283)	49 (37-63)	43 (31-57)	<0.001	45 (33-57)	44 (31-57)	36 (26-48)	<0.001
3-year eGFR (n=3119)	50 (37-65)	45 (32-60)	<0.001	46 (34-61)	46 (33-61)	40 (28-54)	<0.001
5-year eGFR (n=2066)	50 (36-65)	43 (30-59)	<0.001	43 (31-60)	46 (30-61)	40 (29-51)	0.04

Data expressed as median (interquartile range). eGFR expressed as mL/min/1.73 m^2.

[a] Immediate graft function versus DGF of any duration. [b] DGF < 7 days versus DGF 7-14 days versus DGF >14 days

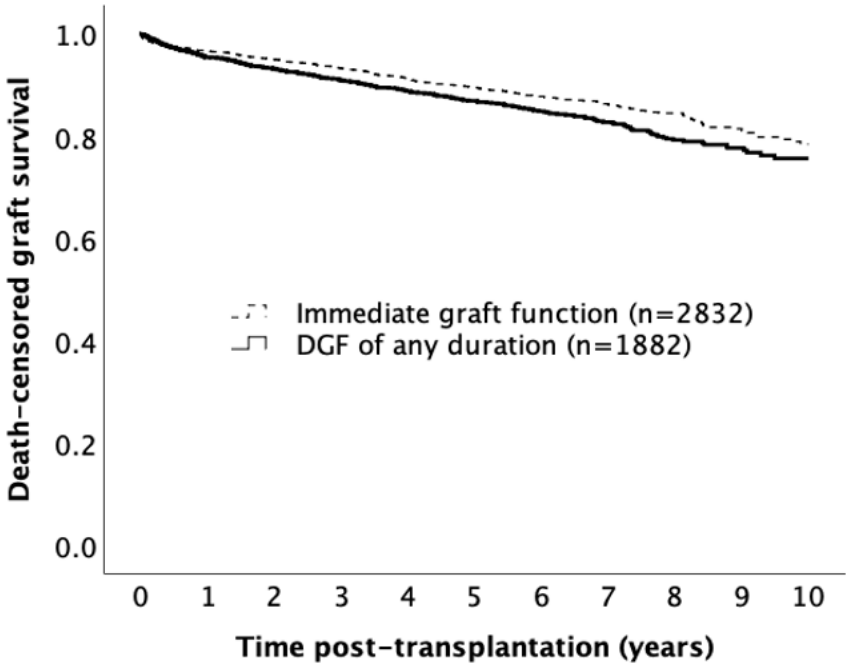
When adjusting for key donor and recipient risk factors (listed in Section 3.3.3), multivariable linear regression indicated that DGF was an independent predictor of inferior eGFR after one-year post-transplant (eGFR 4 mL/min/1.73m^2 lower than recipients with immediate function; p<0.001. The incidence of acute rejection within three months of transplantation was also higher in recipients with DGF compared to recipients with immediate graft function (p<0.001; Table 26).

Table 26 - Frequency of biopsy-proven acute rejection within three months post-transplantation, by early graft function. Immediate graft function versus delayed graft function of any duration, p<0.001.

Early graft function	Acute rejection, number (%)
Immediate graft function	175 (7.6%)
Delayed graft function	275 (16.1%)
Duration of delayed graft function	
<7 days	89 (10.4%)
7-14 days	104 (20.2%)
>14 days	78 (24.8%)

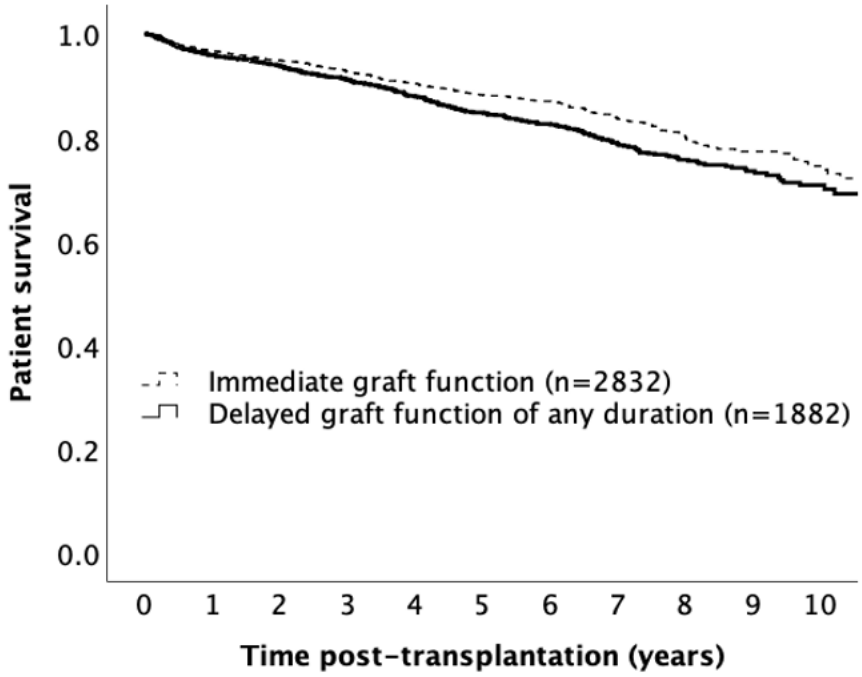
Unadjusted survival analysis showed that the presence of DGF was associated with inferior DCGS (p=0.006; Figure 24) and patient survival (p=0.005; Figure 25)

Figure 24 - Death-censored graft survival in recipients of kidneys from controlled DCD donors, by the presence of immediate graft function or delayed graft function (DGF) of any duration. Number of events in each group: immediate graft function (280 events)



Number at risk											
Immediate graft function	2832	2468	2020	1635	1295	997	764	577	398	239	117
DGF of any duration	1882	1620	1393	1206	994	803	624	474	310	180	84

Figure 25 - Patient survival in recipients of kidneys from controlled DCD donors, by the presence of immediate graft function or delayed graft function (DGF) of any duration. Number of events in each group: immediate graft function (333 events), DGF of any duration (306 events).



Number at risk											
Immediate graft function	2832	2501	2057	1672	1337	1036	798	606	418	257	125
DGF of any duration	1882	1646	1421	1237	1031	839	654	498	332	202	99

However, after adjustment for risk factors, Cox regression analysis suggested that there was no difference in death-censored graft loss in kidneys with DGF compared to kidneys that had immediate graft function (adjusted hazard ratio (aHR) 1.1 (95% confidence interval (CI) 0.9-1.4), p=0.19; Table 27)

Table 27 - Multivariable Cox regression for death-censored graft loss in donation after circulatory death donor kidney transplantation

Variable	Adjusted hazard ratio	95% confidence interval			p value
Graft function					
Immediate graft function	Reference				-
Delayed graft function (any duration)	1.1	0.9	to	1.4	0.19
Donor age (years)					
<40	Reference				-
40-49	1.2	0.9	to	1.7	0.28
50-59	1.9	1.4	to	2.5	<0.001
60-69	2.4	1.8	to	3.3	<0.001
≥70	2.7	1.8	to	4.1	<0.001
Donor sex					
Male	Reference				-
Female	0.9	0.8	to	1.1	0.39
Donor cause of death					
Trauma	Reference				-
Stroke	0.8	0.5	to	1.1	0.17
Other	1.5	1.2	to	2.0	0.001
Donor ethnicity					
White	Reference				-
Black	1.2	0.3	to	4.9	0.81
Asian	1.5	0.8	to	2.8	0.22

Other	1.5	0.7	to	3.4	0.33
Recipient age (years)					
<40	Reference				-
40-49	0.6	0.5	to	0.9	0.007
50-59	0.6	0.5	to	0.9	0.003
60-69	0.5	0.4	to	0.7	<0.001
≥70	0.7	0.5	to	1.0	0.07
Recipient sex					
Male	Reference				-
Female	1.0	0.9	to	1.3	0.55
Recipient ethnicity					
White	Reference				-
Black	1.3	1.0	to	1.9	0.08
Asian	1.1	0.8	to	1.5	0.46
Other	0.3	0.1	to	0.8	0.02
Recipient diabetes mellitus	1.3	0.9	to	1.7	0.12
Recipient hypertension	1.1	0.8	to	1.5	0.67
Calculated reaction frequency (%)					
<85	Reference				-
≥85 (highly sensitized)	1.2	0.6	to	2.6	0.49
Dialysis modality					
Peritoneal dialysis	Reference				-
Hemodialysis	1.3	1.0	to	1.6	0.02
HLA mismatch level*					
Level 1	Reference				-
Level 2	1.1	0.5	to	2.3	0.78
Level 3	1.5	0.7	to	3.0	0.27
Level 4	1.2	0.6	to	2.6	0.60

Cold ischemia time (hours)					
<12	Reference				-
≥12 and <18	1.2	1.0	to	1.5	0.08
≥18 and <24	1.4	1.1	to	1.8	0.01
≥24	1.4	0.9	to	2.2	0.12
Recipient anastomosis time (mins)					
≤40	Reference				-
>40	1.1	0.9	to	1.3	0.27
Organ preservation method					
Static cold storage alone	Reference				-
Hypothermic machine perfusion	1.0	0.7	to	1.2	0.83
Normothermic regional perfusion	1.1	0.3	to	4.6	0.86

Number of cases included in the Cox Regression model n=4325, events=477.

*Defined according to the UK allocation policy 2017 for deceased donor kidneys and based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was 000 mismatch; level 2 0 HLA-DR and 0/1 HLA-B mismatch; level 3 0 HLA-DR and 2 HLA-B mismatch, or 1 HLA-DR and 0/1 HLA-B mismatch; and level 4 was 2 HLA-DR or 1 HLA-DR and 2 HLA-B mismatch.

This multivariable analysis also showed that that increasing donor or recipient age and hemodialysis prior to transplantation were independently associated with poorer graft survival. Although CIT did not appear to be associated with inferior graft survival in this model, when DGF status was removed from the model, it effect on graft survival was restored (inferior graft survival with CIT >12 hours; aHR 1.2 (95% CI 1.02-1.51), p=0.03). Sensitivity analyses demonstrated no difference in DCGS when stratified by donor asystolic time quartile or whether data were missing (p>0.05 for both). Interaction tests demonstrated that the presence of DGF did not appear to modify the effect of donor age, CIT, and recipient anastomosis time on DCGS (Table 28).

Table 28 - Interaction tests for risk factors in the Cox regression model for death-censored graft survival

Risk factor		Hazard ratio (95% confidence interval)		p value for interaction
		Immediate graft function	Delayed graft function	
Donor age (years)	<40	1.0	1.0	0.09
	40-49	1.0 (0.7 - 1.6)	1.6 (0.9 - 2.6)	
	50-59	1.6 (1.1 - 2.3)	2.5 (1.5 - 4.0)	
	60-69	2.1 (1.4 - 3.0)	3.2 (2.0 - 5.2)	
	≥70	2.4 (1.4 - 4.2)	3.4 (1.9 - 6.1)	
Cold ischemia time (hours)	<12	1.0	1.0	0.42
	≥12 and <18	1.2 (0.9 - 1.7)	1.2 (0.9 - 1.7)	
	≥18 and <24	1.5 (1.0 - 2.1)	1.3 (0.9 – 2.0)	
	≥24	1.2 (0.6 - 2.3)	1.6 (0.9 - 3.0)	
Recipient anastomosis time (mins)	≤40	1.0	1.0	0.16
	>40	1.0 (0.8 - 1.2)	1.3 (1.0 - 1.6)	

Interaction test = modifier effect test; p value >0.05 suggests donor age, CIT and recipient anastomosis time do not modify the effect of delayed graft function on death-censored graft survival

After risk adjustment, there was no difference in patient survival between the DGF and immediate graft function groups (aHR 1.1 (95% CI 0.9-1.3), p=0.36, Table 29).

Table 29 - Multivariable Cox regression for patient death in donation after circulatory death donor kidney transplantation

Variable	Adjusted hazard ratio	95% confidence interval			p value
Graft function					

Immediate graft function	Reference				-
Delayed graft function (any duration)	1.1	0.9	to	1.3	0.36
Donor age (years)					
<40	Reference				-
40-49	1.3	0.9	to	1.8	0.11
50-59	1.4	1.0	to	1.8	0.05
60-69	1.6	1.2	to	2.2	0.002
≥70	1.6	1.1	to	2.3	0.02
Donor sex					
Male	Reference				-
Female	1.1	0.9	to	1.3	0.40
Donor cause of death					
Trauma	Reference				-
Stroke	0.8	0.6	to	1.1	0.14
Other	1.0	0.8	to	1.3	0.99
Donor ethnicity					
White	Reference				-
Black	1.0	0.3	to	4.1	0.98
Asian	0.9	0.4	to	2.1	0.83
Other	1.7	0.9	to	3.5	0.12
Recipient age (years)					
<40	Reference				-
40-49	1.8	1.1	to	3.0	0.02
50-59	2.8	1.7	to	4.3	<0.001
60-69	5.0	3.2	to	7.8	<0.001
≥70	7.1	4.2	to	11.5	<0.001
Recipient sex					
Male	Reference				-

Female	1.0	0.9	to	1.2	0.79
Recipient ethnicity					
White	Reference				-
Black	0.9	0.6	to	1.3	0.68
Asian	0.7	0.5	to	1.0	0.03
Other	0.9	0.5	to	1.6	0.67
Recipient diabetes mellitus	1.5	1.2	to	1.9	0.002
Recipient hypertension	1.1	0.8	to	1.4	0.74
Calculated reaction frequency (%)					
<85	Reference				-
≥85 (highly sensitized)	1.0	0.5	to	2.2	0.92
Dialysis modality					
Peritoneal dialysis	Reference				-
Hemodialysis	1.7	1.4	to	2.1	<0.001
HLA mismatch level*					
Level 1	Reference				-
Level 2	1.6	0.8	to	3.2	0.18
Level 3	1.6	0.8	to	3.1	0.18
Level 4	1.9	1.0	to	4.0	0.05
Cold ischemia time (hours)					
<12	Reference				-
≥12 and <18	1.1	0.9	to	1.3	0.48
≥18 and <24	1.2	0.9	to	1.5	0.17
≥24	1.1	0.7	to	1.6	0.82
Recipient anastomosis time (mins)					
≤40	Reference				-
>40	1.0	0.8	to	1.2	0.92

Organ preservation method					
Static cold storage alone	Reference				-
Hypothermic machine perfusion	1.1	0.8	to	1.2	0.92
Normothermic regional perfusion	0.7	0.1	to	4.7	0.68

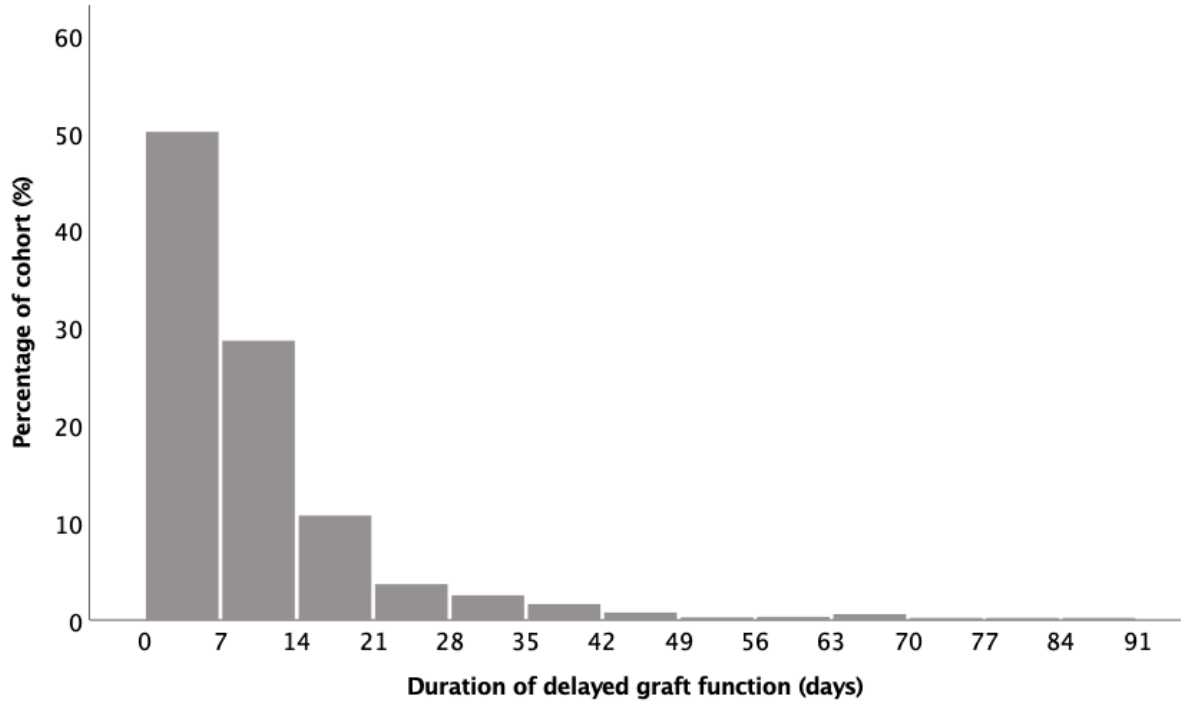
Number of cases included in the Cox Regression model n=4322, events=564.

*Defined according to the UK allocation policy 2017 for deceased donor kidneys and based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was 000 mismatch; level 2 0 HLA-DR and 0/1 HLA-B mismatch; level 3 0 HLA-DR and 2 HLA-B mismatch, or 1 HLA-DR and 0/1 HLA-B mismatch; and level 4 was 2 HLA-DR or 1 HLA-DR and 2 HLA-B mismatch.

3.4.3 Duration of DGF

The distribution of delayed graft function duration is shown in Figure 26. Of the 1882 patients with DGF post-transplant 35 recipients had no DGF duration recorded and had to be excluded from further analyses.

Figure 26 - Histogram showing the distribution of delayed graft function duration



Recipients with DGF were categorised into DGF duration groups. Of the 1847 kidney transplant recipients with DGF duration data recorded, 926 (50.1%) had DGF duration <7 days, 576 (31.2%) had DGF of 7-14 days, and 345 (18.7%) had DGF duration >14 days. Differences in baseline characteristics when DGF was stratified by duration are shown in *Table 30*.

Table 30 - Baseline donor, operative, immunological, and recipient characteristics of kidney transplants from controlled DCD donors by duration of delayed graft function (DGF). Data presented as number (%) or median (interquartile range).

Variable	**DGF <7 days (n=926)**		**DGF 7-14 days (n=576)**		**DGF >14 days (n=345)**		**p value**
Donor age (years)	54	(43-63)	54	(43-63)	56	(47-66)	0.003
<40	182	(19.7%)	96	(16.7%)	45	(13.0%)	<0.001
40-49	172	(18.6%)	123	(21.4%)	64	(18.6%)	
50-59	247	(26.7%)	167	(29.0%)	94	(27.2%)	
60-69	244	(26.3%)	148	(25.7%)	95	(27.5%)	
≥70	81	(8.7%)	42	(7.3%)	47	(13.6%)	
Donor sex							<0.001
Male	547	(59.1)	396	(68.8)	235	(68.1)	
Female	379	(40.9)	180	(31.3)	110	(31.9)	
Cause of death							0.58
Stroke	700	(75.6)	426	(74.0)	261	(75.7)	
Trauma	65	(7.0)	54	(9.4)	28	(8.1)	
Other	161	(17.4)	96	(16.6)	56	(16.2)	
Donor ethnicity							0.61
White	889	(96.0)	555	(96.4)	334	(96.8)	
Black	9	(1.0)	3	(0.5)	1	(0.3)	
Asian	20	(2.2)	9	(1.6)	3	(0.9)	
Other	8	(0.9)	9	(1.6)	3	(0.9)	
Donor diabetes	50	(5.7)	47	(8.6)	28	(8.5)	0.07
Donor hypertension	247	(28.4)	149	(27.9)	112	(34.1)	0.10
Donor body mass index (kg/m^2)	26	(24-30)	27	(24-30)	27	(24-30)	0.12

Kidney Donor Risk Index	1.51	(1.20-1.90)	1.52	(1.22-1.89)	1.59	(1.28-2.00)	0.03
UK Kidney Donor Risk Index	1.12	(1.00-1.54)	1.11	(1.02-1.53)	1.38	(1.03-1.57)	0.005
Terminal creatinine (micromol/L)	70	(56-94)	70	(55-98)	72	(53-101)	0.91
Preservation method							0.14
Cold storage only	712	(82.4)	473	(87.3)	283	(86.3)	
Hypothermic machine perfusion	149	(17.2)	68	(12.5)	44	(13.4)	
Normothermic regional perfusion	3	(0.3)	1	(0.2)	1	(0.3)	
Recipient age (years)	56	(47-64)	55	(46-63)	59	(50-65)	0.003
<40	122	(13.2%)	79	(13.7%)	31	(9.0%)	0.11
40-49	167	(18.0%)	110	(19.1%)	54	(15.7%)	
50-59	286	(30.9%)	163	(28.3%)	98	(28.4%)	
60-69	278	(30.0%)	170	(29.5%)	126	(36.5%)	
≥70	73	(7.9%)	54	(9.4%)	36	(10.4%)	
Recipient sex							0.50
Male	637	(68.8)	411	(71.4)	245	(71.2)	
Female	289	(31.2)	165	(28.6)	99	(11.6)	
Recipient ethnicity							0.55
White	687	(74.6)	418	(73.1)	245	(71.2)	
Black	77	(8.4)	55	(9.6)	40	(11.6)	
Asian	128	(13.9)	86	(15.0)	51	(14.8)	
Other	29	(3.1)	13	(2.3)	8	(2.3)	
Primary renal disease							0.19
Diabetes mellitus	112	(12.1)	63	(10.9)	46	(13.3)	
Hypertension	67	(7.2)	58	(10.1)	29	(8.4)	
Glomerulonephritis	213	(23)	104	(18.1)	61	(17.7)	

Polycystic kidney disease	168	(18.1)	106	(18.4)	64	(18.6)	
Other	366	(39.5)	245	(42.5)	145	(42.0)	
Recipient body mass index (kg/m²)	26.9	(23.8-29.9)	26.5	(24.0-30.5)	26.7	(24.0-30.7)	0.58
Dialysis modality							0.26
Hemodialysis	740	(79.9)	443	(76.9)	264	(76.5)	
Peritoneal dialysis	186	(20.1)	133	(23.1)	81	(23.5)	
HLA mismatch level							0.17
Level 1	22	(2.4)	13	(2.3)	5	(1.4)	
Level 2	224	(24.2)	124	(21.5)	61	(17.7)	
Level 3	536	(57.9)	356	(61.8)	218	(63.2)	
Level 4	144	(15.6)	83	(14.4)	61	(17.7)	
Calculated reaction frequency >85% (HLA sensitisation)	14	(1.5)	10	(1.7)	5	(1.4)	0.001
Induction immunosuppression agent **							0.15
ATG/ALG/OKT3	1	(0.3%)	4	(0.8%)	1	(0.3%)	
Other antibody	833	(99.9%)	513	(99.2%)	310	(99.7%)	
Warm ischemia times (min)							
Donor asystole time	13	(11-15)	13	(11-15)	13	(11-15)	0.22
Recipient anastomosis time	40	(32-49)	39	(32-47)	40	(32-49)	0.43
≤40	497	(55.2%)	338	(60.2%)	176	(53.3%)	0.08
>40	403	(44.8%)	223	(39.8%)	154	(46.7%)	
Cold ischemia time (hours)	14.2	(11.1-17.6)	14.3	(11.3-17.7)	15.3	(12.3-18.9)	0.001
<12	289	(31.7%)	168	(29.4%)	75	(22.0%)	0.01
≥12 and <18	415	(45.5%)	268	(46.9%)	162	(47.5%)	
≥18 and <24	167	(18.3%)	116	(20.3%)	83	(24.3%)	
≥24	41	(4.5%)	20	(3.5%)	21	(6.2%)	

Missing data: donor diabetes 92 (5.0%), donor hypertension 113 (6.1%), donor body mass index 53 (2.9%) UKKDRI 64 (3.5%), donor terminal creatinine 220 (11.9%), preservation method 113 (6.1%), induction immunosuppression agent 657 (13.4%), recipient body mass index 479 (25.9%), donor asystole time 858 (46.5%), recipient anastomosis time 56 (3.0%), cold ischemia time 22 (1.2%).

*Defined according to the UK allocation policy 2017 for deceased donor kidneys and based on donor-recipient differences at HLA-A, HLA-B and HLA-DR loci: level 1 was 000 mismatch; level 2 0 HLA-DR and 0/1 HLA-B mismatch; level 3 0 HLA-DR and 2 HLA-B mismatch, or 1 HLA-DR and 0/1 HLA-B mismatch; and level 4 was 2 HLA-DR or 1 HLA-DR and 2 HLA-B mismatch.

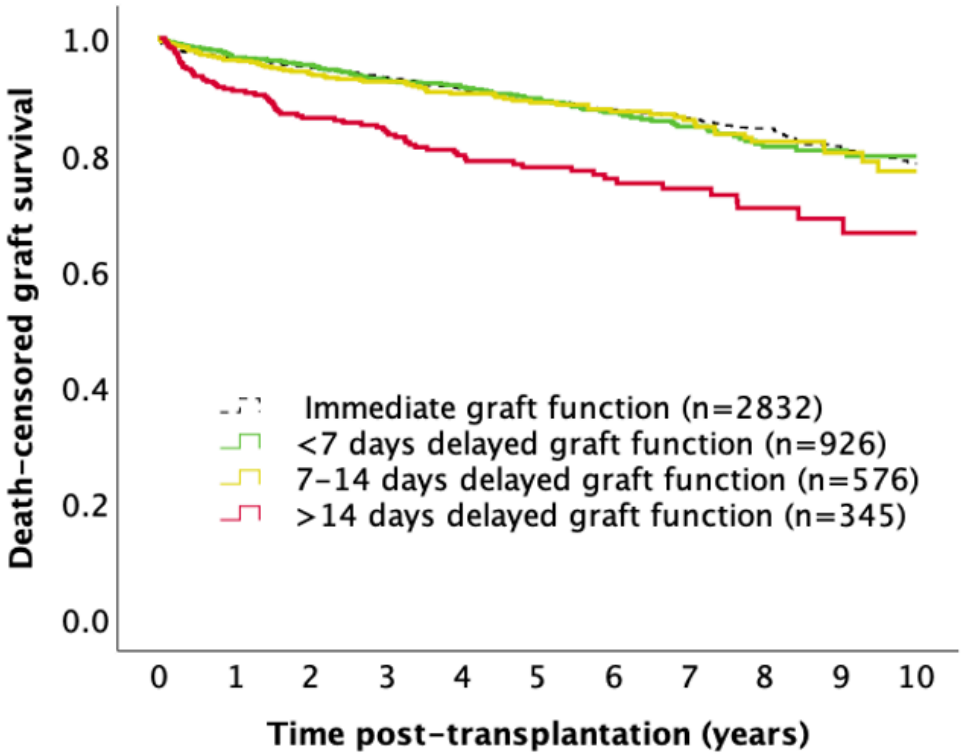
** This is how NHSBT recorded induction immunosuppression data at the time

This univariate analysis indicated that DGF of >14 days was associated with older donors and recipients, kidneys with increased KDRI and UKKDRI, and kidneys exposed to longer CITs.

3.4.4 Death-censored graft survival

Prolonged DGF of duration >14 days was associated with worse transplant function compared to shorter duration DGF at one-, three- and five-years post-transplant ($p<0.001$, $p<0.001$ and $p=0.04$ respectively; Table 25). On multivariable linear regression analysis, increasing DGF duration was an independent predictor of poorer graft function one-year post-transplantation, with a 7 mL/min/1.73m^2 reduction in eGFR in kidneys with >14 days DGF relative to kidneys with immediate function ($p<0.001$). Prolonged DGF >14 days was associated with a 2.5 times higher rate of biopsy-proven acute rejection within three months of transplantation compared to those with DGF lasting <7 days (Table 26). There was no difference in death-censored graft survival (DCGS) between patients with immediate graft function and short duration DGF (<7 days, $p=0.85$), or medium duration DGF (7-14 days, $p=0.61$, Figure 27). However, there appeared to be a marked threshold effect when DGF duration exceeded 14 days, with a significantly inferior DCGS compared to all other groups ($p<0.001$).

Figure 27 - Death-censored graft survival in recipients of kidneys from controlled DCD donors, by the duration of delayed graft function (DGF). Recipients with more than two weeks of delayed transplant function had significantly worse graft survival (p<0.001). Number of events in each group: immediate graft function (280 events); <7 days DGF (104 events); 7-14 days DGF (69 events); >14 days DGF (72 events).



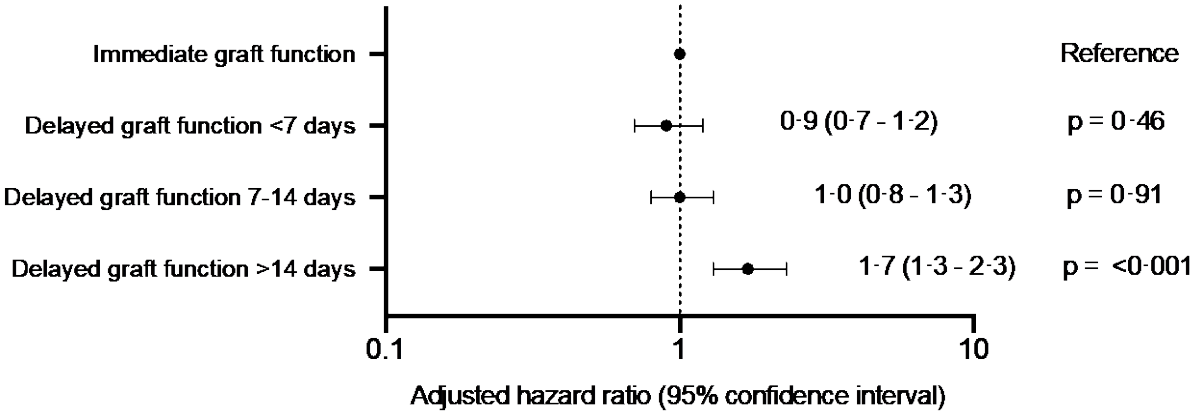
Number at risk

Immediate graft function	2832	2468	2020	1635	1295	997	764	577	398	239	117
DGF <7 days	926	816	711	613	507	407	321	237	155	88	45
DGF 7-14 days	576	508	442	319	322	255	202	165	106	63	30
DGF >14 days	345	269	224	200	159	137	98	70	47	28	9

Using immediate graft function as a reference group, there was no increased adjusted risk of death-censored graft loss in recipients with DGF duration <7 days (aHR 0.9 (95% CI 0.7-1.2), p=0.46), or

DGF duration 7-14 days (aHR 1.0 (95% CI 0.8-1.3), p=0.91) on multivariable analysis (Figure 28). The threshold effect observed in the univariate survival analysis was also seen in the multivariable analysis. DGF exceeding 14 days was independently associated with almost double the risk of graft loss compared to immediate graft function (aHR 1.7 (95% CI 1.3-2.3), p=<0.001).

Figure 28 - Forest plot showing the adjusted hazard ratio and 95% confidence intervals of the association between DGF duration and death-censored graft loss in donation after circulatory death donor kidney transplantation



Adjusted for donor age, sex, ethnicity and cause of death, recipient age, sex, ethnicity, dialysis modality, diabetes, hypertension, HLA mismatch level, HLA sensitisation, cold ischemia time, recipient anastomosis time and organ preservation method.

Other covariates associated with death-censored graft loss in DCD donor kidney transplantation are shown in Table 31. Of note, donor age showed a strong association with graft loss, with donors aged over 70 years with aHR of 2.6 (95% CI 1.7-3.9, p<0.001).

Table 31 - Multivariable Cox regression analysis for death-censored graft loss with delayed graft function (DGF) stratified according to duration in donation after circulatory death (DCD) kidney transplantation.

Variable	Adjusted hazard ratio	95% confidence interval			p value
Graft function					
Immediate graft function	Reference				-
DGF <7 days	0.9	0.7	to	1.2	0.46
DGF 7-14 days	1.0	0.8	to	1.3	0.91
DGF >14 days	1.7	1.3	to	2.3	<0.001
Donor age (years)					
<40	Reference				-
40-49	1.2	0.9	to	1.7	0.25
50-59	1.9	1.4	to	2.5	<0.001
60-69	2.4	1.8	to	3.4	<0.001
≥70	2.6	1.7	to	3.9	<0.001
Donor sex					
Male	Reference				-
Female	0.9	0.8	to	1.1	0.46
Donor cause of death					
Trauma	Reference				-
Stroke	0.8	0.5	to	1.1	0.13
Other	1.4	1.1	to	1.8	0.002
Donor ethnicity					
White	Reference				-
Black	1.4	0.3	to	5.6	0.65
Asian	1.5	0.8	to	2.8	0.22
Other	1.5	0.7	to	3.4	0.32
Recipient age (years)					

<40	Reference				-
40-49	0.7	0.5	to	0.9	0.01
50-59	0.6	0.5	to	0.8	0.001
60-69	0.5	0.4	to	0.7	<0.001
≥70	0.7	0.5	to	1.1	0.09
Recipient sex					
Male	Reference				-
Female	1.1	0.9	to	1.3	0.57
Recipient ethnicity					
White	Reference				-
Black	1.3	0.9	to	1.8	0.13
Asian	1.1	0.8	to	1.4	0.42
Other	0.4	0.1	to	0.8	0.02
Recipient diabetes mellitus	1.3	1.0	to	1.7	0.08
Recipient hypertension	1.1	0.8	to	1.5	0.65
Calculated reaction frequency (%)					
<85	Reference				-
≥85 (highly sensitized)	1.3	0.7	to	2.7	0.43
Dialysis modality					
Peritoneal dialysis	Reference				-
Hemodialysis	1.3	1.0	to	1.6	0.02
HLA mismatch level*					
Level 1	Reference				-
Level 2	1.1	0.5	to	2.2	0.85
Level 3	1.5	0.7	to	2.9	0.30
Level 4	1.2	0.6	to	2.5	0.66
Cold ischemia time (hours)					
<12	Reference				-

≥12 and <18	1.2	1.0	to	1.5	0.12
≥18 and <24	1.4	1.1	to	1.8	0.02
≥24	1.3	0.9	to	2.1	0.15
Recipient anastomosis time (mins)					
≤40	Reference				-
>40	1.1	0.9	to	1.3	0.33
Organ preservation method					
Static cold storage alone	Reference				-
Hypothermic machine perfusion	1.0	0.8	to	1.3	0.99
Normothermic regional perfusion	1.2	0.3	to	4.6	0.96

Number of cases included in the Cox Regression model n=4291, events=465.

Risk-adjusted mediation analysis showed that acute rejection accounted for 26.1% of the effect between DGF duration and DCGS.

Sensitivity analysis: altering the categories of DGF duration

A sensitivity analysis was undertaken to determine whether the grouping strategy of DGF duration altered the DCGS. DGF duration was regrouped in 5-day bins and risk-adjusted survival re-examined (Table 32). This showed that a threshold for significantly worse DCGS remains after 2 weeks of DGF.

Table 32 - Delayed graft function (DGF) duration grouping and influence on risk-adjusted death-censored graft survival.

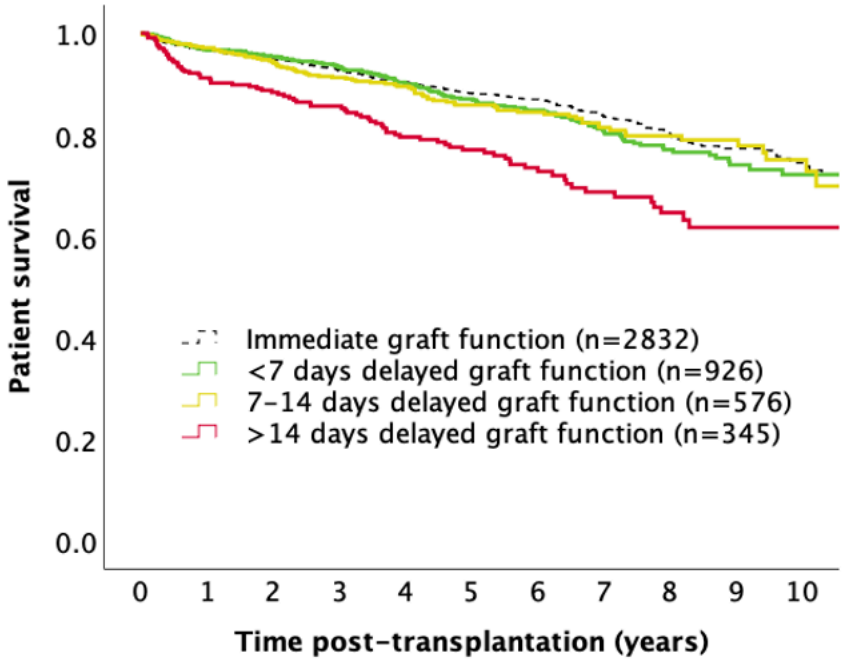
	Adjusted hazard ratio (95% confidence interval)	p value
DGF <5 days	0.9 (0.7-1.2)	0.52
DGF 5-10 days	1.0 (0.8-1.4)	0.87
DGF 10-15 days	1.0 (0.7-1.5)	0.98
DGF >15 days	1.7 (1.3-2.4)	<0.001

Adjusted for donor and recipient age, sex, and ethnicity, donor cause of death, recipient diabetes and hypertension, dialysis modality, HLA mismatch, HLA sensitisation, preservation method, and cold ischemia and anastomosis time.

3.4.5 Patient survival

The effect of DGF duration on patient survival followed a similar pattern to its effect on DCGS. DGF duration of >14 days was associated with inferior patient survival compared to the other DGF duration groups (p<0.001;Figure 29).

Figure 29 - Patient survival in recipients of kidneys from controlled DCD donors, by the duration of delayed graft function (DGF). DGF of more than two weeks was associated with significantly worse patient survival (p<0.001. Number of events in each group: immediate graft function (333 events); <7 days DGF (134 events); 7-14 days DGF (85 events); >14 days DGF (82 events).



Number at risk

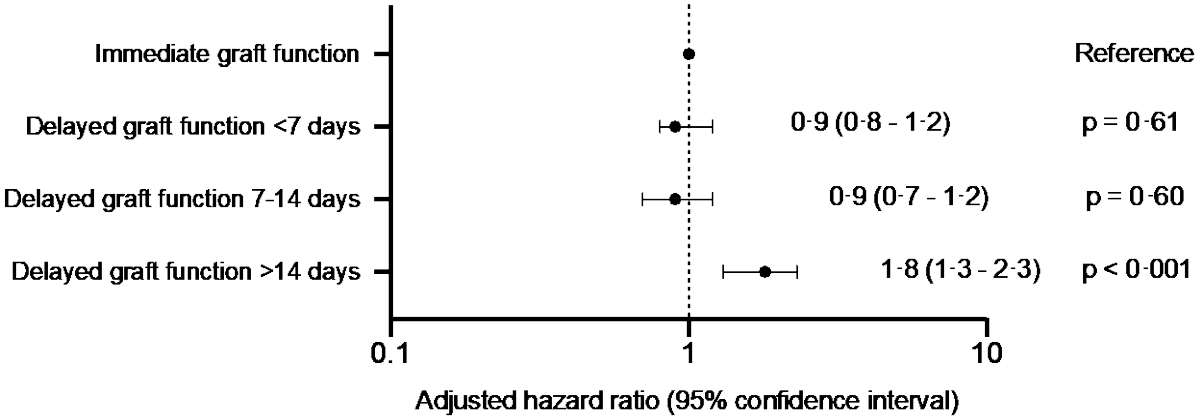
Immediate graft function	2832	2501	2057	1672	1337	1036	798	606	418	257	125
DGF <7 days	926	829	724	629	525	424	336	247	163	96	51
DGF 7-14 days	576	512	448	389	332	264	211	174	116	71	34
DGF >14 days	345	275	231	206	165	143	102	73	50	31	11

There was no difference in patient survival between recipients with immediate graft function and DGF duration of less than or equal to 14 days. Patients DGF duration of >14 days were an outlying group with significantly inferior survival.

After adjusting for donor and recipient risk factors, there was no added risk of patient death in recipients with immediate graft function compared to DGF duration <7 days (aHR 0.9, 95% CI 0.8-1.2, p=0.61) or DGF duration 7-14 days (aHR 0.9, 95% CI 0.7-1.2, p=0.60). The 14-day DGF threshold was

again observed, with almost double the risk of patient death relative to those with immediate graft function (aHR 1.8, 95% CI 1.3-2.3, p<0.001; Figure 30).

Figure 30 - Forest plot showing the adjusted hazard ratio with 95% confidence interval of the association between DGF duration and patient death in donation after circulatory death donor kidney transplantation



Adjusted for donor age, sex, ethnicity and cause of death, recipient age, sex, ethnicity, dialysis modality, diabetes, hypertension, HLA mismatch level, HLA sensitisation, cold ischemia time, warm anastomosis time and organ preservation method

Other covariates associated with patient death after kidney transplantation from controlled DCD donors are shown in Table 33.

.

Table 33 - Multivariable Cox regression for patient death, with delayed graft function (DGF) stratified by duration in donation after circulatory death (DCD) kidney transplantation

Variable	Adjusted hazard ratio	95% confidence interval			p value
Graft function					
Immediate graft function	Reference				-
DGF <7 days	0.9	0.8	to	1.2	0.61
DGF 7-14 days	0.9	0.7	to	1.2	0.60
DGF >14 days	1.8	1.3	to	2.3	<0.001
Donor age (years)					
<40	Reference				-
40-49	1.3	0.9	to	1.8	0.10
50-59	1.4	1.0	to	1.8	0.05
60-69	1.6	1.2	to	2.2	0.002
≥70	1.5	1.0	to	2.2	0.03
Donor sex					
Male	Reference				-
Female	1.1	0.9	to	1.3	0.36
Donor cause of death					
Trauma	Reference				-
Stroke	0.8	0.5	to	1.1	0.11
Other	1.0	0.8	to	1.3	0.95
Donor ethnicity					
White	Reference				-
Black	1.2	0.3	to	4.6	0.84
Asian	0.9	0.4	to	2.0	0.76
Other	1.8	0.9	to	3.6	0.11

Recipient age (years)					
<40	Reference				-
40-49	1.8	1.1	to	3.0	0.01
50-59	2.7	1.7	to	4.3	<0.001
60-69	5.0	3.1	to	7.8	<0.001
≥70	7.2	4.4	to	11.7	<0.001
Recipient sex					
Male	Reference				-
Female	1.0	0.9	to	1.2	0.76
Recipient ethnicity					
White	Reference				-
Black	0.9	0.6	to	1.3	0.57
Asian	0.7	0.5	to	1.0	0.03
Other	0.9	0.5	to	1.6	0.67
Recipient diabetes mellitus	1.5	1.2	to	1.9	0.002
Recipient hypertension	1.0	0.7	to	1.4	0.96
Calculated reaction frequency (%)					
<85	Reference				-
≥85 (highly sensitized)	1.1	0.5	to	2.3	0.87
Dialysis modality					
Peritoneal dialysis	Reference				-
Hemodialysis	1.7	1.4	to	2.1	<0.001
HLA mismatch level					
Level 1	Reference				-
Level 2	1.6	0.8	to	3.1	0.19
Level 3	1.5	0.8	to	3.0	0.21
Level 4	1.9	1.0	to	3.8	0.07

Cold ischemia time (hours)					
<12	Reference				-
≥12 and <18	1.1	0.9	to	1.3	0.54
≥18 and <24	1.2	0.9	to	1.5	0.23
≥24	0.9	0.6	to	1.5	1.00
Recipient anastomosis time (mins)					
≤40	Reference				-
>40	1.0	0.8	to	1.2	1.00
Organ preservation method					
Static cold storage alone	Reference				-
Hypothermic machine perfusion	1.0	0.8	to	1.3	0.80
Normothermic regional perfusion	0.6	0.1	to	4.5	0.65

Number of cases included in the Cox Regression model n=4288, events=559.

3.4.6 Predicting prolonged DGF pre-transplantation

DGF >14 days appears to be strongly associated with inferior graft and patient survival. Risk factors for DGF > 14 days were determined, as they may be a predictor of inferior long-term outcomes. All risk factors considered in Section 3.3.3 were included in a binary logistic regression model to predict prolonged DGF.

Older donor age, donor male sex, pre-transplant hemodialysis, and longer organ cold ischemia and recipient anastomosis times were independent predictors of prolonged DGF >14 days (Table 34). Although recipient anastomosis time is not strictly a pre-transplant risk factor, kidneys with multiple arteries and/or veins, or recipient anatomical factors (such as recipient BMI or iliac artery atherosclerosis on pre-transplant imaging), would be expected to lead to longer anastomosis times. Hypothermic machine perfusion (HMP) was associated with reduced risk of prolonged DGF when compared to static cold storage alone.

Table 34 - Multivariable binary logistic regression analysis showing factors independently predictive of prolonged delayed graft function (>14 days)

Variable	Odds ratio	95% confidence interval	p value
Donor age (years)			
<50	Reference		-
50-59	1.4	1.0 to 1.9	0.04
60-69	1.5	1.1 to 2.1	0.007
≥70	2.5	1.7 to 3.6	<0.001
Donor male sex	1.5	1.2 to 1.9	0.002
Recipient dialysis modality			
Peritoneal dialysis	Reference		-
Hemodialysis	1.4	1.0 to 1.8	0.02
Cold ischemia time (hours)			
<12	Reference		-
≥12 and <18	1.6	1.2 to 2.2	0.002
≥18 and <24	2.0	1.4 to 2.9	<0.001
≥24	2.9	1.7 to 5.0	<0.001
Organ preservation method			
Static cold storage alone	Reference		-
Hypothermic machine perfusion	0.7	0.5 to 0.9	0.02
Normothermic regional perfusion	0.4	0.01 to 3.4	0.43
Recipient anastomosis time (mins)			
≤40	Reference		-
>40	1.3	1.1 to 1.7	0.01

3.4.7 Comparison with donation after brain death (DBD) donors

This analysis focused on the effect of DGF on controlled DCD donor kidney transplantation. However, a logical next question would be to consider how the results of this analysis compare to DBD donor kidney transplants during the same study period. With the exception of donor type, inclusion and exclusion criteria were the same.

During the study period, 7327 recipients of DBD donor kidneys were eligible for inclusion in this additional analysis. 5829 (79.6%) of recipients had immediate graft function, 1498 (20.4%) of recipients had DGF. Of the 1498 patients with DGF, 38 patients had missing DGF duration data and were therefore not included in the DGF analysis (0.5%). Of the DBD donor kidney transplants with DGF duration data, 934 (20.4%) had <7 days DGF, 344 (4.7%) had 7-14 days DGF and 182 (2.5%) had >14 days DGF.

In DBD kidney transplantation, and in contrast to DCD kidney transplantation, even short periods of DGF duration were associated with reduced DCGS when compared to grafts without immediate function (Figure 31). DGF duration <7 days, and 7-14 days had significantly inferior DCGS compared to those with immediate graft function on univariate analyses ($p<0.001$ for both). Prolonged DGF lasting >14 days furthermore had inferior DCGS compared to all other groups ($p<0.001$ throughout). When adjusting for risk factors, this effect was seen again. Cox regression showed that risk of graft loss in DBD donor kidney transplantation increased according to DGF duration (DGF <7 days: OR 1.3, $p=0.002$, DGF 7-14 days: OR 1.4, $p=0.004$, DGF >14 days: OR 2.7, $p<0.001$) when immediate graft function was used as the reference group.

On univariate analysis, immediate graft function in DBD kidney transplantation was associated with superior patient survival compared to recipients with DGF of any duration ($p<0.001$ throughout, Figure 32). However, DGF lasting longer than 14 days was associated with significantly inferior patient survival compared to all other groups ($p<0.001$).

These data indicate that whilst DGF is more common in DCD kidney transplantation, DCD kidneys are more tolerant of DGF compared to DBD kidneys. More than 14 days of DGF is necessary to negatively impact graft survival in DCDs, whilst in DBDs even short duration DGF negatively impacts DCGS.

Therefore, whilst predictors of DGF exceeding 14 days is clinically relevant in DCD transplantation, predictors of DGF of any duration in DBD transplantation is more clinically relevant.

Figure 31 - Death-censored graft survival in recipients of kidneys from DBD donors, by the duration of delayed graft function (DGF)

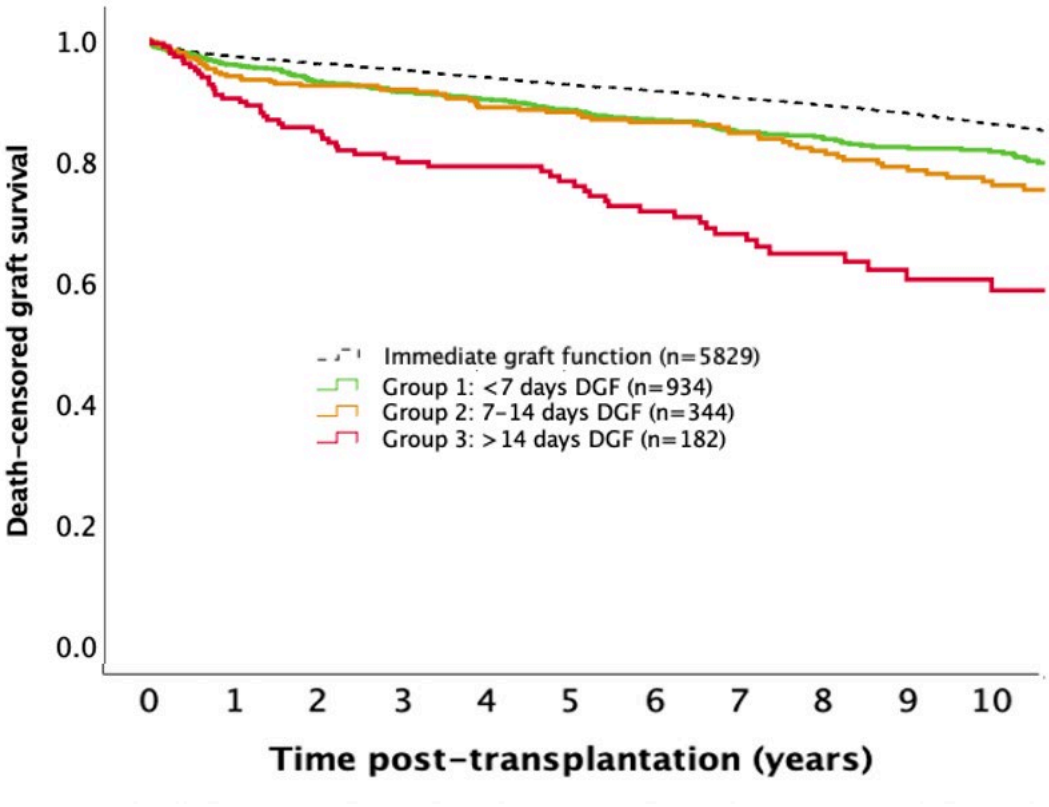
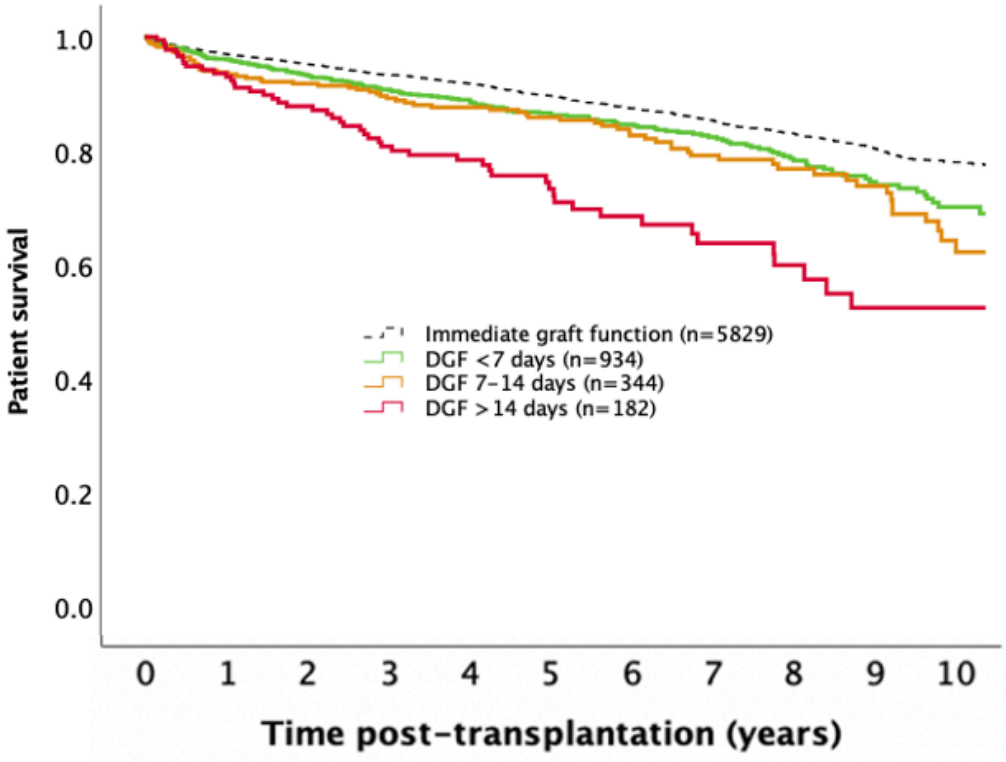


Figure 32 - Patient survival in recipients of kidneys from DBD donors, by the duration of delayed graft function (DGF)



3.5 Discussion

3.5.1 Study Findings and their implications in transplantation

This large national cohort study demonstrates for the first time that prolonged DGF has a long-lasting impact on graft and patient survival after kidney transplantation from controlled DCD donors. DGF lasting less than 14 days has no association with adverse longer-term outcomes. DGF lasting more than 14 days was associated with almost double the risk of graft failure and patient death. This threshold identifies a previously undefined group of transplant recipients who are at risk of serious adverse long-term outcomes. These findings challenge previous and contemporaneous landmark work suggesting that DGF in controlled DCD kidney transplantation does not translate to inferior long-term graft outcomes (6, 138, 242). This larger study providing a more nuanced understanding of the impact of DGF after DCD donor kidney transplantation from this important source of organs.

Deceased donor kidneys undergo a series of insults during donor death, organ retrieval, transport in cold storage, and during implantation and reperfusion within the recipient. These deleterious processes include hypoperfusion, hypoxia, pro-inflammatory cytokine release, ischemia-reperfusion injury (IRI), and damage via the alloimmune response (245). The duration of DGF likely reflects the magnitude of these injuries and the propensity of the kidney to recover. The benefit of stratifying according to DGF duration is that it is possible see when these protective mechanisms are overcome, and decompensation occurs. This study suggests that DCD donor kidneys may sustain acute injury without leading to longer-term compromise until a critical tipping point is reached, manifesting as DGF beyond 14 days. In contrast to DCD donor kidneys, this analysis showed that in DBD donor kidneys, any duration of DGF translates to a fall in graft and patient survival. This many suggest that DCD kidneys are able to tolerate some degree of acute injury before conceding to graft failure, possibly through up-regulation of resilience-enhancing pathways, which are less well developed in brain-death donors. De Kok *et al* demonstrated that a number of molecular upstream regulators, which are associated with resilience to injury, are upregulated preferentially in DCD donor kidneys relative to DBD donor grafts (242). These protective pathways include those previously associated with renal development, organogenesis, and

stem cell maintenance. DCD donor kidneys also demonstrate down-regulation of pro-inflammatory pathways compared to DBD. These proinflammatory pathways share roles in tumour suppression (e.g., p53 gene expression) and cardiovascular disease (e.g., heat shock protein expression). The mechanism of donor death therefore plays an important role in subsequent gene expression, which alter the way in which organs behave following transplantation. These findings are of significant interest to the transplant community as it directly influences organ utilisation and, more specifically appropriate longevity matching between an organ and a potential recipient.

Patients with DGF lasting 14 days or more had higher risk of patient death. The cause of death in this group would be of significant interest. It is possible that higher donor and recipient age played a part in this, however this was adjusted for in multivariable analyses. It is possible that prolonged DGF suggests significant injury to the kidney, with subsequent impact on graft function. With reduced graft function, patients are likely to have returned to dialysis, and hence exposed to high rate of morbidity and mortality. Biopsies of kidneys from recipients in this study would have been on interest when considering the processes underlying graft loss.

It should be acknowledged that differing surgical, anaesthetic and fluid replacements strategies and the use of dialysis in the first week of transplantation reflect local practices meaning that DGF in the first week of transplantation may not truly reflect acute kidney injury. Potentially, more prolonged dialysis (e.g., greater that 14 days) may reflect more clinically relevant acute graft injury with implications on longer-term graft outcomes. How one defines DGF is also likely to influence the observed impact on clinical outcomes (145, 246). Defining DGF as the need for dialysis in the first seven days of transplantation does not adequately capture the injury sustained in patients who are not yet established on haemodialysis, since their native kidney function is likely to mask this outcome. For this reason, pre-emptive patients receiving a transplant could not be examined in this study. Biochemical markers of graft function are needed to include such patients in future studies in this field.

Given that DGF duration of more than 14 days was found to be strongly associated with worse graft and patient outcomes after DCD donor kidney transplantation, clinical variables independently

predictive of prolonged periods of dialysis-dependency were identified using binary logistic regression. This is intended to identify modifiable risk factors for prolonged DGF in order to improve outcomes for patients. The use of donors aged ≥70 years was strongly associated with prolonged DGF, with almost triple the risk of graft loss. However, these risks should be weighed against the risks of remaining on the waiting list and realistic chance of receiving a kidney from a much younger donor. This emphasises the importance of longevity matching and careful patient counselling. The new UK kidney offering scheme was introduced in September 2019 and was developed to match donor and recipient more appropriately (247). Older recipients are therefore expected to be offered organs from roughly their age group.

Organ CIT was strongly associated with longer periods of DGF, with a marked dose-dependent effect. Previous studies have demonstrated the differential effect of prolonged organ CIT on DCD versus DBD donor kidneys,(6, 68) and it is important that this modifiable risk factor is minimised in order to achieve improved graft outcomes post-transplant. It is notable that graft survival after DCD donor kidney transplantation in the United States are inferior to those from DBD donors, even after risk-adjustment. This may be due to longer average organ CITs within the US geography, when compared to those in the UK travel distances (237). DCD donor kidney allocation and offering schemes should reflect the need for shorter CITs.

These data also suggest that HMP may reduce the risk of prolonged DGF. Preservation technique is a clear modifiable factor which may have an impact on patient outcomes. Indeed evidence suggests that HMP is likely to reduce DGF in both DBD and DCD donor kidneys (248). Howevr, we note that HMP had no statistically significant effect on DCGS or patient survival. Greater uptake of HMP may be needed for this to be formally examined in registry analyses. Furthermore, the use of normothermic machine perfusion remains an area of interest. Urinary biomarkers that may predict DGF may be used in future studies in this field – these include IL-6, TNF-a, endothelin-1 and neutrophil gelatinase-associated lipocalin (NGAL) (163, 199).

There are a number of clinical trials investigating novel pharmacological and immunosuppressive agents (243, 244, 249-251) as well as organ perfusion technologies (182, 201, 252) in order to reduce

the incidence of DGF. DGF is commonly used outcome measure in trials as it is easy to measure, with a short follow-up period necessary. For this reason, clinical trials often target DCD kidneys, as DGF is more common in these donor types, meaning fewer participants are needed for the trial to be adequately powered. If the longer-term significance of DGF is in question, the value of these studies is uncertain. However, the findings of this study justify treatments aimed at reducing DGF in DCD kidneys, since long-term adverse outcomes may be modulated by these therapies. Since DGF as binary outcome measure provides poor granularity, the duration of DGF likely provide a better scale, or level of detail in a set of data, necessary to detect efficacy of novel interventions in reducing DGF.

The manifestation of DGF results from a number of pathological processes, including IRI and acute rejection. These two processes are not independent of each other. IRI can increase organ immunogenicity through the activation of antigen presenting cells (114) and promotion of alloantibody production (115), thus leading to a higher likelihood of acute rejection. For this reason, the interaction between DGF and acute rejection had to be considered in this study. Separating the independent and inter-dependent effects of DGF and acute rejection is challenging with a retrospective observational study. However, a novel mediation analysis developed by VanderWeele TJ *et al* indicated that acute rejection acts as a partial mediator between DGF duration and subsequent graft loss. These finding are consistent with comparable work by Lim *et al* (253).

3.5.2 Limitations

There are a number of limitations that are recognised in the study finding, which are inherent to the design of a retrospective registry analysis. These include reporting errors and missing data. Transplant centres in the UK submit mandatory data to the registry, however reporting errors and omissions may occur when data is submitted by recipient centres to the transplant registry. There are also limitations in the type of data requested and held by the UK transplant registry. For example, the registry does not currently capture pertinent data on donor haemodynamic stability following withdrawal of life sustaining treatment, machine perfusion at the recipient hospital and pre-implantation

kidney biopsies, which may have provided important perspectives into the pathogenesis, treatment, and predictability of DGF. Another limitation of the study was that recipients of second and multiple previous kidney transplants were not addressed in this research, which are known to be risk factors for DGF. This could be examined further in future work, as it remains relevant to patients in this category.

3.5.3 Summary

In summary, DCD donor kidneys are a valuable source of organs. The relatively higher rate of DGF in DCD donor grafts should not deter transplant programmes from utilising these organs, given that recovery from IRI appears to be complete in organs with up to 14 days of DGF. However, therapies that reduce IRI and promote organ recovery should be aimed at organs likely to have prolonged DGF beyond 14 days.

4 Chapter 4: The safely, feasibility and efficacy of ex vivo normothermic perfusion in the early transplant period

Work from this chapter has been published in the following peer-reviewed journals:

- Chandak P, **Phillips BL***, Uwechue R, et al. Dissemination of a novel organ perfusion technique: ex vivo normothermic perfusion of deceased donor kidneys. Artificial Organs. 2019;43(11):E308-E319. doi:10.1111/aor.13499 **Joint first authorship / contributed equally*

- **Phillips BL**, Chandak P, Uwechue R, van Nispen Tot Pannerden C, Hemsley C, Callaghan CJ. Microbial Contamination During Kidney Ex Vivo Normothermic Perfusion. Transplantation. 2018;102(4):e186-e188. doi:10.1097/TP.0000000000002076

- **Phillips BL**, Chandak P, Uwechue R, van Nispen Tot Pannerden C, Hemsley C, Callaghan C. The Authors' Reply. Transplantation. 2018;102(9):e399-e400. doi:10.1097/TP.0000000000002303

Work from this chapter has been presented at the Southwest and East Kidney Society (SWEKS) conference 2018, and I was awarded the first prize for the presentation.

Funded by Guy's and St Thomas' Charity:

4.1 Abstract

Static cold storage is currently the gold standard method of organ preservation prior to transplantation. EVNP is emerging as a novel method of organ preservation, which may enable organ improved viability assessment and amelioration of early graft function prior to deceased donor kidney transplantation. However, its safety and feasibility have not yet been fully determined. In particular the infectious complications of EVNP are largely unknown. Likewise, the efficacy of EVNP in the reduction in DGF has not yet been evaluated in clinical trials.

This is a prospective case series examining the safety and feasibility of EVNP prior to deceased donor kidney transplantation, and its efficacy in reducing DGF compared static cold storage alone. Human deceased donor kidneys undergoing EVNP at a single centre were enrolled in an observational study. The incidence and clinical significance of bacterial growth in EVNP perfusate samples was recorded, as well as early graft function and incidence of DGF. No antibiotics were administered during EVNP. Contralateral kidneys undergoing SCS alone were used as a comparator group.

Fourteen kidneys underwent EVNP during the study period. Twelve kidneys were implanted into 10 recipients. Two pairs of kidneys were implanted as dual grafts and one kidney was implanted simultaneously with a pancreas. The remaining seven kidneys were transplanted as single allografts following EVNP. Contralateral kidneys from the same donor, all underwent static cold storage prior to transplantation at another centre.

Of the twelve kidneys undergoing EVNP, three kidneys did not undergo warm perfusate culture due to sampling errors. Five of the 9 EVNP perfusate cultures (56%) had positive bacterial growth. Two cold transport fluid cultures had positive growth, but with no consistency with the organisms grown from the EVNP perfusates. None of the EVNP perfusate-cultured organisms were implicated in episodes of recipient infection post-transplantation.

Using contralateral kidneys undergoing SCS alone as a comparator group, early graft function and the rate of DGF were examined in kidneys that were implanted as single grafts. Although there was

significantly longer CIT in the EVNP group compared to kidneys undergoing SCS ((mean (SD) 14.0 (1.8) vs. 10.1 (2.8) hours; p=0.01), recipient and operative characteristics were comparable. The rate of DGF was 43% in kidneys undergoing SCS alone (n=3) and 14% (n=1) in those undergoing EVNP. This difference did not reach statistical significance (p=0.56). No difference in kidney transplant function was observed between the two groups at one week and one, three-, six- and 12-months post transplantation (p=63, p=0.58, p=0.71, p=0.74, p=0.22, respectively). This study did not demonstrate a reduction in DGF in kidneys implanted as single grafts undergoing EVNP, compared to static cold storage. Despite a high rate of positive growth in EVNP perfusate culture, there were no clinically significant infections in recipients of EVNP kidneys. This study demonstrates safety and efficacy of EVNP as a means of organ preservation.

4.2 Introduction

4.2.1 Organ preservation and perfusion

Static cold storage remains the most commonly practice method of organ preservation in the UK, as well as internationally (254). Although cold preservation allows the cellular metabolic rate to decrease, prolonged cold storage eventually results in depletion of intracellular adenosine triphosphate (ATP) (194). Cold ischaemia time is an independent predictor of DGF. In a risk-adjusted US cohort study involving over 90,000 kidney transplant recipients, cold ischaemia time was found to be an independent predictor of DGF, regardless of donor age (255). Normothermic machine perfusion allows the restoration of normal metabolic activity in the presence of aerobic respiration and has shown to replete ATP in a porcine model of transplantation (194). Only one study has shown that EVNP may reduce DGF compared to static cold storage alone, although this study used historical controls as the comparator group, and it was only conducted in extended criteria donors (200). To-date, there have been no published, prospectively conducted studies examining the use of EVNP in the reduction of DGF (184), although one trial is underway (201).

4.2.2 Immediate graft function, delayed graft function and primary non-function

In the literature, it is well established that immediate kidney function following transplantation is associated with improved early outcomes, namely reduced length of hospital (147), lower risk of acute rejection (148, 149) and less chance of needing invasive investigations such as transplant biopsy (150). Furthermore, Chapter 3 of this thesis has shown that DGF has a detrimental long-term impact on deceased donor kidney transplantation; DGF of any duration is associated with inferior patient and graft survival in DBD donor kidney transplants while in DCD donor kidney transplantation, DGF lasting more than 14 days is associated with significantly inferior graft and patient survival.

DGF is one of the clinical manifestations of ischaemia-reperfusion injury and is defined as the need for dialysis in the first week of transplantation, to avoid fluid overload, uraemia, hyperkalaemia, and

death. DGF has become increasingly common, given the changes in the donor demographics over the past decade. Increasing donor age and co-morbidities has contributed to the increasing incidence of DGF. Furthermore, since kidneys donated after circulatory death are twice as likely to encounter DGF, the increased utilisation of kidneys from this donor pool has naturally made DGF more common in kidney transplant recipients. Ameliorating IRI, and reducing DGF, has therefore become a target for intervention. Given the adverse long-term implications of DGF on graft and patient survival in both DBD and DCD kidney transplantation, research efforts into reducing DGF have become justified. DGF has been identified as a risk factor for antibody-mediated rejection in observational studies (256). Indeed, DGF may provoke acute rejection by promoting alloantibody production (115), possibly by due to severe IRI leading to increased activation of donor antigen-presenting cells. It is therefore possible that reduction or avoidance of DGF may also help to prevent acute rejection episodes. Patients with *both* DGF and acute rejection have significantly poorer graft survival comparted to other cohorts (34% graft survival at 5 years post-transplantation) (152). Primary non-function, in which a graft never works, irrespective of cause, has significant long-term detrimental sequelae including perioperative mortality without a working graft, increased HLA antibody reactive frequency for subsequent grafts and reduced long-term patient survival (136).

Although a number of modifiable risk factors exist for PNF, there is no evidence to indicate that preservation technique influences its incidence. A recent meta-analysis found that amongst seven published studies in the literature, hypothermic machine perfusion did not affect the risk of PNF, when compared to static cold storage (RR 1.05, 95% CI 0.37-3.02, p=0.92) (184). A propensity-score analysis found that normothermic regional perfusion had no impact on PNF rates in DCD donor kidney transplant (255). However, the impact of EVNP has not been explored to-date.

4.2.3 Complications of ex vivo normothermic perfusion

Transplantation of a kidney following EVNP was first performed in humans in 2011 (196). Since the commencement of this study, there have only been five published studies examining the use of EVNP

following kidney transplantation in humans (197, 198, 200, 257, 258). The safety profile of EVNP has not yet been extensively reported.

The incidences of adverse events such as acute rejection, renal artery or vein thrombosis, complications of graft biopsy and hospital admissions will be examined as part of the large multicentre RCT (201). However, instances of infective complications are not specifically being gathered by the RCT. Given the paucity of information on this potential complication, and the fact that infections are the most common cause of non-cardiovascular death after kidney transplantation (259), it is a particular interest in this chapter

Contamination of the kidney with microorganisms may occur during organ retrieval, organ transportation within cold preservation fluid or during the implementation of EVNP. Kidney transplant recipients are particularly vulnerable to infection, with induction immunosuppression, the presence of ureteric stents, urinary catheters and invasive vascular lines for monitoring, and recipient comorbidities such as diabetes mellitus and advanced age (259). The most prevalent infection in a renal transplant recipient is a urinary tract infection, with *Escherichia coli* being the predominant causal organism (260).

Although no infections complications attributable to EVNP have been reported in these studies, it is possible that the warm, blood-based perfusate may enhance bacteria growth. EVNP is not performed in a closed circuit.

Prior to this research, there was no consensus as to whether antibiotics should be routinely added to the perfusate during EVNP as a preventative measure against bacterial infection (personal communication with M. L. Nicholson (Cambridge) and C Wilson (New Castle)).

The aim of this study was first to determine the safety and feasibility of EVNP in deceased donor kidney transplantation at Guy's Hospital. Given that the infectious complications of EVNP are largely unknown, I aimed to investigate the incidence of recipient infection post-transplantation. The secondary aim of this study was to determine whether EVNP reduced DGF in a paired kidney analysis.

4.3 Methods

4.3.1 Clinical governance

The implementation of EVNP was discussed and approved by the NHSBT kidney advisory group, given the possibility that kidneys undergoing EVNP may be subsequently declined by an EVNP centre, and transplanted at a non-EVNP centre. Stringent processes were developed to enable appropriate tracing of blood products used during EVNP. Clinical governance approvals were gained prior to the implementation of EVNP in transplant recipients. This involved approvals from local transfusion services for the use of O negative packed red blood cells, microbiology services, medical physics, operating staff, and anaesthetic colleagues. Support from our local patient representative group was also gained. Local approvals were gained to undertake a service evaluation of the EVNP programme. As a service evaluation of an existing treatment, local research ethical and HRA approvals were not required.

Kidneys were retrieved from deceased donors in the UK by the National Organ Retrieval Service on behalf of NHSBT. Consent was gained by specialist nurses of organ donation from patients' next-of-kin prior to organ retrieval.

4.3.2 Study population and design

Donor and recipient baseline characteristics, and clinical outcome data, were recorded prospectively from consecutive kidneys undergoing EVNP prior to transplantation. Inclusion and exclusion criteria of this observational study are shown in Table 35.

Table 35 - Inclusion and exclusion criteria

Inclusion criteria
Deceased donor kidney accepted for transplantation at Guy's Hospital
Accepting surgeon requested ex vivo normothermic perfusion
Favourable arterial anatomy
Exclusion criteria
Inclusion in another clinical study involving ex vivo normothermic perfusion
HIV positive donor
Hepatitis B surface antigen positive donor
Hepatitis C IgG antibody positive donor

In order to provide a comparator group, data from the contralateral kidney from the same donor was obtained from NHS Blood and Transplant. Kidneys undergoing EVNP, and their contralateral organ, from April 2016 to July 2017 were included. Study follow-up ended on 1 May 2018.

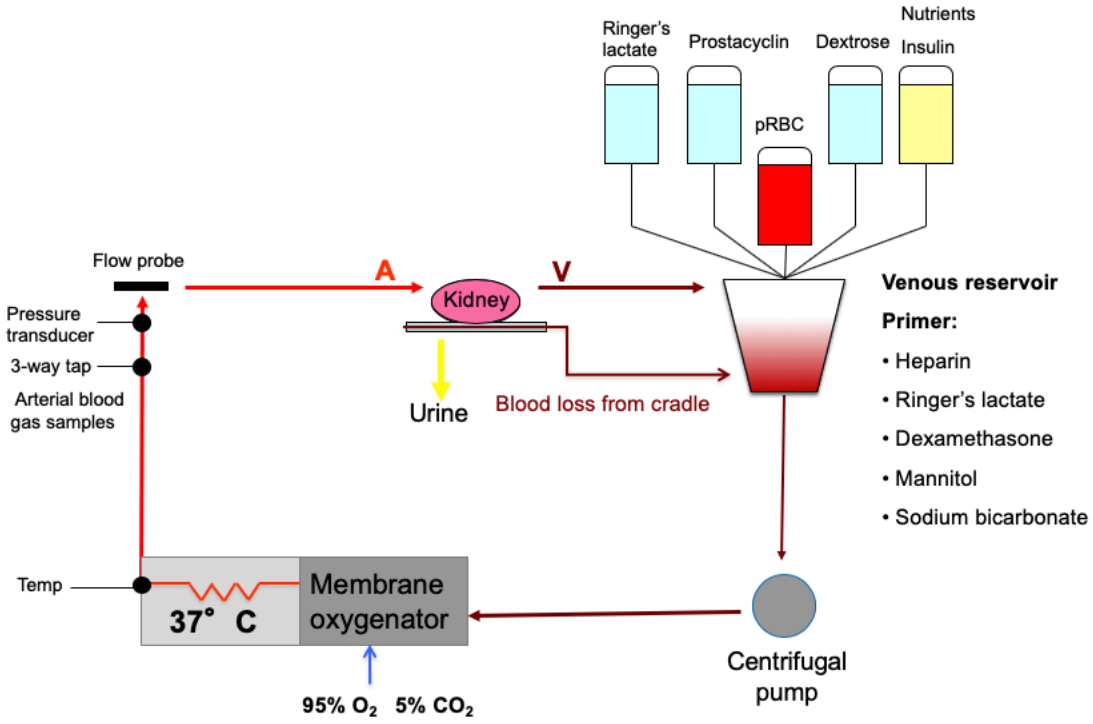
4.3.3 Ex vivo normothermic perfusion

All kidneys underwent static cold storage during transport. Kidneys undergoing EVNP were prepared in the usual way for transplantation prior to EVNP. The EVNP circuit consists of a paediatric cardiopulmonary bypass centrifugal pump (Medtronic Watford, UK) which generates continuous flow of

fluid through a clinical grade ¼ inch sterile polyvinyl chloride tubing (Medtronic) (see Figure 33). This allowed the perfusate to pass through a heat exchanger (target temperature 37°C), affinity membrane oxygenator (95% O_2 and 5% CO_2 mix, 0.1L/min). The circuit also comprises a venous reservoir which also acts as bubble and clot filter. The contents of the perfusate were added to the circuit, oxygenated, and tested to ensure acceptable temperature pH, partial pressures of oxygen and carbon dioxide. The circuit was then cut to allow inclusion of the kidney, which sat in a sterile cradle (Precision Surgical). The renal artery was cannulated with 10-14F steel wire enforced cannulae (Medtronic Watford, UK). Where possible, an aortic patch clamp was used instead of direct renal artery cannulation to allow perfusion via the renal ostia to avoid instrumentation of the renal artery. Perfusate returns to the reservoir via a venous catheter, and if present, bleeding around the organ via the organ cradle. The perfusate flow, pressure and temperature and are monitored using a TX50P flow transducer and manometer (Medtronic) and temperature probe (Cole-Parmer, London, UK).

Kidneys underwent EVNP at body temperature (35-37°C) at a target mean arterial pressure of 75mmHg for one hour. Organ resistance was determined by the perfusate flow and pressure (Ohm's law).

Figure 33 - Ex vivo normothermic perfusion circuit, once the kidney has been included



pRBC: packed red blood cells. A: Arterial catheter. V: venous catheter

4.3.4 Composition of perfusate use of blood products

The liquid perfusate was comprised of an initial primer, present at the beginning of perfusion, as well as infusions which entered circulation at a fixed rate during perfusion. The primer solution was comprised of 250mL Hartmann's solution (sodium chloride 6g/L, potassium chloride 0.4g/L, calcium chloride dihydrate 0.27g/L, sodium lactate 3.2g/L), human packed red blood cells (one unit, approximately 250-350mL), 10% mannitol 15mL. Sodium bicarbonate 8.4% was added to the primer solution and titrated to a target pH 7.35-7.45 (typically 35mL) to reach a physiological pH of human blood. The circuit was fully heparinised to avoid clot formation (3000 units unfractionated heparin).

Infusion of Hartmann's solution was added during perfusion at a rate determined by the urine output of the kidney. A fixed rate of nutrition (amino acids, vitamins, and glucose present in synthamin, cernavit and 5% dextrose solution, respectively) was infused during EVNP.

4.3.5 Use of blood products

Clinical-use third-party group O packed red blood cells units were used in the EVNP perfusate and were provided by Guy's and St. Thomas' NHS Foundation Trust Transfusion services. Group O ensured that there would be no blood compatibility issues if a kidney underwent EVNP but was subsequently and unexpectedly offered to an alternative recipient (e.g., if the initial intended recipient was not able to proceed with transplantation). The use of group O also meant that EVNP could be initiated for organ viability assessment before the recipient was selected. Likewise, Rhesus D antigen (RhD) negative blood was used to avoid the risk of alloimmunisation in RhD-negative female recipients of childbearing age.

4.3.6 Static cold storage

Contralateral kidneys undergoing static cold storage were used as a comparator group. Kidneys undergoing static cold storage alone were kept at 4^{o}C in sterile Belzer University of Wisconsin storage solution (Bridge to Life Ltd). fluid until removal at the time of implantation.

4.3.7 Clinical outcomes

DGF was defined as the need for dialysis within the first week of transplantation regardless of cause (145). Duration of DGF was defined as the number of days from transplantation to the last day of dialysis. Primary non-function was defined as failure of the graft to ever function following transplantation, regardless of cause. Graft failure was defined as return to dialysis or graft nephrectomy, whichever occurred first, and was censored for death. CIT was defined as the period from the start of cold perfusion in the donor to reperfusion with blood within the recipient (i.e., including the EVNP duration). Following transplantation, four-variable MDRD estimated glomerular filtration rate were recorded

prospectively seven days at seven days, and one, three, six, and 12 months. These data were also gained from NHSBT for those kidneys undergoing SCS alone at other centres.

Where one kidney from a donor was implanted after EVNP and the paired kidney was transplanted after static cold storage only, characteristics and post-transplant outcomes were compared. Groups were compared using Fisher's exact and chi-squared tests for nominal data, the Mann-Whitney test for ordinal or non-parametric continuous data, and Student's t-test for parametric continuous data.

4.3.8 Infectious complications

Cold preservation fluid from the organ transport bag was also cultured before EVNP was performed. EVNP was performed in a strictly sterile environment (operating theatre), with sterile circuitry, perfusate fluids and medications. After the kidney had undergone EVNP for 40-50 minutes, 20ml of warm perfusate fluid was placed in aerobic and anaerobic blood culture bottles containing soybean-casein digest broth 3%. No antibiotics were given during EVNP. All recipients received induction antibiotics, in keeping with local microbiology practice. Since kidneys undergoing EVNP were implanted at one centre, recipients of these kidneys received a single dose of intravenous amikacin 7mg/kg at induction of anesthesia. All kidneys are transported in cold Belzer University of Wisconsin storage solution (Bridge to Life Ltd) or Soltran (Marshall's hypertonic citrate, Baxter Healthcare Ltd) preservation fluid, which was also sent for microscopy and culture.

Data on infected perinephric collections within three months of transplantation were recorded in kidneys undergoing EVNP. All other infectious complications were recorded prospectively. All positive warm and cold fluid culture results were discussed with a consultant microbiologist and given antibiotics only if recommended.

4.4 Results

4.4.1 Baseline characteristics

Between 1 March 2016 to 31 July 2017, EVNP was performed on 14 kidneys from 12 donors. Two kidneys that underwent EVNP were not implanted as one performed poorly during EVNP and was deemed untransplantable, and the other was discarded after prolonged cold ischaemia time as the prospective recipient had an anaesthetic complication resulting in cancellation of the operation.

Of the 14 kidneys that underwent EVNP, 12 organs were implanted into 10 recipients. Two pairs of kidneys were implanted as dual grafts and one kidney was implanted simultaneously with a pancreas. The remaining seven kidneys were transplanted as single allografts following EVNP and were included in the analysis. Contralateral kidneys, from the same donor, all underwent static cold storage prior to transplantation at another centre, providing a comparator group.

The cohort of organs undergoing pair analysis included DCD donor kidneys (n=8, 57%) with a median (IQR) donor age of 51 (21-64) years, male donor sex (n=6, 43%), and death from cerebrovascular accident (n=6, 43%). There were no statistically significant differences in recipient demographics between kidneys that underwent EVNP compared to those that remained in static cold storage prior to transplantation (Table 36). Recipients were comparable in terms of age, sex, and mode of dialysis ($p>0.05$ throughout). However, those organs undergoing EVNP had significantly longer cold ischaemia time compared to static cold storage kidneys (mean (SD) 14.0 (1.8) vs. 10.1 (2.8) hours; $p=0.01$).

Table 36 - Baseline characteristics for kidneys undergoing ex vivo normothermic perfusion compared to the contralateral paired kidney undergoing static cold storage

Variable	EVNP kidneys n=7	Static cold storage n=7	p value
Recipient age (years)	48 (29-61)	57 (31-61)	1.00
Recipient male sex	5 (71%)	5 (71%)	1.00
Pre-transplant mode of dialysis **Not on dialysis / pre-emptive** **Haemodialysis** **Peritoneal dialysis**	 1 (14%) 5 (72%) 1 (14%)	 0 (0%) 5 (60%) 2 (40%)	0.51
Cold ischaemia time (hours)	14.0 (1.8)	10.1 (2.8)	0.01

Data expressed as mean (SD), median (IQR), number (%)

4.4.2 Early graft function

There were no instances of primary non-function in kidneys undergoing EVNP. One kidney (14%) that underwent SCS only had primary non-function, however this difference did not reach statistical significance (p=0.99, Table 37).

The rate of DGF was 43% in kidneys undergoing SCS alone (n=3) and 14% (n=1) in those undergoing EVNP. This difference did not reach statistical significance (p=0.56). Statistical analysis of DGF duration could not be performed, as there was only one episode of DGF in the EVNP group.

No difference in kidney transplant function was observed between the two groups at one week and one, three-, six- and 12-months post transplantation (p=63, p=0.58, p=0.71, p=0.74, p=0.22, respectively).

Table 37 - Outcomes of kidneys undergoing ex vivo normothermic perfusion compared to the contralateral paired kidney undergoing cold storage

Variable	**EVNP n=7**	**Static cold storage n=7**	**p value**
Primary non-function	0 (0%)	1 (14%)	0.99
Delayed graft function	1 (14%)	3 (43%)	0.56
eGFR (mL/min/1.73m²)			
1 week	24 (18)	30 (31)	0.63
1 month	47 (14)	53 (24)	0.58
3 months	46 (14)	50 (20)	0.71
6 months	51 (14)	48 (19)	0.74
12 months	59 (15)	53 (21)	0.64
Death-censored graft survival at 1 year	7 (100%)	6 (86%)	0.31

Data presented as n(%) or mean(SD)

4.4.3 Infectious complications in the ex vivo normothermic perfusion group

Twelve kidneys underwent EVNP prior to transplantation into ten recipients (including two dual kidney transplants). EVNP perfusate cultures were not analysed in three kidneys due to a processing error. Five of the 9 EVNP perfusate cultures (56%) had positive bacterial growth (Table 38). In four cases the organisms were coagulase-negative *Staphylococcus* species and were not treated. Methicillin-sensitive *Staphylococcus aureus* was cultured once, and the asymptomatic recipient was treated with intravenous flucloxacillin for 7 days from day 1 post-operatively. Two cold transport fluid cultures had positive growth, but with no consistency with the organisms grown from the EVNP perfusates. None of the EVNP perfusate-cultured organisms were implicated in episodes of recipient infection during the early post-transplant period (Table 38). All 10 patients remained infection free, with no incidences of infected perinephric collections or early sepsis at follow-up (6 – 15 months post-EVNP).

Table 38 - Donor and recipient demographic data, warm and cold culture results, and post-operative infectious episodes

Graft type	Donor age (years)	Donor type	Pre-mortem donor cultures and treatment *	Recipient age (years)	EVNP duration (min)	EVNP warm perfusate culture	Cold kidney transport fluid culture	Recipient infections and organisms
Dual	69	DCD	None	60	68 (right) 60 (left)	No growth (right) No growth (left)	*Candida albicans*	None
Single	66	DCD	Sputum: *Enterobacter cloacae* (not treated)	54	55	Culture not taken	No growth	None
Dual	66	DCD	None	58	53 (right) 65 (left)	Culture not taken	No growth	Sterile lymphocele (secondary infection following repeated

								drainage) treated with IV flucloxacillin Superficial wound infection – *Pseudomonas stutzeri* treated with ciprofloxacin UTI – *E. coli* treated with co-amoxiclav
Single	72	DCD	None	62	62	*Staph. haemolyticus* *Staph. warneri* *Staph. epidermidis*	No growth	None
Single	53	DCD	Sputum: no growth	51	60	*Staph. capitis* *Staph. warneri*	No growth	None
Single	17	DBD	None	28	60	*Staph. aureus*	No growth	None
Single	55	DBD	Urine: *pneumococcal* antigen positive Treated with piperacillin and tazobactam	54	60	No growth	*Acinetobacter spp.*	None
Single	28	DCD	None	57	60	*Staph. warneri*	No growth	None
Single	45	DCD	Blood: *Streptococcus* (not treated)	53	60	No growth	No growth	Wound infection – *Staph. Aureus* treated with co-amoxiclav UTI – *Klebsiella pneumoniae treated with co-*amoxiclav Diarrhoea – *Clostridium difficile (untreated)*
Single	48	DCD	Sputum: *Staph. aureus* *H. influenza*, Moraxella catarrhalis,	69	45	*Staph. epidermidis* *Staph. capitis* *Staph. hominis*	No growth	Wound infection – *Klebsiella pneumoniae* treated with co-amoxiclav

			(treated with piperacillin and tazobactam, and co-amoxiclav)					

4.5 Discussion

4.5.1 Safety and feasibility

This study demonstrates the safety and feasibility of short-duration EVNP prior to deceased donor kidney transplantation, with a particular focus on infective complications. Although this study demonstrated a high rate of apparent skin commensal contamination of the warm perfusate during EVNP of kidneys prior to transplantation, there were no observed clinical implications in post-transplant infectious outcomes. Microbial growth within the perfusion fluid of EVNP may have been introduced either during EVNP or the culture-taking process. Non-concordance between identification of organisms in the cold transport fluid and the warm EVNP perfusate suggests that EVNP did not support growth. Organisms found in the warm perfusate but not the transport fluid are unlikely to have originated from the organ retrieval process. However, an alternative explanation for these findings may be explained by the fact that the cold transport fluid underwent direct plate culture, whilst the warm perfusate underwent enhanced culture in 3% soybean-casein digest broth, which would likely be more effective at promoting organism growth. It is therefore possible that the organisms grown from the EVNP perfusate originated prior to organ perfusion. The absence of post-operative infectious episodes, where growth of these organisms has been demonstrated, is reassuring. This lack of clinically detectable infection in the transplant recipients may be due to the routine use of antibiotics at the time of organ retrieval and implantation, or the coverage of antibiotics used for other infections in the early transplant period.

The risks and benefits of the use of antibiotics during ex vivo organ perfusion has not been thoroughly investigated in the literature. Antibiotics have been used in other forms of normothermic perfusion technologies, such as NRP, cardiopulmonary bypass and extracorporeal membrane oxygenation (ECMO) (261, 262). Although some studies found no difference in antibiotic

pharmacokinetic parameters between ECMO and matched control patients (263), other studies have found variation in these parameters depending on the agent used (264) as well as the mode of propulsion (i.e. roller-pump versus centrifugal pump) (265). None-the-less, ex vivo lung and liver perfusion have routinely included prophylactic antibiotics within the perfusate (266, 267). In EVNP, there is a risk of nephrotoxicity to the graft and the potential to impair early and late graft function. Given the absence of significant recipient infection following EVNP, this study does not justify the use routine use of prophylactic antimicrobials during EVNP. As a result of this work, all other UK EVNP centres have stopped using antibiotics during EVNP (personal communication: Mr. Colin Wilson (Newcastle), Mr. Gabriel Oniscu (Edinburgh) and Dr Sarah Hosgood / Prof. Mike Nicholson (Cambridge)). The use of antimicrobial agents to treat active or suspected infection in the donor as a means of facilitating and enabling transplantation should be investigated in clinical trials.

4.5.2 DGF reduction

This is the first case series examining the use of EVNP to reduce DGF post-transplantation, against paired contralateral kidneys undergoing SCS alone. The study design enabled kidneys from the same donor, undergoing different interventions, to be compared. This meant that measured and unmeasured donor factors would be comparable between the intervention and the control group. However, variation in preservation, operative and recipient factors varied between the two groups. Indeed, the mean cold ischaemia time was statistically more prolonged in the EVNP group, by four hours. Although cold ischaemia time is an independent predictor of DGF, DGF rates between paired kidneys undergoing EVNP versus cold storage were comparable. It is possible that EVNP off-set the effect of greater cold ischaemia subjected to the EVNP group. Early kidney transplant function was comparable between kidneys undergoing EVNP and static cold storage up to one year post-transplantation. There were no instances of patient death in either group during the study period. This study corroborates the findings of a recently published case series of EVNP from Canada (268). Selzner et al published the outcomes of 13 kidneys undergoing EVNP, compared to a matched control group of 26 kidneys. A key difference between my

work and Selzner et al, was that all kidneys had undergoing SCS prior to EVNP in the UK cohort, whereas all kidneys had undergone HMP in the Canadian cohort. Although, Selzner et al did not demonstrate statistically significant differences in DGF rates (EVNP group 30.8% vs HMP alone 46.2%, p=0.51), they were able to demonstrate safety and feasibility of EVNP at their centre.

The limitations of my study are acknowledged. In particular, the study is likely to be underpowered to detect a difference in DGF rates. We are currently undertaking a multicentre RCT examining the effect of EVNP on DGF rates in DCD donor kidney transplantation (201). In this RCT, a power calculation was performed indicating that even in a sample of DCD donor kidneys, in which DGF is more common that DBD donor kidneys, a sample size of 370 recipients would be required to detect a difference DGF rates. Furthermore, Hosgood et al based their power calculation on an effect size estimated from a case series of only 18 kidneys undergoing EVNP in 2013. If the effect of EVNP on DGF is smaller than expected, or if DGF is more common in the more recent population of donor kidneys, then a larger sample size may be required. The sample size of our study was therefore likely significantly underpowered. There are several other limitations of this study that should be acknowledged. Although the presence of DGF is an outcome of interest to nephrologists and transplant surgeons, it has its drawbacks as an outcome measure in clinical studies. The presence of DGF is thought to imply acute kidney injury of the transplant. In reality, the decision to undergo dialysis in the first week of transplantation is influenced by local practices pertaining to anaesthesia, intraoperative fluid and blood product management and approaches to early post-operative care, meaning that it may not truly reflect kidney injury. Furthermore, patients not established on dialysis prior to transplantation, or those with native urine output, have early graft dysfunction without needing dialysis. Other definitions of DGF exist and include those that are not dependent on the use of dialysis, but rather the change in serum creatinine (consider in Section 1.4.2, summarised in Table 5). It may be helpful for clinical studies reporting on DGF consider more than one definition within sensitivity analyses. Although this study included DGF duration as an outcome measure, since only one kidney experienced DGF, DGF duration could not be included in the analysis. DGF duration may provide greater granularity and better reflect the spectrum of kidney injury. This is explored in Chapter 3 of this thesis. Another weakness of this study was that there was a significant difference in CIT

between the groups. This meant that any difference in outcome observed between the interventions may have been confounded by the statistically significant difference in CIT. Registry analyses with large sample sizes may be able to adjust for these in multivariable analyses.

5 Chapter 5: The utility of ex vivo normothermic perfusion in amelioration of post-reperfusion syndrome

This work represents a subgroup analysis of an already existing RCT which I am a co-investigator in.

In this chapter, I screened and recruited participants on the day of their deceased donor kidney transplant. EVNP was performed by me prior to the recipient proceeding with transplantation by the clinical team (occasionally also me). All data was collected and analysed by me. Advice and guidance regarding intraoperative parameters was gained by two anaesthetic consultants.

Funded by Kidney Research UK:

5.1 Abstract

In transplantation, reperfusion of the organ within the recipient can be associated with cardiovascular instability, termed "post-reperfusion syndrome" (PRS). This has been shown to be associated with inferior outcomes in liver transplantation, but relatively little work has been performed to assess its frequency or effects in kidney transplantation. Likewise, EVNP prior to liver transplantation has been shown to reduce the incidence of PRS, however this has not yet been investigated in kidney transplantation. As part of an existing RCT examining outcomes of EVNP prior to DCD donor kidney transplantation, a single-centre subgroup exploratory analysis was undertaken to compare the incidence of PRS following EVNP versus static cold storage. PRS was defined as reduction in recipient mean arterial pressure ≥15% within five minutes of reperfusion, lasting more than one minute. Secondary outcome measures included intraoperative inotrope and vasopressor use, volume of intraoperative intravenous fluid and blood transfusion requirements, and rise in post-operative inflammatory markers. During the study period, twenty-five kidneys were randomised to EVNP and 24 were randomised to SCS alone. Four recipients (16.7%) met the criteria for post-reperfusion syndrome in the static cold storage group, and no recipients (0%) in the EVNP group had post-reperfusion syndrome. This did not reach statistical significance following intention-to-treat analysis (p=0.05). Following reperfusion, there was no difference in the use of inotrope/vasopressor infusions (12 (48%) versus 11 (45.8%), p=0.89). A rise in post-transplant inflammatory markers was observed in both groups and was comparable (p>0.05 throughout). In conclusion, EVNP kidneys did not demonstrate improved intraoperative haemodynamics compared to static cold storage, though the study was likely to have been underpowered. Further work is needed to determine whether EVNP use prior to kidney transplantation leads to reduced incidence of PRS.

5.2 Introduction

PRS is a pathological phenomenon involving severe cardiovascular dysfunction following perfusion of recipient blood into a transplanted organ (discussed in detail in Section 1.3.4). Early evidence suggests that ex situ NMP of the donated liver prior to transplantation may ameliorate PRS in liver transplantation, relative to static cold storage (269). In a landmark RCT by Nasralla *et al* examining ex situ liver NMP and static cold storage (SCS), PRS was less common following NMP (21% reduction, $p<0.001$), and with reduced vasopressor requirements in recipients of NMP livers (266).

In a porcine model of kidney transplantation, NMP has been shown to upregulate protective mechanisms such as nitric oxide synthase production (270), which is hypothesised to protect the renal vasculature during organ reperfusion, which may improve the recipient's haemodynamic response (271). However, the effect of NMP on recipient cardiovascular stability and post-transplant inflammatory response following kidney reperfusion in humans has not yet been investigated, and this data is currently not being recorded in the kidney EVNP RCT (201).

The aim of this work is to determine whether kidney EVNP ameliorates recipient post-reperfusion syndrome compared to static cold storage in humans undergoing kidney transplantation. Following transplantation, changes in clinically relevant markers of inflammation, namely C-reactive protein, white cell count and platelet count, will be examined in order to compare differences in post-operative inflammatory response following EVNP and static cold storage.

5.3 Methods

5.3.1 Study design and population

The Data Monitoring Committee of the EVNP RCT authorised an additional single-centre analysis examining the incidence of PRS within an existing RCT between recipients of kidneys that underwent static cold storage alone versus one hour of EVNP prior to kidney transplantation (trial registration

number ISRCTN15821205) (201). The inclusion and exclusion criteria are detailed in Table 39. The study period was October 2016 – July 2019. Follow-up period was 3 days post-transplantation.

Table 39 - Inclusion and exclusion criteria for the randomised control trial of EVNP versus cold storage in post-reperfusion syndrome

Inclusion criteria
Kidneys from donation after controlled circulatory death (DCD) donors
Donors aged ≥18 years old
Recipients who have given informed written consent
Recipients undergoing single kidney transplantation
Kidney transplant recipients aged ≥18 years old
Recipients undergoing their first or second kidney transplant
Exclusion criteria
Uncontrolled DCD donors
Donors aged <18 years old
Donors undergoing other forms of machine perfusion prior to transplantation e.g., normothermic regional perfusion or hypothermic machine perfusion
Recipients aged <18 years old
Recipients undergoing multi-organ transplantation e.g., simultaneous pancreas and kidney or dual kidney transplantation
Hepatitis C IgG antibody positive donor

The primary outcome of this subgroup analysis was the incidence of PRS as defined by reduction in mean arterial pressure ≥15% within five minutes of reperfusion, lasting more than one minute.

Secondary outcome measures included intraoperative inotrope and vasopressor use, volume of intraoperative intravenous fluid and blood transfusion requirements, and rise in post-operative inflammatory markers, namely white cell count, C-reactive protein, and platelet count in the first three days post-transplant.

Since the definition of post-reperfusion syndrome is not widely established in the literature, sensitivity analyses were conducted to determine whether altering the definition of PRS influenced its incidence between the two interventional groups:

- reduction in mean arterial pressure ≥15% within ten minutes of reperfusion, lasting more than one minute
- reduction in mean arterial pressure of ≥10% within five minutes of reperfusion, lasting more than one minute

5.3.2 Randomisation

Eligible recipients underwent a verbal explantation of the EVNP RCT followed by sufficient time to read the patient information sheet. Once informed consent was gained, participants were randomly allocated to EVNP or cold storage alone in a 1:1 ratio using the online randomisation service sealedenvelope.com, as per the EVNP RCT (see Section 1.5.3 - Ex vivo normothermic perfusion randomised controlled trial) The study was not blinded to investigators, clinicians or participants.

5.3.3 Cold storage group

Kidneys randomised to cold storage were retrieved, preserved in static cold preservation fluid at 4^{o}C, transported and transplanted according to local practice.

5.3.4 Ex vivo normothermic perfusion group

Kidneys randomised to EVNP were retrieved and initially preserved in static cold preservation fluid at 4^{o}C during transport to the implanting centre. Kidneys underwent cannulation of the renal artery, or an aortic patch clamp was used to allow perfusion via the renal ostia. Kidneys underwent EVNP using the standard method described by Hosgood *et al* (201). The process of EVNP is outlined previously in Chapter 4, Section 4.3.3 - Ex vivo normothermic perfusion. After EVNP, kidneys were flushed with cold Soltran (Marshall's hypertonic citrate, Baxter Healthcare Ltd) to enable static cold storage until implantation in the recipient. Likewise, the composition of the perfusate and the use of blood products has be outlined previously in Chapter 4, Sections 4.3.4 and 4.3.5.

5.3.5 Anaesthesia and peri-operative care practices

Routine monitoring included oxygen saturations, continuous electrocardiography, non-invasive blood pressure and capnography. Arterial line was not used routinely. Core temperature was monitored using an oesophageal temperature probe. Urinary catheterisation was performed with urine output monitored following reperfusion of the graft. Measurement of central venous pressure (CVP) was standard practice, to optimise of intravascular volume. The volume and choice of crystalloid varied according the individual circumstances and individual anaesthetists, but the latter was typically 0.9% sodium chloride, Ringer's lactate, 5% dextrose. Cardiac output monitoring was gained using oesophageal Doppler.

Propofol with remifentanil or alfentanil were used routinely as induction agents. Remifentanil was also used for maintenance. The airway was secured with an endotracheal tube and a rapid sequence induction. Nerve stimulator was used to ensure full paralysis throughout.

Patients received 30–40 ml/kg of crystalloid intra-operatively. A target CVP of 8–12 mmHg and normotension within 20% of baseline was the agreed goal. Vasopressor was used if required to maintain blood pressure, at the discretion of the anaesthetist. Post-operatively, IV Plasma-Lyte 148 was used to

replace urine output fluid losses at a 1:1 ratio as a standard IV fluid regimen, following review by a nephrologist in recovery.

Patients routinely returned to a level 1 bed on a dedicated renal transplant ward unless there was a specific indication for high dependency.

5.3.6 Statistical analysis

Intraoperative data was gained prospectively from operative and anaesthetic records. All variables were tested for normality using the Shapiro-Wilk test. EVNP scores were given to each kidney based on characteristics described in Table 8. Differences in demographic or clinical characteristics between groups were examined using the independent Test for parametric continuous data, Kruskal-Wallis test for non-parametric continuous data and the Chi-squared test/Fisher's exact test for categorical data. CIT was defined as the period from the start of cold perfusion in the donor to reperfusion with blood within the recipient (i.e., including the EVNP duration). Donor asystole phase WIT was defined by the time from circulatory arrest to perfusion with cold fluid. Recipient anastomosis phase WIT was defined as the time from kidney removal from ice to perfusion with recipient blood.

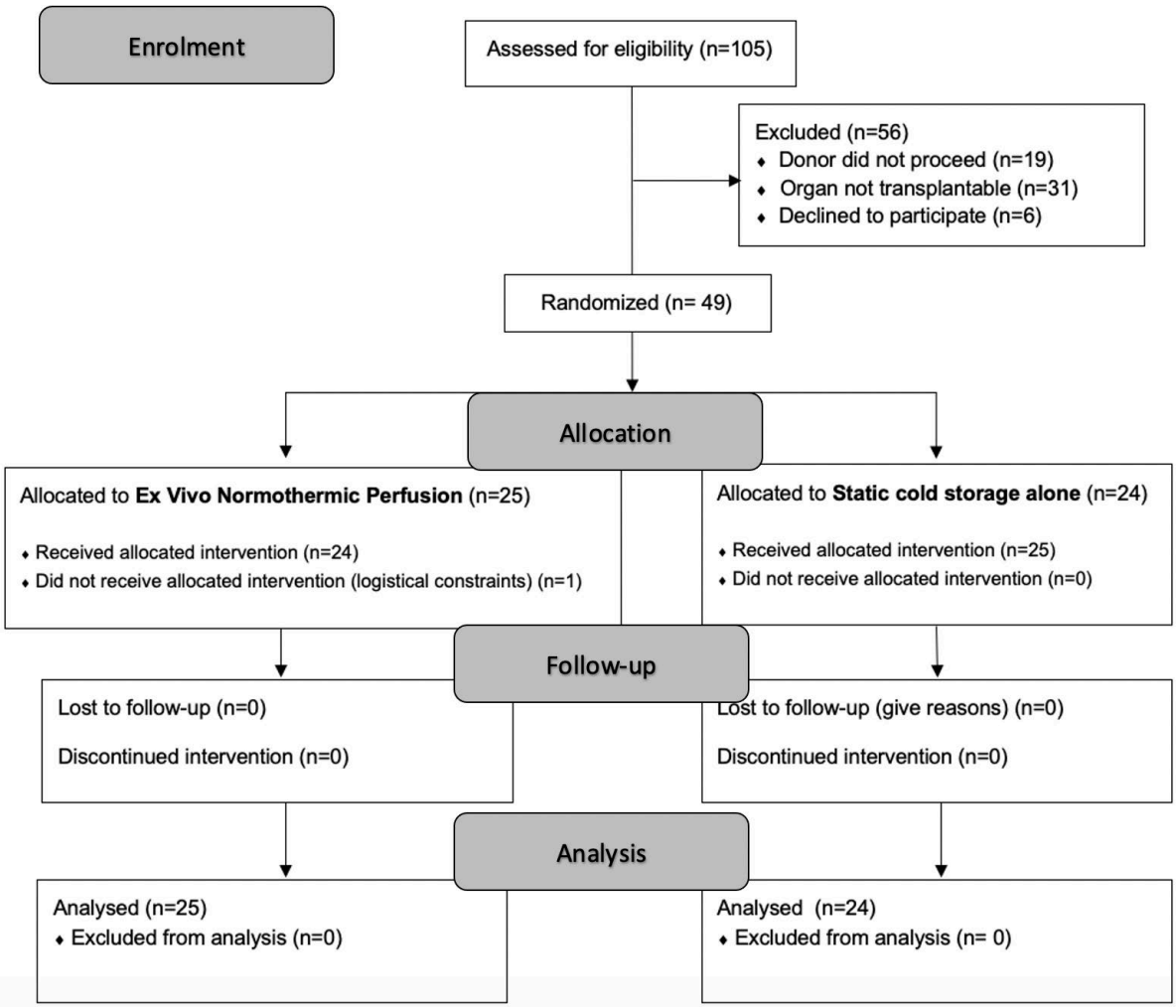
The proportion of recipients who encounter PRS in each group (EVNP versus static cold storage alone) were examined using the Fisher's exact test. Differences in mean white cell, CRP and platelet count between the groups were interrogated using the unpaired Student t test. An intention-to-treat analysis was performed, and crossover in treatment between each group recorded. Analysis per-protocol (i.e., the treatment actually received) will also be undertaken. Two-sided tests were conducted and $p<0.05$ was considered statistically significant. Data were analysed using IBM SPSS Statistics for Macintosh version 25 (IBM, Armonk, NY, USA) and Prism 8.3, GraphPad Software LLC.

5.4 Results

5.4.1 Baseline characteristics

During the study period 105 kidneys were assessed for eligibility, with 49 kidneys meeting the inclusion criteria (Figure 34). Twenty-five kidneys were randomised to EVNP and 24 were randomised to SCS alone. One kidney crossed over from the EVNP group to SCS alone, due to clinical staff shortage. All patients were analysed and there were no patients lost to follow-up.

Figure 34 - CONSORT diagram for the flow of kidneys and participants through the clinical trial



The two groups demonstrated comparable donor and recipient characteristics. Baseline demographics donor and recipient no significant difference between the two groups (p>0.05 throughout, Table 40). However, CIT demonstrated a marked difference between the two groups: approximately three hours more in the SCS group. This may have been due to the additional surgeons available to prepare the recipient and the organ when the EVNP pathway was activated (generally contributing 1-3 more surgeons) which may have improved time efficiency.

The two groups had comparable induction immunosuppressive agents, either with alemtuzumab or basiliximab (p=0.16). Whilst, the mean anastomosis time did not differ between the groups (p=0.79), kidneys randomised to EVNP demonstrated a statistically shorter mean cold ischaemia time compared to kidneys randomised to SCS alone (789 minutes versus 982 minutes, p=0.01), despite the CIT including EVNP duration.

Table 40 - Donor, recipient, and retrieval demographic information between the ex vivo normothermic perfusion group and the static cold storage group

	Static cold storage only	**Ex vivo normothermic perfusion**	**p value**
	n = 24	n = 25	
Donor age	53 (±14)	59(±14)	0.13
Donor sex			
Male	17 (70.8%)	13 (52.0%)	0.18
Female	7 (29.2%)	12 (48.0%)	
Donor cause of death			
Stroke	16 (72.7%)	21 (84.0%)	0.62
Trauma	2 (9.1%)	1 (4.0%)	
Other	4 (18.2%)	3 (12.0%)	
Donor BMI	27 (24-31)	25 (20-31)	0.20
Donor type			
Donation after circulatory death (controlled)	24 (100%)	25 (100%)	1.00
Donation after brain death	0 (0%)	0 (0%)	

Recipient age	56(±14)	60(±7)	0.24
Recipient sex			
Male	17 (70.8%)	15 (60.0%)	0.56
Female	7 (29.2%)	10 (40.0%)	
Recipient ethnicity			
Black	6 (25.0%)	10 (40.0%)	0.52
White	15 (62.5%)	13 (52.0%)	
Other	3 (12.5%)	2 (8.0%)	
Recipient cause of renal disease			
Diabetes mellitus	4 (16.7%)	6 (24.0%)	0.32
Hypertension	3 (12.5%)	5 (20%)	
Glomerulonephropathy	6 (25.0%)	2 (8.0%)	
Polycystic kidney disease	6 (25.0%)	6 (24.0%)	
Recipient induction Immunosuppression **Basiliximab** **Alemtuzumab**	 24 (100%) 0 (0%)	 23 (92%) 2 (8.0%)	 0.16
Cold ischaemia time (minutes)*	982 (±299)	789 (±185)	0.01
Asystole phase warm ischaemia time	14 (13-17)	13 (12-15)	0.48
Recipient anastomosis time (minutes)	44 (34-52)	41 (38-45)	0.79

*Cold ischaemia time includes duration of EVNP

5.4.2 Ex vivo normothermic perfusion parameters

Of the 25 kidneys randomised to EVNP, 24 kidneys actually received EVNP, for a median (IQR) perfusion time of 60 (58-60) minutes. 15 (62.5%) kidneys were given an EVNP score of 1 (excellent quality perfusion characteristics), 8 (32%) of kidneys were given a score of 2, and one kidney was given a score of 4, before going on to transplantation. During EVNP mean (SD) renal blood flow index was 74.8 (38) mL/min/100g and total urine output was 97 (70-294) mL.

Table 41 - Perfusion characteristics of the kidneys undergoing EVNP prior to transplantation

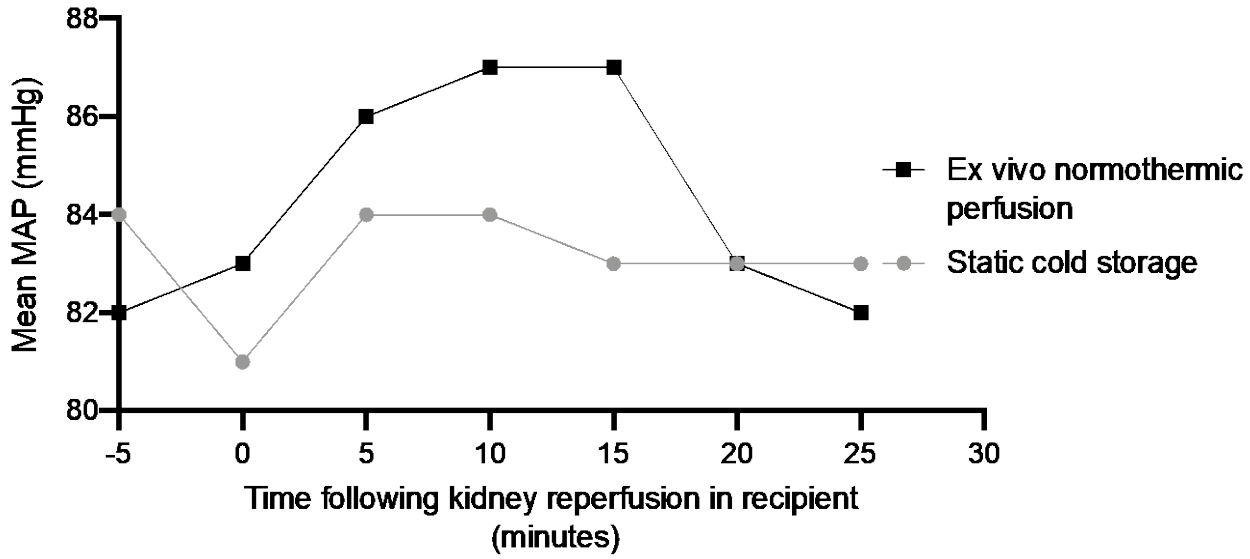
EVNP parameters	**Output**
EVNP score (1 best – 5 worse)	1 (1-2)
Mean renal blood flow index (mL/min/100g)	74.8 (38)
Total urine output (60 minutes)	97 (70-294)
Time on EVNP	60 (58-60)

Data expressed as mean (SD), median (IQR).

5.4.3 Reperfusion haemodynamics

Following reperfusion of the graft with the recipient blood, both groups demonstrate changes in recipient mean MAP over time (Figure 35). Recipients in the SCS group demonstrate a fall in mean MAP at reperfusion, but with recovery to baseline within five minutes, after which MAP appears to remain stable over 30 minutes. Recipients in the EVNP group do not demonstrate any drop in mean MAP after reperfusion, but instead demonstrate a steady increase in MAP before falling again over a 30-minute period.

Figure 35 - Change in mean MAP over time between the two groups



Standard deviation bars not shown (overlapping between the groups at all time points)

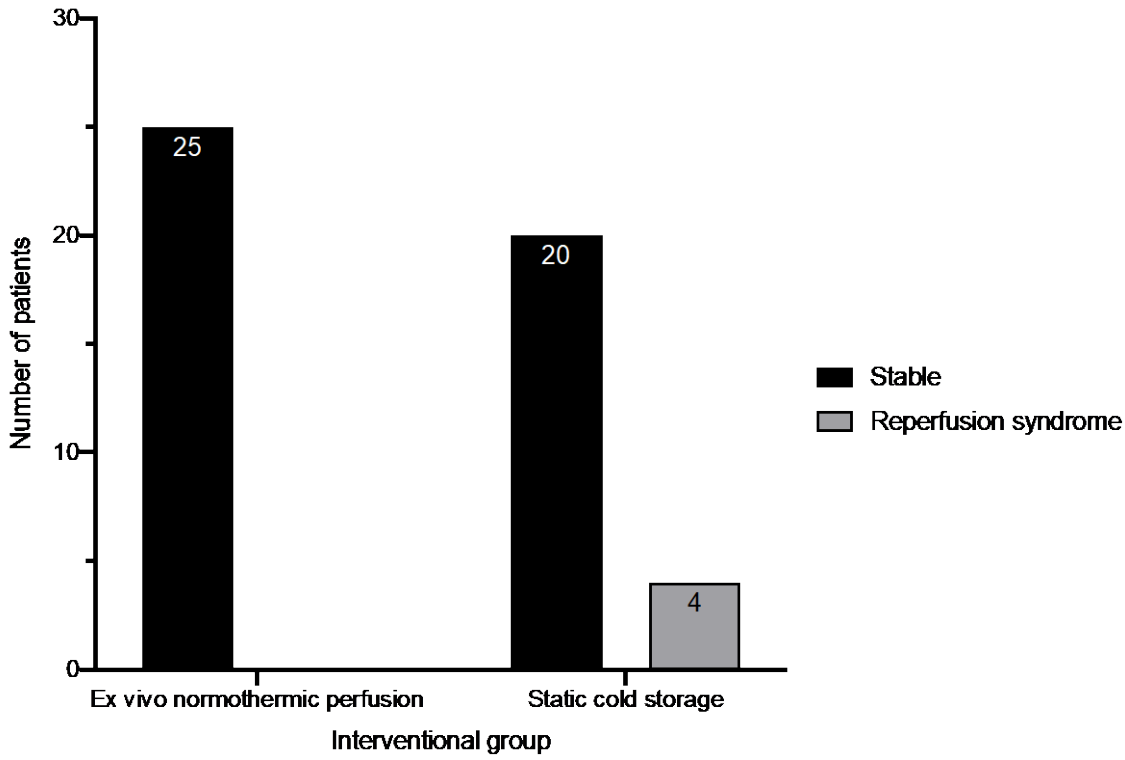
5.4.4 Intra-operative inotropes, vasopressors, fluids, and blood transfusions

Prior to reperfusion, there was no difference in the use of vasopressors or inotropes between the EVNP group and the SCS group (12 (50%) versus 13 (52%), p=0.89). Following reperfusion, there was no difference in the use of inotrope/vasopressor infusions (12 (48%) versus 11 (46%), p=0.89) or boluses (3 (12.0%) versus 6 (25.0%), p=0.29) between the EVNP and SCS groups. Mean (SD) volume of fluid resuscitation (excluding blood products) was 4.1 (1.1) litres for recipients of EVNP kidneys and 4.3 (1.2) litres for SCS kidneys (p=0.46). There was no significant difference in blood transfusion rates between the EVNP and SCS groups (2 (8.0%) versus 1 (4.2%), p=0.58). Only one recipient (4%) in the SCS group received, and two recipients (8%) in the EVNP group, received an intraoperative blood transfusion (SCS group: 1 unit of packed red blood cells, EVNP group: 1-2 units of packed red blood cells).

5.4.5 Post-reperfusion syndrome

Four recipients (16.7%) met the criteria for post-reperfusion syndrome in the static cold storage group, and no recipients (0%) in the EVNP group had post-reperfusion syndrome (Figure 36) This did not reach statistical significance following intention-to-treat analysis (p=0.05) or following per-protocol (i.e., the treatment actually received) (p=0.12). In the case of the four recipients with PRS, none had significant bleeding (<500mL), none required blood products, and all had between 4000-6000mL IV fluid intraoperatively. None of the patients with RPS required admission to a high dependency unit.

Figure 36 - Number of patients with stable post-reperfusion syndrome in each group



Sensitivity analyses

Sensitivity analyses were conducted to determine whether changing the definition of PRS to a >10% within 5 minutes would alter the incidence of PRS within the groups. The incidence of a >10% fall in mean arterial pressure, lasting greater than one minute, of post-reperfusion was 1(4%) versus 4 (16.7%) in the EVNP and SCS groups, respectively (p=0.19). The incidence of a 15% fall in mean arterial

pressure within 10 minutes of post-reperfusion was 0 (0%) versus 1 (4.2%) in the EVNP and SCS groups, respectively (p=0.99). Altering the definition of PRS to include a smaller fall in mean arterial pressure, or a longer duration post reperfusion did not identify a difference in the efficacy of EVNP in the amelioration of PRS.

5.4.6 Post-operative inflammation

Baseline CRP and platelet count prior to transplantation were comparable between the groups (Table 42; $p<0.05$). However, baseline pre-transplant white cell count was higher in the static cold storage group (p=0.01). Following transplantation, a rise in serum white cell count and CRP was observed in both groups, generally reaching its highest point on the second post-operative day. A fall in platelet count was demonstrated in both groups, with its lowest point between the second and third post-operative day. However, there was no significant difference in the white cell count, CRP or platelet counts between the two interventional groups on days 1-3 post-transplantation. Likewise, there was no correlation between the use of EVNP and changes in white cell count, CRP, or platelet count post operatively (Table 43).

Table 42 - Mean change in inflammatory markers post-transplant between the two groups

		Static cold storage only	**Ex vivo normothermic perfusion**	**p value**
		n = 24	n = 25	
White cell count	Pre-transplant	7.3 (±2.1)	5.9 (±12.1)	0.01
	Day 1	11.6 (±3.3)	10.4 (±3.9)	0.21
	Day 2	11.5 (±3.9)	10.9 (±5.9)	0.71
	Day 3	10.8 (±3.6)	9.6 (±4.7)	0.32

C-reactive protein	Pre-transplant	5.7 (±5.1)	5.2 (±4.2)	0.68
	Day 1	18.9 (±9.6)	23.0 (±15.0)	0.31
	Day 2	29.6 (±24.1)	33.1 (±29.1)	0.66
	Day 3	26.0 (±26.4)	30.0 (±24.1)	0.69
Platelet count	Pre-transplant	191.0 (±48.0)	184.3 (±56.4)	0.55
	Day 1	165.2 (±49.8)	160.3 (±50.1)	0.67
	Day 2	146.7 (±37.4)	154.4 (±62.2)	0.74
	Day 3	146.8 (±38.5)	140.0 (±49.4)	0.45

Data expressed mean (standard deviation)

Table 43 - Correlation between the use of ex vivo normothermic perfusion and inflammatory markers before and after transplantation

	White cell count				**C-reactive protein**				**Platelet count**			
	Pre-transplant	***Day 1***	***Day 2***	***Day 3***	***Pre-transplant***	***Day 1***	***Day 2***	***Day 3***	***Pre-transplant***	***Day 1***	***Day 2***	***Day 3***
Correlation coefficient*	-0.4	-0.2	-0.1	-0.1	-0.1	0.2	0.1	0.1	-0.1	-0.1	0.1	-0.1
p value	0.01	0.21	0.71	0.32	0.68	0.31	0.66	0.69	0.55	0.67	0.74	0.45

* Pearson's correlation coefficient (-1 to +1)

5.5 Discussion

5.5.1 Study findings and their implications in transplantation

The use of EVNP prior to transplantation to improve early graft function is currently being investigated in a multicentre, open-label RCT (201). The study in this chapter represents a single-centre retrospective subgroup analysis of that trial to determine whether EVNP reduces haemodynamic instability intraoperatively, known as PRS. During the trial, there were no instances of reperfusion syndrome in any kidney which was randomised to EVNP. The incidence of post-reperfusion syndrome in kidneys randomised to SCS was 16.7%. However, the difference in post-reperfusion syndrome rate between the two interventions did not reach statistical significance. This may represent a type II statistical error in which the trial was under-powered to detect this particular outcome, and the main analysis of the wider RCT was not indented to be powered for PRS. Furthermore, there were no differences in circulatory support or blood transfusion requirements between the EVNP and SCS groups. Participants who received kidneys randomised to EVNP showed comparable inflammatory responses in the first three days of transplantation, compared to SCS. In sensitivity analyses, alteration of the definition of post-reperfusion syndrome to include a smaller fall in mean arterial pressure, or a longer duration following reperfusion, did not significantly alter the outcomes of the study.

The incidence of PRS in deceased donor kidney transplantation undergoing SCS in this study was four times higher than previously demonstrated by Bruhl SR et al in 2012 (16.7% versus 4%[†]) (123). This may be due to differences in donor and recipient demographics between the two studies. Indeed, donor age was significantly lower in the study performed by Bruhl et al (<40 years) compared to the

[†] In Bruhl et al, 4 recipients of deceased donor kidney transplants encountered reperfusion syndrome, compared to 95 recipients who did not. The 51 living donor kidney transplants are not included in this figure.

donors included in this study (>50 years), with only included one kidney (0.6%) from a DCD donor. The difference in incidence of PRS may also vary according to anaesthetic practices, and variations in anaesthetic, fluid and blood transfusion usage. Since the publication of Bruhl SR et al in 2012, the donor risk profile has evolved, with the use of older and more comorbid donors. The incidence of post-reperfusion syndrome could be rising. Currently the incidence of PRS is not reported to the national transplant registry. This should be considered as it would allow a greater understanding of the impact of post-reperfusion syndrome on short and long-term outcomes and allow detailed analyses of the risk factors for its development.

The pathogenesis of post-reperfusion syndrome is complex and is mediated through a variety of immune pathways, including overlap with ischaemia reperfusion injury (IRI), and interaction with the circulatory system. EVNP has been shown to ameliorate post-reperfusion syndrome following liver transplantation (266), though it is not understood the mechanisms of its action in this setting. It could be hypothesised that PRS is ameliorated when IRI is reduced.

PRS in OLT appears to be associated a larger drop in recipient blood pressure, which has not been demonstrated in kidney transplantation. This is likely to be due to several factors. The liver's regional blood flow is relatively higher than the kidney, meaning that it not only demands a greater proportion of the recipient's cardiac output, but there is a higher volume of preservation fluid dumped into circulation upon reperfusion. This is further compounded by significantly higher expected blood loss during liver transplantation, due to portal hypertension, the need to remove the native liver and the coagulopathy associated with liver failure. A hyperdynamic circulation is also associated with advanced cirrhosis (125). Post-reperfusion syndrome may therefore not only be more common in liver transplantation, but also more profound. None-the-less, its impact in kidney transplantation needs to be better defined.

5.5.2 Limitations

There are several limitations of this study. Firstly, the clinical trial was not designed to answer questions regarding PRS. Therefore, the study was not powered to detect a difference in PRS. This should

ideally be considered in a RCT of its own, with an *a priori* power calculation. This study did not consider an alternative treatment to EVNP and SCS, namely hypothermic machine perfusion. Since these three preservation methods are competing options, these should be considered in future clinical trials, in which data from this study may inform power calculations and sample size. Based on the incidence of PRS observed in EVNP and SCS groups (0% vs 16.7%), and assuming a power (1-beta) of 0.80 and the probability of a type 1 error (alpha) of 0.05, a power calculation indicates that 42 patients would be required in each group (1:1 enrolment ratio) to detect a difference in PRS between SCS and EVNP groups. Finally, the impact of a longer CIT in the SCS group may have influenced the results and should be taken into account.

Greater information on the clinical impact of RPS, in terms of short-term morbidity, ITU admission, blood product sensitisation and adverse events, as well as longer-term graft and patient survival, is needed, in order to understand the relevance of RPS in kidney transplantation.

5.5.3 Summary

EVNP is a new technology under clinical evaluation, and this study provides further insight into its safety. Although this study did not show superiority relative to static cold storage, inferiority was not demonstrated either. Even if there are potentially comparable rates of PRS, there may be other benefits of EVNP over SCS which have not yet been considered, such as organ viability assessment, avoidance of unnecessary organ discard and improvements in early graft function. Furthermore, the form of EVNP in this study does not represent a 'finished product'. The inclusion of white cell filters, to reduce ischaemia reperfusion injury, is considered in Chapter 6 of this thesis. Further optimisation of EVNP perfusate, including its ingredients, temperature and pressure, may reduce IRI and recipient cardiovascular dysfunction (272). Likewise, delivery of additional immunomodulatory agents during EVNP to reduce IRI is still being considered in other studies (213, 214, 273). The application of EVNP as an evolving therapy in the reduction of PRS should still be considered as an endpoint in future studies.

6 Chapter 6: The use of white cell filtration during ex vivo normothermic perfusion as a means of ameliorating early graft parameters

Within this Chapter, I screened all potential UK declined donor kidney offers. Kidneys were surgically prepared and perfused using EVNP by me. All samples were taken and analysed by me. Single cell suspension, cell labelling, and flow cytometry were performed by me.

This work has been submitted to the British Transplant Society congress 2022 for presentation.

Funded by GSTT KPA:

6.1 Abstract

Passenger leukocytes (PLs) are donor-derived organ resident white blood cells found within transplanted organs which are strongly implicated in IRI and acute rejection. Populations of PLs have been shown to be mobilised into the circulation during EVNP in animal models, with depletion demonstrated by application of a white cell filter. This study aimed to demonstrate the presence of PLs mobilised from human kidneys during EVNP and to determine whether these could also be depleted using white cell filters. Finally, after a period of static cold storage, kidneys with and without leukocyte depletion were reperfused with whole blood as a model for transplantation, and early allograft outcomes were compared between the two interventional groups. This study demonstrated that PLs are present in the perfusate during EVNP in large numbers, predominantly comprising cytotoxic T cells and T helper cells. White cell filtration appeared to reduce the number of PLs in circulation. However, this did not translate into superior renal blood flow, oxygen consumption or urine output when reperfused with whole blood. This may be due to underpowering of the study. PLs may be a potential target for intervention during EVNP in larger studies.

6.2 Introduction

6.2.1 Mechanisms underpinning graft dysfunction

The mechanisms underpinning graft dysfunction and subsequent graft loss are complex, multifactorial, and vary depending on the duration post-transplant. Pathological processes include ischaemia-reperfusion injury (IRI) (hours to days post-transplant), early acute rejection (weeks to months post-transplant), and chronic rejection (years post-transplant). Passenger leukocytes (PLs), which are donor-derived organ resident white blood cells (274), are strongly implicated in IRI and early acute rejection, and are a key target for intervention.

6.2.2 Passenger leukocytes

Prior to deceased organ donation, it is recognised that the donor's kidneys undergo a series of injuries, e.g., due to hypotension, sepsis, or systemic inflammation resulting from raised intracranial pressure and brain death. As a response to these injuries, PLs within kidneys from deceased donors develop a pro-inflammatory phenotype (275) with high numbers of infiltrating T lymphocytes and macrophages (276). PLs are likely to play many different pathogenic roles post-transplant, as multiple donor white cell populations with varying functions are found within a transplanted organ including dendritic cells, tissue-resident macrophages, neutrophils, and lymphocytes. PLs play a key role in promoting both innate and adaptive immune responses in the transplant recipient (88).

Leukocytes in general, and PLs specifically, have been widely implicated in IRI. T- or B-cell deficient animals have markedly reduced IRI responses (111), and lung transplant models have demonstrated that IRI is mediated by PLs (113, 277). In the murine kidney, resident dendritic cells have been shown to be the predominant secretors of TNF after IRI (278), and to increase antigen presentation capabilities (279). After IRI, activated leukocytes release reactive oxygen species, nitric oxide, and an array of proinflammatory cytokines and chemokines, including IL-1β, IL-6, IL-8, IL-11, IFN-γ, and TNF-α. Many other pathways have also been implicated in IRI as a result of T and B cell behaviours, including

activation of the complement cascade (280). Natural killer cells (NK) are a population of lymphocytes that form part of the innate immune response. Evidence suggests that NK cells also play a role in immune-mediated graft damage following kidney transplantation (281). NK cells prime the adaptive immune response, promote migration of other immune cells to the graft (especially cytotoxic T cells), and do so without needing exposure to an antigen (282).

PLs also play a role in acute allograft rejection. Three major pathways of allorecognition have been described: direct, semi-direct, and indirect (283). PLs are fundamental to both the direct and semi-direct pathways. Direct allorecognition occurs when recipient T cells recognise intact donor HLA antigens presented on the surface of donor antigen-presenting cells (APCs) contained within the PL population. Direct allorecognition alone can result in acute allograft rejection (87), and is thought to be the dominant pathway by which early graft rejection occurs in a non-sensitised recipient, as recipient natural killer (NK) cells and CD8+ T lymphocytes destroy PLs soon after implantation (284). In semi-direct allorecognition, recipient APCs acquire intact HLA molecules from donor cells and present these to recipient T cells. There is emerging evidence in mouse models that recipient APCs trigger T cell responses after transplantation, having 'borrowed' donor cell-surface HLA molecules (285, 286), and that donor-derived DCs play a crucial role in this process (285).

Depletion of donor-derived T, B and NK cells before transplantation is a potential strategy in improving early allograft function and reducing rates of acute rejection and subsequent graft loss. In contrast, there may be certain populations of PLs that may inhibit or modulate inflammatory processes. T regulator cells are thought to have immunosuppressive properties and actively promote recipient tolerance to kidney allograft (287). Evidence also suggests that donor-derived T regulator cells also play a beneficial role in suppressing the allogeneic immune response, with reduction of the incidence of chronic allograft vasculopathy (288).

6.2.3 Ex vivo normothermic perfusion as a means for studying early graft dysfunction

EVNP is currently under clinical evaluation as a pre-transplant intervention with the aim of reducing DGF post-transplant, so-called organ 'pre-conditioning' (considered in Chapter 4). EVNP also has the potential to administer novel interventions directly on the organ of interest prior to transplantation, whilst simultaneously assessing the function of the organ. The benefit of this approach is that it enables functioning organs to be studied in 'real-time' in a system that is easily manipulated, e.g., by the addition or removal of elements in the circuit, changes to organ perfusate flow, pressure, or oxygenation.

The same system can also be used experimentally to study organ pathophysiology in the hours following revascularization by perfusing the organ with whole blood. This acts as a model for transplantation, by mimicking the conditions of reperfusion in a transplant recipient, so called 'transplant mode' EVNP. This study utilises both 'pre-conditioning' and 'transplant' mode EVNP, to deliver an intervention as well as examine its potential effect following transplantation, respectively.

6.2.4 Ex vivo normothermic perfusion and passenger leukocytes

Porcine models using autologous whole blood to perfuse kidneys have demonstrated that the addition of a white cell filter to the EVNP circuit reduced IRI and improved kidney function during EVNP (289). A subsequent study confirmed that white cell depletion of the EVNP kidney perfusate leads to reduction of myeloperoxidase-expressing cells into the kidney and decreased renal tubular apoptosis, caspase-3 activity, and IL-1β activation (290).

As a result of these findings, the perfusate used in clinical kidney EVNP uses packed red blood cells (i.e., depleted of white blood cells, complement, and platelets), and early clinical series suggest that rates of DGF are lower in kidneys undergoing EVNP (200). A multi-centre RCT is currently underway in the UK to investigate the ability of EVNP to reduce the rates of DGF in kidneys from DCD donors (201), though without the use of a white cell filter in the EVNP circuit.

Crucially, Nicholson's animal experiments are unable to differentiate between the role of donor and recipient leukocytes in renal IRI, as autologous blood was used as a perfusate, and a white cell filter in the circuit was not used in addition to pre-EVNP leukocyte-depleted blood. Furthermore, in clinical EVNP, the use of packed red blood cells from the blood bank does not prevent donor PLs from potentially mediating IRI. Notably, use of leukocyte-depleted blood as a perfusate, but without a white cell filter in the EVNP circuit, did not fully ameliorate IRI, and leukocyte counts increased after re-perfusion of porcine kidneys, suggesting that PLs mobilized out of the kidney and entered the perfusate(291).

EVNP provides a unique model in which to study PL mobilization and function in the absence of a recipient immune system, and in a functioning organ. Recent studies have demonstrated that EVNP can be used to study PLs, mainly in animal lung models. PLs, predominantly non-classical monocytes, are known to exit the human lung during EVNP, and differentiate into dendritic cells with an inflammatory phenotype on cell culture (292). Stone *et al* have also shown that EVNP of porcine lungs leads to a reduced PL load within recipient lymphoid tissue after transplantation, and that this is associated with a reduction in recipient T cell priming and graft infiltration (293). This study did not specifically utilise leukocyte filters, however. Noda *et al* has that the use of a leukocyte filter in a rat lung transplant EVNP model led to improved lung function after transplantation, and lower levels of IL-1β, IL-6, and TNF-α within the perfusate (294).

To date, the only study that has assessed PL efflux during kidney EVNP has come from the Manchester group. Using a porcine model, and perfusing with leukocyte-depleted autologous blood, kidneys liberated PLs and proinflammatory cytokines into the circuit after 60 minutes, though the kinetics of cell release and cytokine production varied (295). No interventions were performed to ameliorate the PL or cytokine responses.

The role of PLs in renal IRI and acute allograft rejection, and the ability of EVNP to enable targeting these cells, has therefore not yet been fully addressed. It is not known if PLs in human kidneys behave similarly to those in porcine organs, or if leukocyte filters (or other interventions can inhibit pathogenic PL function.

The aims of this chapter are two-fold:

1. To determine whether populations of PLs are present in the human kidney and are mobilised into the perfusate during EVNP
2. To determine whether populations of PLs can be depleted from the human kidney during EVNP using white cell filtration, resulting in an improvement in early transplant function

6.3 Methods

6.3.1 Study population

Human kidneys intended for transplantation, but subsequently declined by all UK transplant centres were recruited into this basic science study. Generic research consent for the use of human organs declined for clinical transplantation was gained from specialist nurses of organ donation from organ donor families. When kidneys were unexpectedly found to be untransplantable, such as donor malignancy, organs are offered to registered research groups, by NHS Blood and Transplant, via the Research, Innovation, and Novel Technologies Advisory Group (RINTAG; *https://www.odt.nhs.uk/odt-structures-and-standards/clinical-leadership/research-innovation-and-novel-technologies-advisory-group/).* Local research ethical committee (REC) approval was also gained (REC reference 10/LO/0928, IRAS 229654).

Inclusion and exclusion criteria of the study are described in Table 44. No restrictions were made on donor age, donor type (such as donation after circulatory or brain death), warm or cold ischaemia time.

Table 44 - Inclusion and exclusion criteria

Inclusion criteria
Discarded human kidneys declined by all UK kidney transplant centres
Generic consent for research attained from donor families by NHS Blood and Transplant
Favourable arterial anatomy
Exclusion criteria
HIV positive donor
Hepatitis B surface antigen positive donor
Hepatitis C IgG antibody positive donor
Machine perfusion during or after retrieval

HIV and viral hepatitis positive donors were excluded due to local laboratory restrictions on blood and tissue from donors with infectious diseases. Donors and organs already exposed to novel machine perfusion technology were also excluded as this may have impacted the PL population within the kidney. All kidneys were retrieved by the UK's national organ retrieval service (NORS).

Retrieval of organs differed according to donation type. Briefly, DBD donors underwent systemic heparinisation and iliac/aortic cannulation before asystole was induced. DCD donors underwent a super rapid retrieval approach whereby death is confirmed, the donor is left for five minutes, and cannulation of the infra-renal aorta or common iliac artery in the absence of spontaneous cardiac activity. DCD donors did not undergo systemic heparinisation. Organs underwent in situ flushing with cold preservation fluid whilst the abdominal cavity was packed with ice. Following removal of the kidneys, these were packed in sterile bags and transported at a target temperature of 4^{o}C.

6.3.2 Organ preparation

On arrival, kidneys were prepared for perfusion in the same way organs are typically prepared for clinical implantation. In a surgical process known as 'benching', perinephric adipose tissue was removed and small veins in this tissue with 3-0 Vicryl (polyglactin 910, Ethicon) ligature and 5-0 Prolene (polypropylene, Ethicon) suture to prevent bleeding during perfusion. Gonadal and adrenal veins are ligated in the same way. The renal vasculature was isolated (renal arteries and veins) and either directly cannulated or attached to an aortic patch clamp to allow blood to flow in and out of the organ. Organs were weighed after benching.

6.3.3 Ex vivo normothermic perfusion of human organs

The process of EVNP is outlined previously in Chapter 4, Section 4.3.3 - Ex vivo normothermic perfusion. After EVNP, kidneys were flushed with cold Soltran (Marshall's hypertonic citrate, Baxter Healthcare Ltd).

6.3.4 Composition of perfusate

The composition of the perfusate has be outlined previously in Chapter 4, Sections 4.3.4. Human packed red blood cells units, compatible with the donor kidney, were provided by Guy's and St. Thomas' NHS Foundation Trust Transfusion services. These were intended for clinical transfusion but were not used due to reaching expiration date for clinical use. All units of packed red blood cells were used within 1 week of their expiration date and visually examined for quality.

Prior to perfusion of the kidney, leukocyte numbers within each unit of packed red blood cells were analysed. This was performed in the same way that EVNP perfusate samples were assessed for leukocyte numbers, described in Sections 6.3.6 and 6.3.7 - *Sampling process for EVNP perfusate*. The circuit was heparinised to avoid clot formation (3000 units unfractionated heparin).

Infusion of Hartmann's solution was added during perfusion at a rate determined by the urine output of the kidney. Infusions of nutrition (amino acids, vitamins, and glucose present in synthamin, cernavit and 5% dextrose solution, respectively) were commenced during perfusion.

6.3.5 Leukocyte mobilisation during EVNP

The first part of this study examined the mobilisation of passenger leukocyte populations from kidneys during EVNP. Discarded human kidneys underwent EVNP with one unit of ABO-compatible packed red blood cells, and samples of the perfusate were examined at baseline (before inclusion of the kidney) and every 30 minutes up to 120 minutes. Samples underwent cell staining and flow cytometry to determine numbers of specific PL subpopulations. The PL populations of interest, and their associated fluorochrome-labelled primary antibodies, are listed in Table 46..

Table 46. This included CD4+ and CD8+ T cells, CD19+B cells, CD25+ T regulatory cells and NK cells, broadly representing clinically relevant white cells of interest.

6.3.6 Leukocyte depletion during EVNP

The second part of this study examined the use of white cell filters (Leukcoguard® LG6 arterial filter, Pall Medical) during EVNP as a means of depleting PL numbers. Kidneys that had undergone EVNP *without* white cell filtration (from Section 6.3.5) were used as a comparator group. The relative number of white cell populations could therefore be compared between kidneys undergoing white cell filtration and those without white cell filtration.

During this experiment, EVNP was performed in two modes: 'pre-conditioning' mode and 'transplant mode'. Pre-conditioning mode intends to revive the organ in the absence of a recipient immune system (packed red blood cell based perfusate), in order to ameliorate graft function. In pre-conditioning mode, the presence or absence of leukocyte filters is being investigated. Transplant mode EVNP acts as a model for transplantation (an alternative to reperfusing the organ in a living human), with reconstituted whole blood (packed red blood cells with third-party peripheral leukocytes). Transplant

mode EVNP will be used as a means of assessing the efficacy of leukocyte depletion following pre-conditioning EVNP. The difference between pre-conditioning and transplant mode EVNP is summarised in Table 45.

Table 45 - Different modes of delivering EVNP, and their purpose

EVNP mode	Difference in perfusate composition	Purpose
Pre-conditioning mode	No third-party peripheral leukocytes present	• Organ preservation • Addition of therapies, such as white cell filters
Transplant mode	Third-party peripheral leukocytes present	• Mimic reperfusion in a transplant recipient • Model for transplantation • Alternative to human or animal implantation

Both modes of EVNP include one unit of packed red blood cells

After standard retrieval from the donor, kidneys were transported in cold storage to the laboratory. Kidneys underwent pairwise randomisation (random number generation; www.randomizer.org) and allocated to either white cell depletion or non-depletion EVNP (Figure 37). The position of the white cell filters, relative to the other components of the EVNP circuit is demonstrated in Figure 38.

Since there was only one EVNP machine, each intervention was randomised to determine which would be performed first and which would be performed second (the latter incurring approximately 2 hours of additional cold storage). Kidneys in the non-white cell depletion group are made up of the kidneys that had undergone EVNP *without* white cell filtration (from Section 6.3.5) as well as those that were randomised to EVNP without white cell filtration.

Figure 37 - Experiential plan for intervention and control kidneys

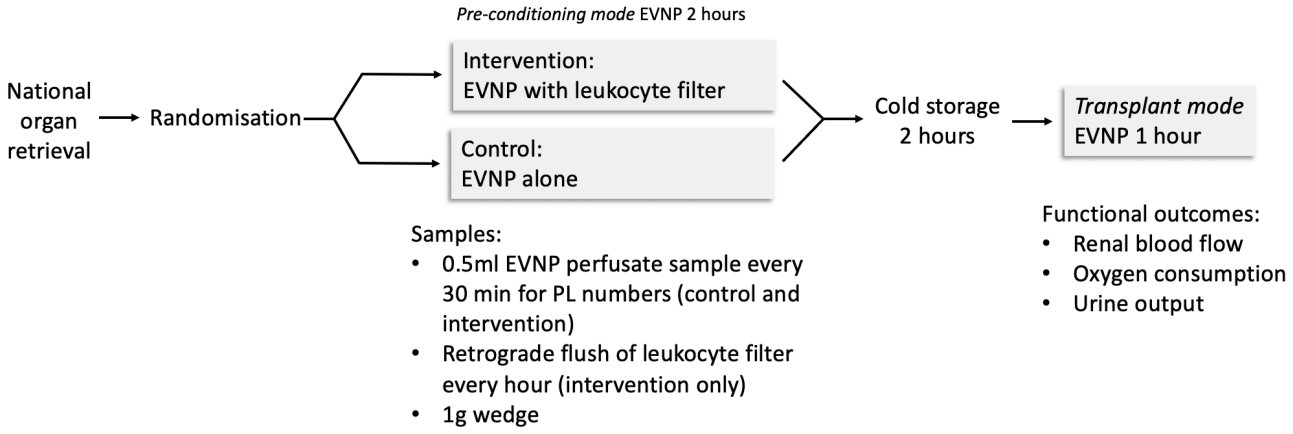
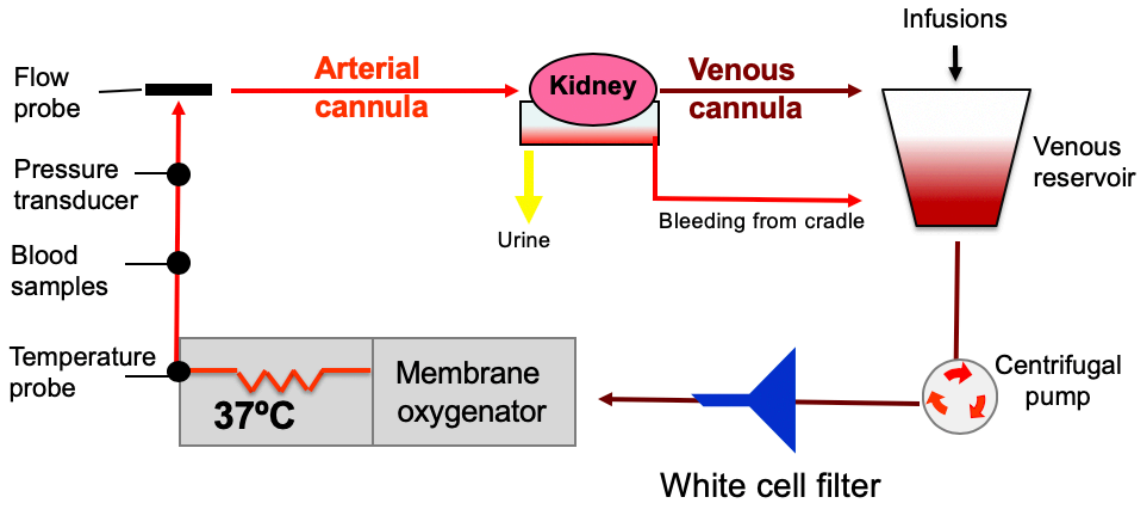


Figure 38 - Ex vivo normothermic perfusion circuit with inclusion of white cell filter for the depletion of donor-derived passenger leukocytes



To prevent saturation of the white cell filters, the filter was be replaced every hour. To enable continuous perfusion of the kidney, two white filters cells were inserted in parallel, and flow directed via the route of the active filter. After one hour of perfusion the first filter was removed from the circuit and

retrogradely flushed with trypsin-EDTA 0.25% solution (Sigma-Aldrich), incubated at 37°C for 5 minutes to allow cells to become dissociated from the filter, and washed with phosphate-buffer saline. After the second hour, the same process was repeated for the second filter. Cells collected from each filter were centrifuged and put in a single cell suspension, stained with fluorochrome labelled antibodies (Table 46..

Table 46) and analysed using flow cytometry. At the end of 'pre-conditioning' EVNP, a wedge biopsy was be taken to estimate the number of PLs remaining in the kidney after two hours of white cell depleting EVNP.

Kidneys were then be flushed with cold Soltran and placed back in static cold storage for two hours before proceeding to 'transplant mode' EVNP. This involved EVNP with allogeneic ABO-compatible packed RBCs replete with human allogeneic peripheral blood leukocytes. Human allogeneic peripheral blood leukocytes were acquired in the form of 'cones', derived from leukocyte reduction filtration from a single platelet apheresis donor, available at request from NHSBT (https://hospital.blood.co.uk/components/non-clinical-issue/nci-products/). These peripheral leukocytes were added to the EVNP perfusate to make approximate physiological concentration of 4000-10000 cells/μL (296). This acted as a model for reperfusion in a human recipient (therefore termed 'transplant mode' EVNP) given that a 'recipient' immune system is present. Previously described EVNP functional parameters were measured, including urine output, renal blood flow and intra-renal resistance. Oxygen consumption was measured by performing arterial and venous cannulae blood gas analysis at 60 minutes of reperfusion with whole blood, using Equation 2.

Equation 2 - Estimation of oxygen consumption

$$= (Difference\ in\ arterial\ and\ venous\ partial\ pressure\ of\ oxygen) \times \frac{Renal\ blood\ flow}{kidney\ weight}$$

These parameters provided a measure of renal function and a functional readout of the degree of IRI that occurred within the graft over 1 hour of 'transplant mode' EVNP, and enabled differences

between PL-depleted and non-PL depleted kidneys to be observed. EVNP perfusate samples at 30-minute intervals (intervention and control kidneys) and retrograde trypsin flush of the white cell filters each hour of perfusion (intervention kidneys only) were analysed using flow cytometry. The PL populations of interest, and their associated fluorochrome-labelled primary antibodies, are listed in Table 46..

Table 46 - Passenger leukocyte population of interest, explanation of their homeostatic role when resident in kidney, and their fluorochrome-labelled primary antibody stain

Passenger leukocyte population	**Fluorochrome-labelled primary antibodies**
All leukocytes	CD45-APC
T helper cells	CD4-PE Texas Red
Cytotoxic T cell	CD8-BV605
B cells	CD19-BB515
T regulatory cells	CD25-BV421
Natural killer cells	CD56-PerCP Cy5.5

A brief explanation of the homeostatic role of tissue resident immune cells within the kidney is found in Section 1.2.3.

Unstained controls were examined using forward and side scatter dot plots in order to determine appropriate voltages for the population. Single stained compensation controls were used to avoid spectral overlap. Fluorescence-minus-one controls were used to build the flow cytometry panel of labelled antibodies in order to avoid significant fluorescence spillage of the controls into other channels, and the gate set accordingly. Cells were stained with all fluorochromes except one, in order to identify the positive population from the negative population. Minimal compensation was needed in this basic panel.

Sampling process for EVNP perfusate

Every 30 minutes of perfusion, 50μL EVNP perfusate and fluorochrome-labelled antibodies (20μL total) were added to BD TruCount™ FACS tubes containing fluorescent counting beads. FACS tubes were then vortexed gently to mix the reagents and incubated at 21°C for 15 minutes to allow antibody binding to cell surface antigens. 450μL of FACS lysing solution is added to make a known volume of 500μL.

Sampling process for kidney wedge biopsy

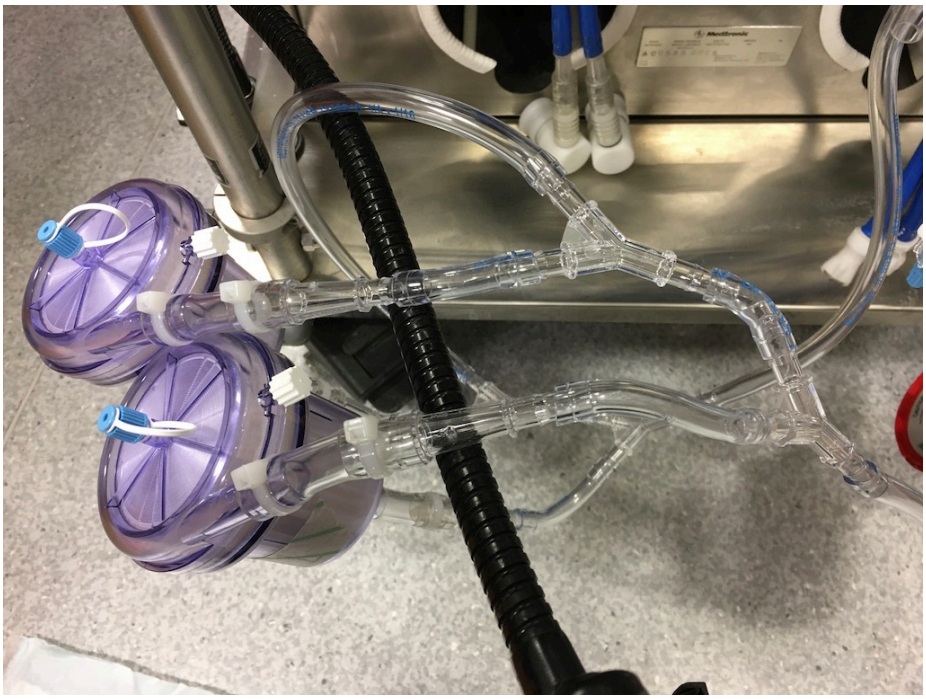
Wedge biopsy, weighing 1g, was excised from the mid-pole of the kidney following pre-conditioning EVNP in both the intervention and control kidneys. To prevent bleeding from the biopsy site, the kidney parenchyma was closed using Prolene sutures (polypropylene, Ethicon). Preparation of the wedge biopsy into a suitable state for fluorescent-activated cell sorting involved four steps. First, biopsies were cut and mechanically broken down using dissecting scissors and forceps to increase the total surface area. Second, digestive enzyme solution was added to the kidney tissue (Trypsin-EDTA 0.25% solution) and incubated at 37°C on an orbital shaker for five hours to digest the connective tissues between the cells. Third, debris and large aggregates were removed using a nylon mesh cell strainer and the single cell suspension was evaluated under a light microscope for cell viability using a live/dead viability dye (Trypan blue solution 0.4%, BioVision Inc.). Finally, the single-cell suspension was added to BD TruCount™ FACS tubes, containing fluorescent counting beads, along with fluorochrome-labelled. FACS tubes were the vortexed and incubated at 21°C for 15 minutes to allow antibody binding to cell surface antigens. 450μL of FACS lysing solution is added to make a known volume of 500μL.

Sampling process for cells trapped in the white cell filter

After each hour of perfusion, the leukocyte filter was changed (total perfusion time 2 hours). To prevent cessation of perfusion, two leukocyte filters were placed in parallel to the EVNP circuit (Figure

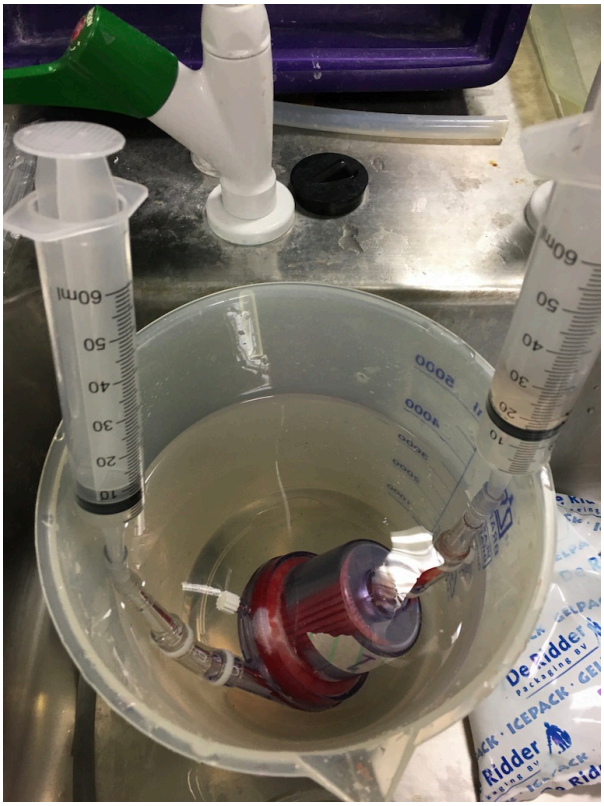
39). Using arterial clamps, the EVNP perfusate could be direct via filter 1, filter 2 or through a route with no filter. Filters could therefore be removed for analysis during active organ perfusion. Dead-space was filled with perfusate prior to organ perfusion to avoid air embolism to the organ or air locking within the circuit.

Figure 39 - White cell filters placed in parallel to the EVNP circuit. Clamps were used to direct blood via filter 1, filter 2 or through a route with no white cell filtration.



After each hour of perfusion, filters underwent retrograde flush with an enzyme solution (Trypsin-EDTA 0.25% solution, Sigma-Aldrich®) within a warm bath (37°C) to remove cells caught during perfusion (Figure 40).

Figure 40 - Retrieval of leukocytes caught in the white cell filter after ex vivo normothermic perfusion by retrograde flush with trypsin solution

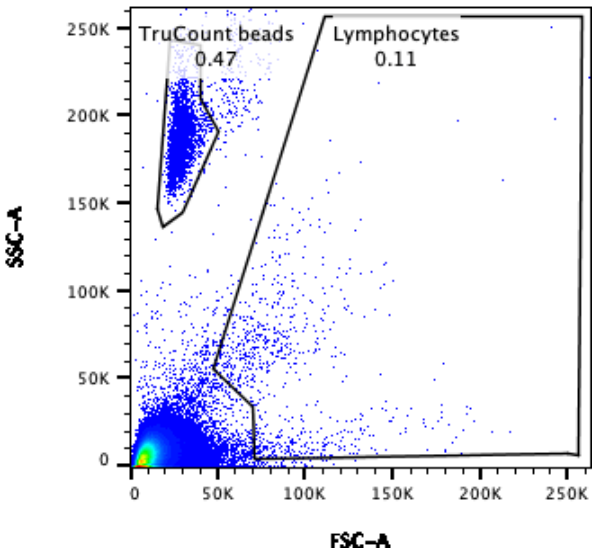


The single-cell suspension was added to BD TruCount™ FACS tubes, containing fluorescent counting beads, along with fluorochrome-labelled as described above for EVNP perfusate samples.

6.3.7 Flow cytometric analysis and gating strategy

A representative gating strategy are shown below. Firstly, BD TruCount™ beads were identified from forward scatter area (FSC-A) vs side scatter area (SCC-A) dot plots (Figure 41). BD TruCount™ FACS tubes contain a known number of counting beads so that absolute numbers of PLs can be determined using a simple formula (Equation 3). The number of cells in each gated population could then be determined based on the number of bead events and the known volume of the FACS tube. Lymphocytes were also gated from FSC-A vs SCC-A.

Figure 41 - Gating strategy: TruCount™ beads and lymphocytes

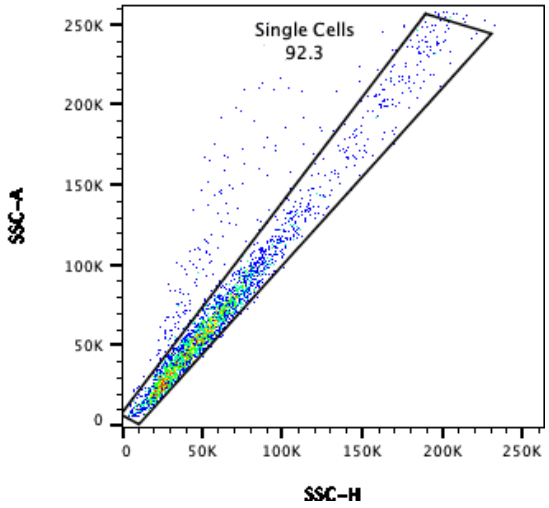


Equation 3 - Determining the absolute number of gated cells on flow cytometry using counting beads

$$Absolute\ number\ of\ cells = \frac{Number\ of\ gated\ cell\ events}{Number\ of\ bead\ events} \times \frac{Number\ of\ beads\ per\ test}{Test\ volume}$$

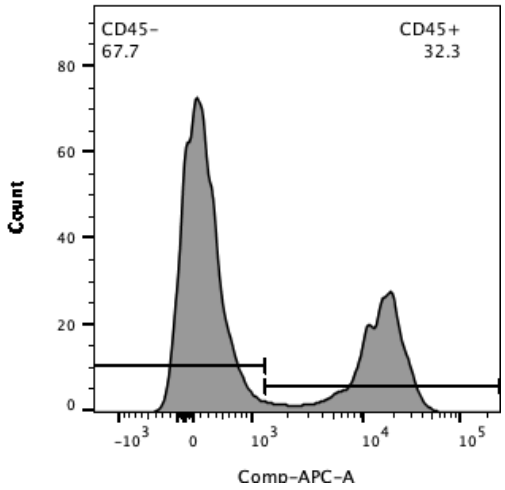
Doublet cells were then excluded by plotting height vs area, allowing only single cells to be gated (Figure 42). Fixable viability dye was not utilised in these studies.

Figure 42 - Gating strategy: doublet exclusion



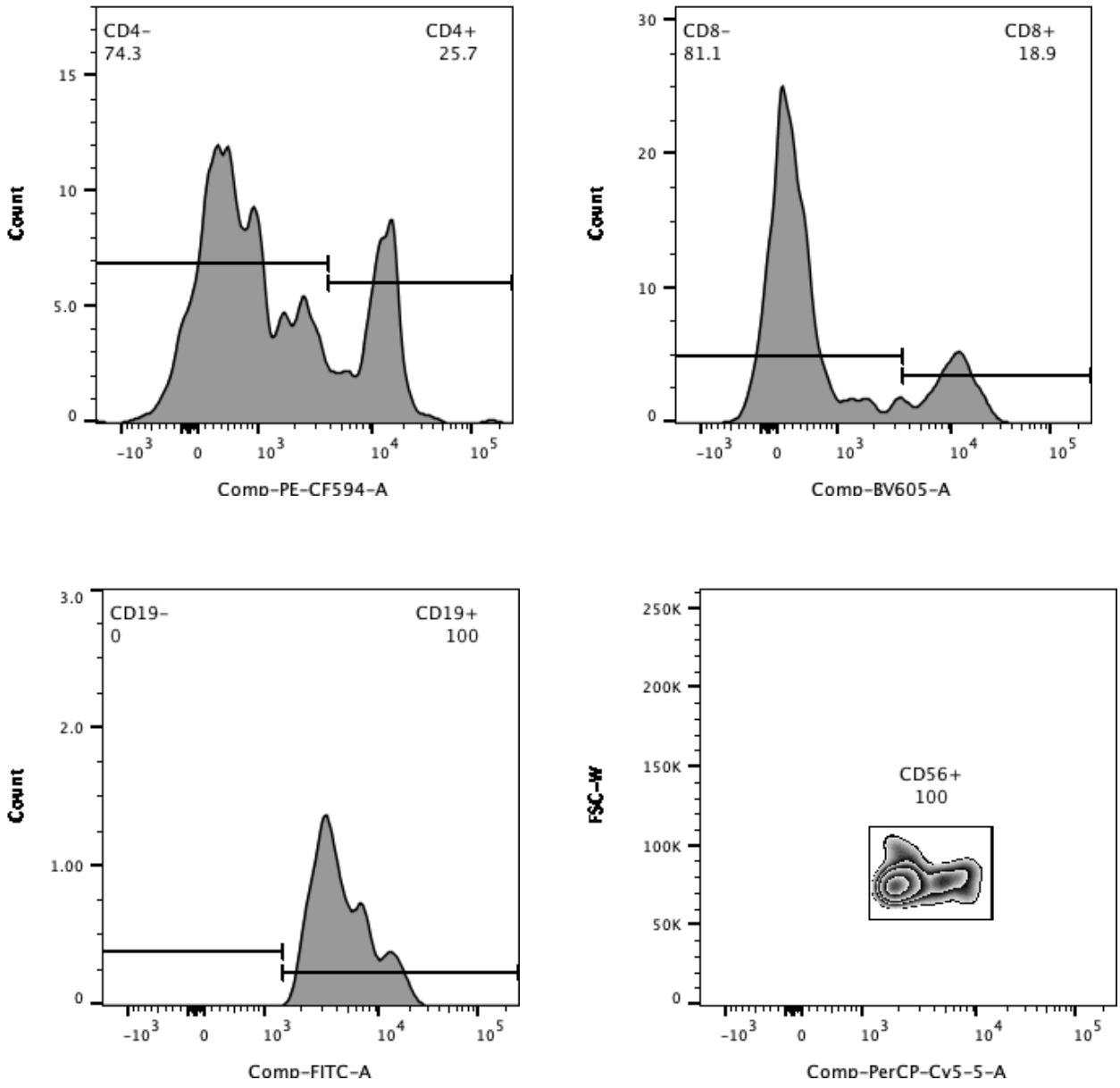
CD45+ and CD45- populations could then be demonstrated on single measurement parameter by plotting histograms of fluorescence (light scatter intensity from the cells) versus the number of events (cell count) (Figure 43).

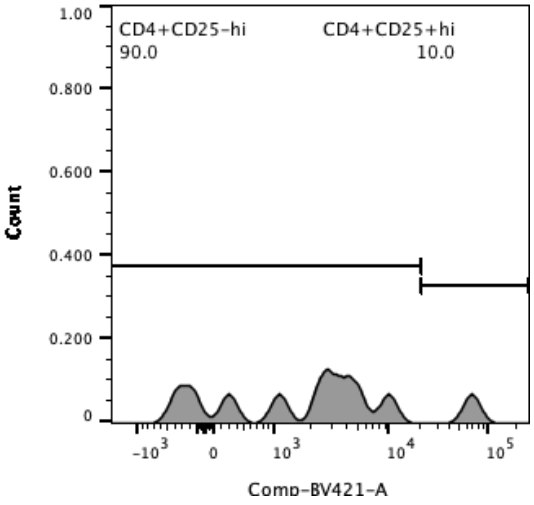
Figure 43 - Gating strategy: APC labelled CD45+ cells distinguishable as a discrete population with high light scatter intensity



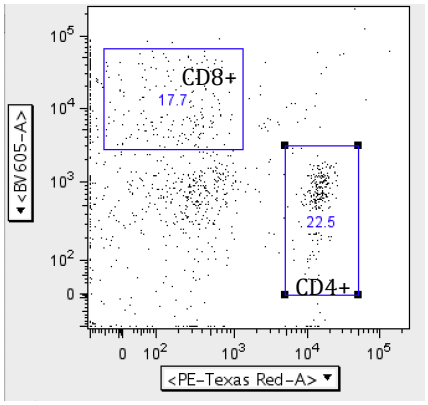
Within the gated CD45+ cell population, individual fluorochrome-labelled cell populations were identified, and demonstrated on scatter plots and histograms (Figure 44).

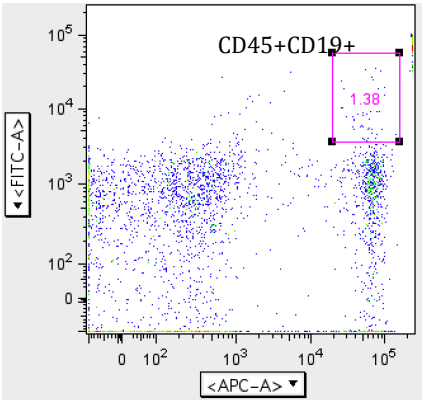
Figure 44 - Gating strategy of fluorochrome-labelled CD4, CD8, CD19, CD4+CD25+ and CD56+ cells.





*Populations of CD4+, CD8+, CD19+ and CD25 were best demonstrated using flourenscence intensity vs count. The population CD56+ was best demonstrated using flourenscence intensity versus forward scatter





The numbers of cells within each cell population were measured during perfusion every 30 minutes for two hours. The volume of perfusate, at each time point, was recorded to allow scaling up of leukocyte numbers in order to determine total numbers of white cells within the EVNP circuit. The numbers of cells within each cell population from filters after retrograde flush were measured each hour.

6.3.8 Statistical analysis

Differences in demographic and clinical characteristics between the two groups were examined using the Kruskal-Wallis test for continuous data or the Chi-squared test/Fisher's exact test for categorical data. All variables were be tested for normality using the Shapiro-Wilk test. CIT was defined as the period from the start of cold perfusion in the donor to EVNP transplant mode (i.e., including the

duration of EVNP pre-conditioning mode). Mobilisation of cell populations over time between filter versus non-filter interventional groups generated two curves which could be compared graphically and interrogated statistically. Comparison of the two curves was analysed using linear regression and the pattern of variation analysed using the F-test. Renal blood flow index was compared over time, allowing area under the curve to be examined and compared statistically using the Kruskal-Wallis test. Two-tailed p values were generated, with $p<0.05$ considered statistically significant. Data in this chapter were analysed using Prism 8.3, GraphPad Software LLC (San Diego, California, USA).

6.3.9 Ethical approval

Ethical approval for this study was granted by the local Research Ethics Committee (REC – study reference number 18/LO/0928), Health Research Authority (HRA – reference number 229654) and RINTAG (ODT study reference number 73). The study was also approved by the local hospital Research and Development at Guy's and St Thomas' NHS Foundation Trust, and the departmental Renal Project Board Steering Committee. The study sought to gain the input and support of patients, and its aims and intentions were presented to the Guy's and St Thomas' Kidney Patients Association (GSTT KPA), who supported the study.

6.4 Results

6.4.1 Baseline characteristics

During the study period, 10 human kidneys were declined for transplantation and met the inclusion criteria of the study.

The most common reason for decline for transplantation into a human recipient was suspected or confirmed malignancy in the donor (n=7) (Table 47). The exact nature of the suspected/confirmed donor malignancy in each group is shown in Table 48. Other reasons for decline by UK transplant centres was prolonged cold ischaemia time (n=1, approximately 20 hours), poor cold perfusion flush (n=1) and

organ damage (n=1, torn capsule of the kidney; repaired with good result). Median (IQR) donor age was 66 (55-69) years. Terminal creatinine in all kidneys was excellent (median 55, IQR 50-83). Median (IQR) CIT was 17 (13-21) hours.

Of the ten kidneys in the study, seven underwent EVNP and PL mobilisation *without* white cell filtration and three kidneys underwent EVNP and PL mobilisation *with* white cell filtration.

The baseline donor demographic information of the kidneys in the two interventional groups is summarised in Table 47. Donor age and sex were comparable ($p>0.05$). There were no statistically significant differences in the presence of clinically relevant comorbidities, including donor diabetes, hypertension, or obesity, ($p<0.05$ throughout). All kidneys came from donors of white ethnicity. There was no statistically significant difference in the reason for organ decline between the two groups ($p=0.36$).

Table 47 - Donor kidney baseline characteristics and reason for decline

Variable	**White cell depletion**		**Non-white cell depletion**		**p value**
	n=3		**n=7**		
Donor age (years)	69	(56-69)	63	(52-69)	0.38
Donor sex					1.00
Male	2	(66.7%)	5	(71.4%)	
Female	1	(33.3%)	2	(28.6%)	
Donor type					0.50
DBD	1	(33.3%)	5	(71.4%)	
DCD	2	(66.7%)	2	(28.6%)	
Cause of death					1.00
Intracranial haemorrhage	2	(66.7%)	5	(71.4%)	
Hypoxic brain injury	1	(33.3%)	2	(28.6%)	
Presence of donor sepsis	2	(66.7%)	2	(28.6%)	0.50

Donor ethnicity					*constant*
White	3	(100%)	7	(100%)	
Other	0	(0%)	0	(0%)	
Donor diabetes mellitus	2	(66.7%)	1	(14.3%)	0.18
Donor hypertension	1	(33.3%)	2	(28.6%)	1
Donor body mass index (kg/m^2)	30	(28-30)	25	(19-35)	0.38
Terminal creatinine (micromol/L)	58	(52-58)	52	(50-83)	0.38
Cold ischemia time (minutes)	949	(822-949)	1211	(761-1376)	0.67
Reason for decline					0.36
Probable or confirmed donor malignancy	2	(66.7%)	5	(71.4%)	
Prolonged cold ischaemia time	0	(0%)	1	(14.2%)	
Poor perfusion	0	(0%)	1	(14.2%)	
Organ damage	1	(33.3%)	0	(0%)	

Data expressed as median (interquartile range) or number (%)

DBD (donation after brain death), DCD donation after circulatory death, ICH (intracranial haemorrhage), CIT (cold ischaemia time)

Table 48 - Nature of the suspected or confirmed malignancy in the donors, within each interventional group

White cell depletion group	**Non-white cell depletion group**

1. Mass of contralateral kidney * 2. Adenocarcinoma of colon	1. Kidney mass * 2. Liver mass * 3. Lung mass * 4. Lung mass * 5. Non-small cell lung cancer

*Unconfirmed malignancy

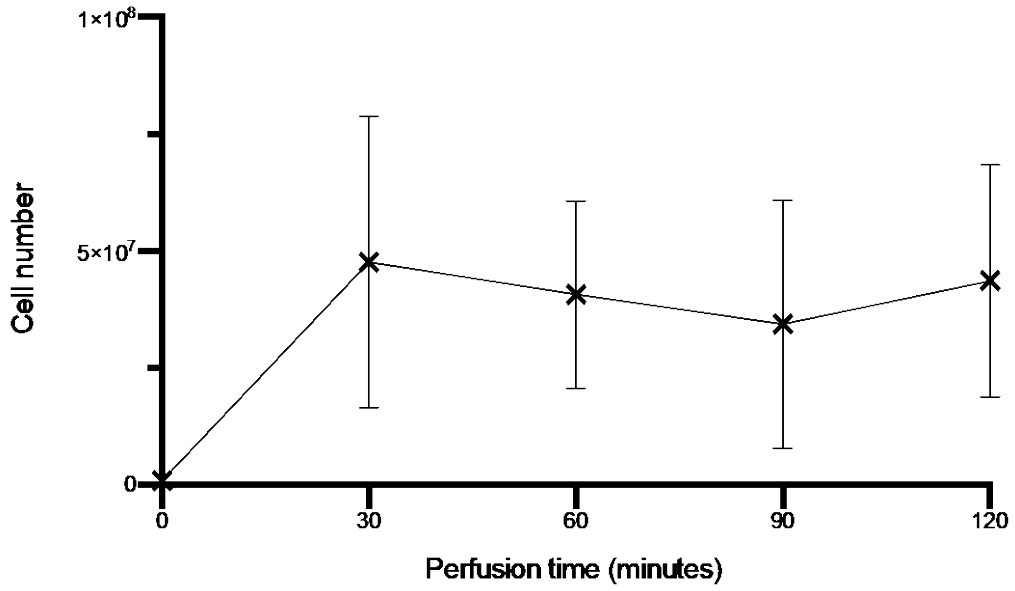
6.4.2 Leukocytes present in packed red blood cells

Prior to inclusion of each kidney onto the EVNP circuit, the EVNP perfusate was analysed for baseline PL numbers. The mean (± standard deviation) number of leukocytes (CD45+) in a single unit of packed red blood cells was low, approximately 70,000 cells in each unit bag (1158 cells/mL). A rise in the number of leukocytes following inclusion of each kidney onto the circuit were attributed to mobilisation of kidney donor-derived leukocytes.

6.4.3 Passenger leukocytes mobilised during ex vivo normothermic perfusion (no white cell filtration

After inclusion of each kidney onto the circuit, total numbers of CD45+ passenger leukocytes were monitored over time, based on the know volume of the perfusate (Figure 45). Mean numbers of leukocytes increased rapidly after 30 minutes of perfusion. After 30 minutes, the number of leukocytes in free circulation appeared to stabilise, at approximately $50x10^6$ cells in <u>total</u> volume of the circuit (this approximates to 83 thousand cells per mL) . This suggests that there may be a net flow of cells exiting and re-entering the organ over time.

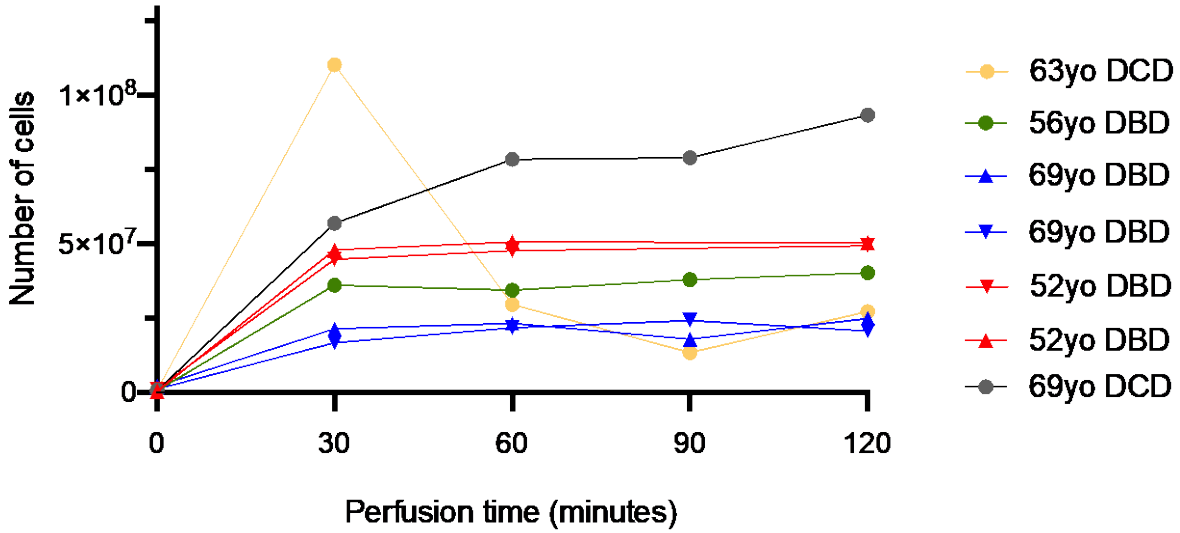
Figure 45 - Mean number (and standard deviation) of CD45+ cells mobilised into circulation during ex vivo normothermic perfusion (n=7)



Cell number represents the total number of CD45+ cells within the EVNP circuit (calculated from the known perfusate volume at each time point).

Significant variability between kidneys from different donors was observed when examining the number of CD45+ cells from individual kidneys (Figure 46). Pairs of kidneys from a single donor appeared to have similar numbers of PL mobilised at each time point. DCD donor kidneys (n=2) appeared to have larger number of PL mobilisation compared to DBD donor kidneys (n=5). However, interpretation is limited with small numbers of organs.

Figure 46 - Total number of CD45+ cells mobilised during EVNP in each kidney. Kidneys from a single donor are marked with the same-coloured line. Number of cells expressed is the total number of cells in the circuit (i.e. not normalised per mL)

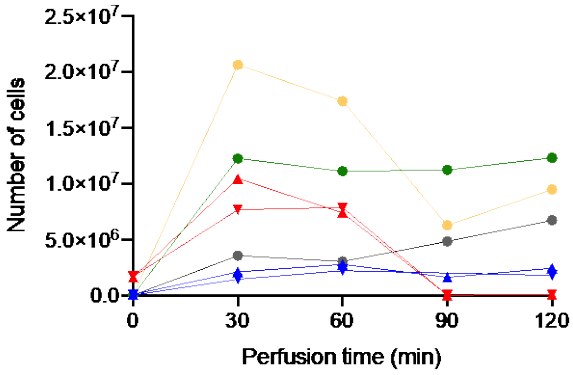


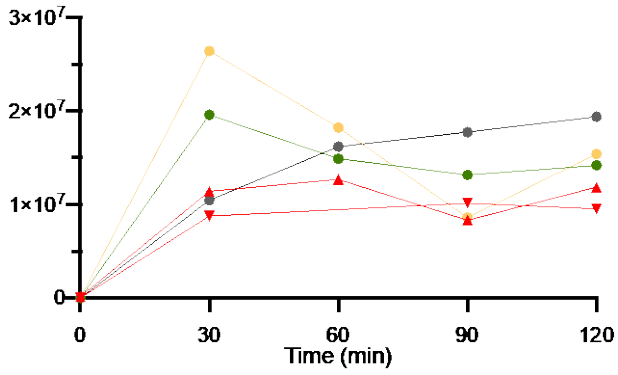
Pairs of kidneys from the same donor are marked in the same colour (i.e., 69yo DBD both marked with blue lines)

Variation in the mobilisation leukocyte subpopulation was observed (Figure 47A-E). Detection of T-helper, cytotoxic T cells, T regulatory cells, B cells and natural killer cells differed between kidneys. T helper, cytotoxic T cell, B cell and natural killer cell mobilisation appeared to be highest in the first 30 minutes in all kidneys. The composition of the PL population during this time was predominantly made up of cytotoxic T cells and T helper cells (Figure 48).

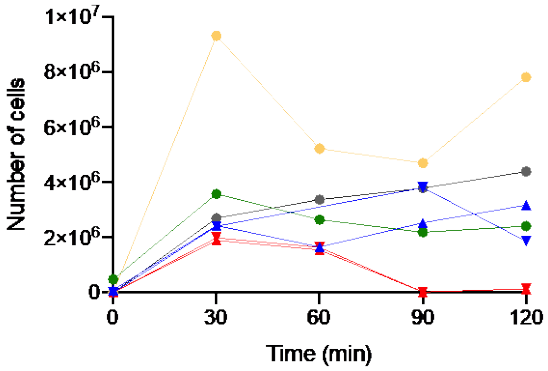
Figure 47 - Number of passenger leukocyte populations mobilised during ex vivo normothermic perfusion over time. Number of cells expressed is the total number of cells in the circuit (ie. not normalised per mL)

A **T helper cells (CD4+)** B **Cytotoxic T cells (CD8+)**

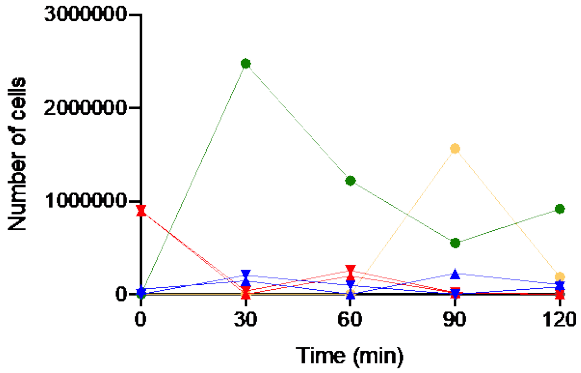




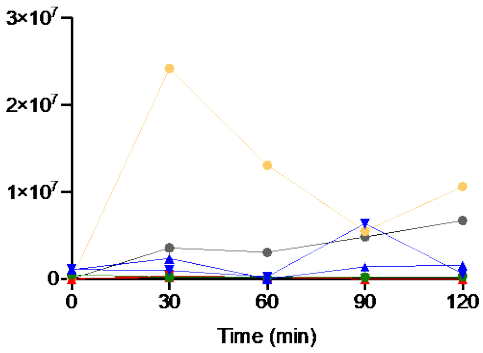
C **B cells (CD19+)**



D **T regulator cells (CD4+CD25+)**



E **Natural killer cells (CD56+)**



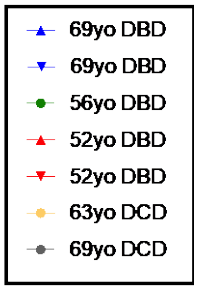
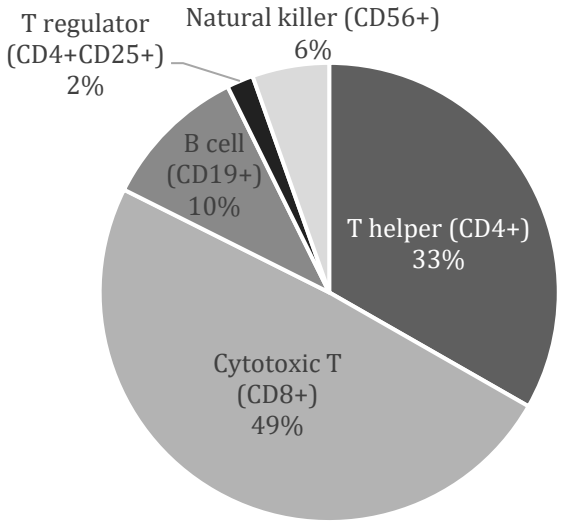


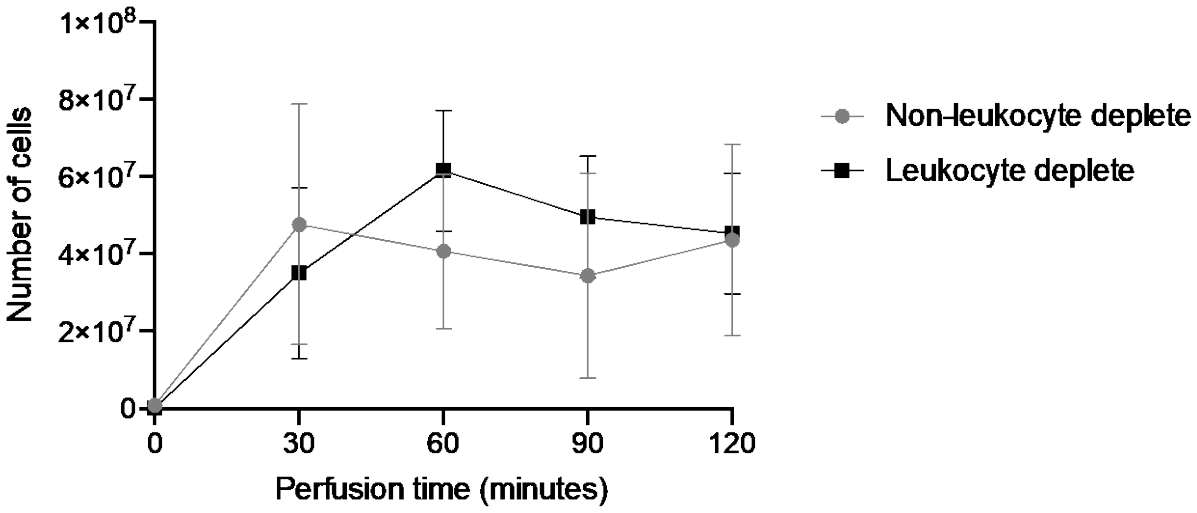
Figure 48 – The composition of the passenger leukocyte population at 30 minutes of perfusion (%)



6.4.4 Passenger leukocyte depletion using white cell filtration during ex vivo normothermic perfusion

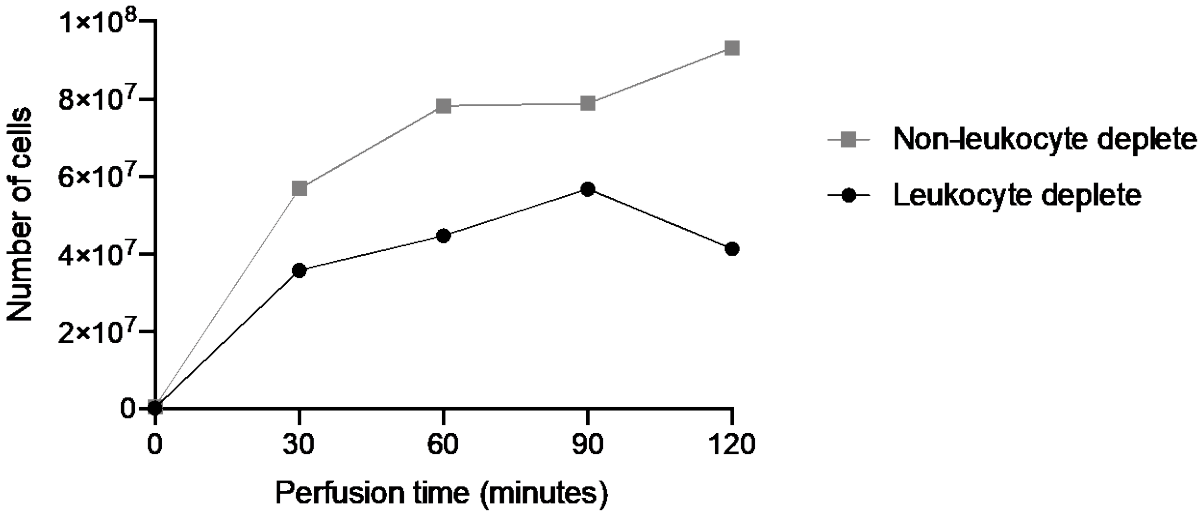
Three kidneys underwent EVNP with white cell filtration and were compared to seven kidneys that had undergone EVNP *without* white cell filtration. This included a single pair of kidneys from one donor, in which one kidney was randomised to leukocyte depletion and the other to EVNP alone. Mobilisation of CD45+ leukocytes between the two interventional groups is demonstrated in Figure 49.

Figure 49 – Mean number of CD45+ leukocytes mobilised during ex vivo normothermic perfusion between the two interventional groups, with standard deviation bars



Regression analysis compared the curves generated by each interventional group over time, however there was no statistical difference in the pattern of variation between each group (F-statistic 0.16, p=0.70). When directly comparing depletion versus non-depletion between two kidneys from the *same* donor, there are fewer CD45+ leukocytes in circulation at all time points (Figure 50). After two hours of perfusion, there are approximately half the number of PLs in free circulation following leukocyte depletion ($4x10^7$ cells versus $9x10^7$ cells). Analysis of the pattern of variation indicates that there are statistically fewer cells mobilised over time following leukocyte depletion compared to its contralateral counterpart (F-statistic 0.80, p=0.04).

Figure 50 - Comparison of the number of CD45+ leukocytes mobilised during ex vivo normothermic perfusion between leukocyte depletion and non-leukocyte depletion (kidneys from the same donor)



Examination of leukocyte numbers remaining in the wedge biopsies following perfusion was intended to provide an estimate for the number of PLs remaining in the kidney parenchyma following EVNP with and without leukocyte depletion. This analysis found that when considering the pair of kidneys from the same donor, demonstrated in Figure 50, there were 32.5% fewer CD45+ cells in a 1g wedge biopsy in the leukocyte deplete kidney compared to the contralateral kidney which did not undergo leukocyte depletion. When scaling up to each kidney's respective mass, there was an estimated $2.2x10^8$ more PLs in the kidney that did not undergo leukocyte depletion ($5.2x10^8$ versus $7.4x10^8$).

6.4.5 Differences in kidney haemodynamics after reperfusion with whole blood

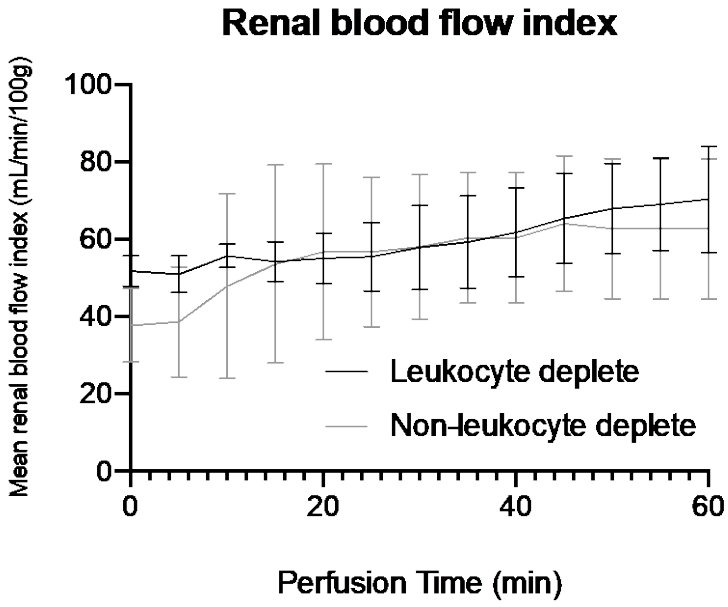
Following pre-conditioning EVNP with and without white cell filtration, kidneys were placed in static cold storage for two hours and reperfused on transplant mode with 'whole blood' in order to replicate implantation within a living human recipient. Outcomes of the two groups were compared.

Renal blood flow index

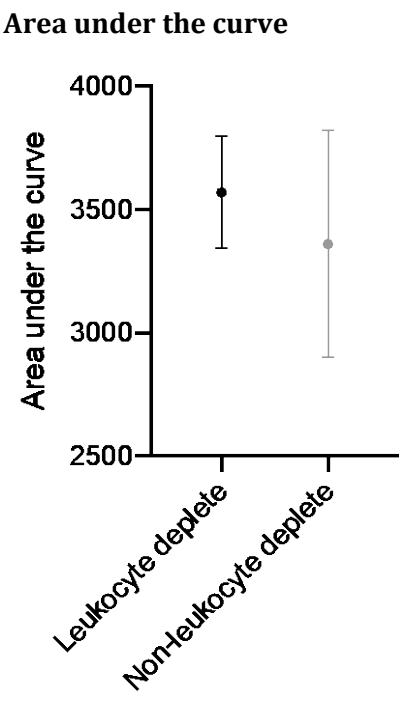
Renal blood flow index was compared between kidneys that underwent leukocyte depletion compared to kidneys that had not. Figure 51-A shows renal blood flow index over time, with both groups showing a rise in renal blood flow over time. Comparing the area under each curve (AUC) allowed changes in flow over time to be analysed (Figure 51-B). There was no significant difference in renal blood flow index AUC observed between the two groups (p=0.55).

Figure 51 - Difference in (A) mean (SD) renal blood flow index between kidneys that underwent white cell depletion compared to kidneys that did not; (B) Renal blood flow index area under the curve between leukocyte deplete and non-deplete kidneys (p=0.55)

(A)



(B)



Oxygen consumption

After 60 minutes of reperfusion, an estimation of oxygen consumption was determined between kidneys undergoing leukocyte depletion versus non-leukocyte depletion. Mean oxygen consumption of

leukocyte depletion kidneys was higher than non-leukocyte deplete kidneys (mean (SD) 42.8 (8.8) versus 23.5 (9.9) kPa.mL/min/g). However, this difference did not reach statistical significance (p=0.79).

Urine output

After 60 minutes of reperfusion, the total urine output was compared between kidneys undergoing leukocyte depletion versus non-leukocyte depletion. Mean urine output was comparable between the two groups (mean (SD) 103 (18) versus 97 (10) mL/hour) (p=0.47).

6.5 Discussion

6.5.1 Study findings and their implications in transplantation

This is the first study examining passenger leukocytes and their depletion in human kidneys using EVNP. The first part of the study demonstrated the presence of PLs in circulation during EVNP, whilst the second part attempted to deplete these cells from the circulation using white cell filtration. Finally, a model for transplantation was used, in which a population of third-party allogeneic peripheral blood leukocytes were added to an EVNP circuit to replicate reperfusion in a transplant recipient. This enabled differences in post-transplantation graft function to be compared between kidneys that had undergone white cell filtration compared to those that had not.

This study demonstrated that large numbers of donor immune cells are mobilised from human deceased donor kidneys during EVNP. PLs reached their highest concentration after 30 minutes of normothermic perfusion. This population of PLs was predominantly made up of cytotoxic T cells and T helper cells, though there were also smaller populations of B cells and NK cells. These cell types have been widely implicated in mediating IRI and acute rejection. Without EVNP prior to transplantation, these donor-derived cells are likely to enter transplant recipients' systemic circulation, providing an opportunity for direct allorecognition.

This study also demonstrated the presence of T regulatory cells being mobilised from kidneys during EVNP. T regulatory cells represented approximately 2% of the population of PLs detected in free circulation during perfusion and were readily removed from circulation, having been found in the leukocyte filters. This corroborates the findings of a recent study of human kidneys undergoing EVNP, in which donor-derived T regulator cells were found in leukocyte filters in similarly small numbers (288). In a murine heart transplant model, depletion of donor-derived T regulatory cells resulting in an increased humoral immune response, and accelerated graft rejection (288). In further experiments, donor and recipient T regulatory cells were re-introduced into transplanted mice. Whilst recipient-derived T regulator cells had no discernible impact on outcome, donor-derived T regulator cells resulted in reduced chronic allograft vasculopathy severity and improved graft survival. This suggests that not all

PL cell types promote inflammation and rejection. Donor-derived T regulator cells may have immunosuppressive properties. It should therefore be considered whether the indiscriminate removal of PL results in both the removal of harmful donor-derived immune cells, as well as beneficial cells.

6.5.2 Limitations

There was significant variation in the total number of PLs between each kidney, as well as the individual PL subtypes. This may have reflected significant variation in human deceased donor kidneys included in this study. This study was not able to control donor and organ characteristics in the same way that animal studies can, but the use of human organs is debatably more pragmatic as this reflects the current reality of deceased donor transplantation. The presence of donor infection, immune stimulatory events, instrumentation of the urinary tract (urinary catherization) or many other variables affecting the donor at the time of their death may influence the numbers of PLs present within the kidney. In particular, the presence of donor cancer, within the kidney or in a distant organ (e.g., lung) may have influenced the result of this study. Although the effect of malignancy, distant to the kidney, on its immune cell composition is not fully known, the presence of cancers within kidney certainly would have altered the immune make-up of the organ. Renal cell carcinoma tumours are known to be heavily infiltrated by T cells, including T regulator cells, and myeloid cells such as macrophages and dendritic cells (297).

This study did not demonstrate an improvement in renal blood flow index, oxygen consumption or urine output when white cell filtration was included in the EVNP circuit. Examination of wedge biopsies indicated that after two hours of EVNP, there remained large numbers of PLs within the kidney parenchyma. Two hours of EVNP, with two white cell filters, may not be sufficient to deplete enough PLs to reduce PL-mediated injury to the kidney. Since this study was small, this may also represent a type II statistical error in which the study may not have been sufficiently powered to detect a difference in renal blood flow, oxygen consumption or urine output. Kidney supply became limited following the SARS-CoV-2 pandemic meaning the numbers of discarded human deceased donor kidneys being enrolled into the study were low (78). Longer periods of EVNP may have also been necessary for PL depletion and could

be examined in further dosimetric studies. The challenges of longer periods of EVNP are being considered by a number of research groups (298, 299).

This study demonstrated a broad understanding of the main subtypes of leukocytes that may be present in circulation during EVNP. This was achieved using only five main cell surface antigens (CD4, CD8, CD19, CD25 and CD56). This therefore represents a relatively basic categorisation of cell types that leaves the opportunity for subpopulations within each to be considered in further work. As the first piece of work to consider PLs in human kidneys during EVNP, this level of simplicity was considered appropriate. However, more complex panels of antibodies/fluorochromes would add greater detail. In particular, CD45/CD4/CD25 positivity alone may not be sufficiently granular in characterising T regulator cells. Additional markers, such as the presence of FoxP3 and the absence of the alpha chain of IL-7R/CD127, may better identify T regulator cell populations. Since the presence of donor-derived T regulator cells may be beneficial in organ transplantation, this may be an interesting line of research that would necessitate this level of detail. Other PL subtypes of interest would be dendritic cells, neutrophils, and monocytes.

In retrospect, a Live/Dead stain would have increased the quality of the data, as this lessens autofluorescence that inevitably occurs. The absence of a Live/Dead stain in the main panel of fluorophores limits the interpretation of the results, especially in cell populations where numbers were low. In further work, a Live/Dead stain will be included.

Another limitation of this study was that there was no comparator group of kidneys that underwent static cold storage alone. This meant that all kidneys that were perfused with whole blood during 'transplant mode' had already undergone some form of EVNP (EVNP with or without white cell filtration). Kidneys that undergo EVNP without white cell filtration still benefited from significant leukocyte depletion, because they still undergo perfusion with fluid that is subsequently removed (i.e., the blood-based perfused is discarded after EVNP, containing circulating PLs, prior to re-perfusion on transplant mode). In future studies, it would be more informative to include kidneys undergoing static cold storage alone, in addition to the two groups that undergo EVNP.

The use of EVNP with whole blood, replete with recipient immune cells ('transplant mode'), had its advantages and disadvantages. An advantage of this system was that it avoids the complexities of a clinical trial involving implantation of declined organs into human recipients. Although 'declined' human kidneys have been implanted into human recipients in other EVNP studies (198), this would have further underpowered the study as only a small fraction of kidneys may have been considered acceptable for transplantation. EVNP in 'transplant mode' also allows for some clinically relevant outcome measures to be recorded, such as urine output. Other measures, such as creatinine clearance, have been used to assess organ function during EVNP and could have been considered in this study (194). However, important early transplant outcomes, such as delayed graft function or acute rejection, could not be examined using this model.

6.5.3 Further work

Further work is necessary to examine the complex immune processes during ex vivo normothermic perfusion. Whilst leukocyte filters reduce the numbers PLs from returning to the organ, it does not remove these cells entirely from circulation until the filter is removed from the circuit altogether. PLs caught in the filter may still be functional and release pro-inflammatory cytokines into the perfusate. Stone *et al* demonstrated that cytokines are present in the perfusate during EVNP after 60 minutes of perfusion and continued to increase up to 180 minutes (293, 295). Addition of cytokine filters may be necessary to reduce IRI during EVNP. A recent study in humans found that EVNP was associated with significant upregulation of immune and inflammatory gene pathways, compared to static cold storage (300). This study demonstrated that cytokine filtration reduced pro-inflammatory gene expression that is typically associated with delayed graft function (300). During ex vivo lung perfusion of rats, in addition to inflammatory cytokines, there was also a rise in DAMPs detected in the perfusate, which are associated with lung graft dysfunction. DAMPs within the perfusate may activate innate immune responses (301) and histological evidence of graft injury (302). However, the targeted removal of DAMPs during organ perfusion has not yet been performed and will be considered in further work. In future work, it would be

of interest to examine the immunogenicity of PLs by looking at the expression of surface MHC class I and II, co-stimulatory molecules (for instance CD80, CD86) and performing mixed lymphocytic reactions.

PLs have also been implicated in the development of other immune-mediated conditions, such as graft versus host disease (GVHD) and passenger lymphocyte syndrome. GVHD occurs when competent PLs, transferred into recipient circulation, initiate a graft-driven immune response again the recipient, resulting in a maculopapular rash and a range of gastrointestinal manifestations (303). Passenger lymphocyte syndrome is a condition in which donor-derived B cells, transferred into recipient circulation, results in the development of antibodies against recipient erythrocytes, resulting in haemolysis (304). Both GVHD and passenger lymphocyte syndrome are most commonly associated with allogeneic haematopoietic cell transplantation and occurs only rarely following kidney transplantation (305, 306). However, solid organ transplantation involving larger numbers of PL transfer from donor to recipient, such as small bowel transplantation, may benefit from ex vivo removal of PLs (307, 308).

Normothermic kidney perfusion provides a promising opportunity to module and manipulate the immune system of an organ to the benefit of its recipient. This work broadly demonstrates that donor-derived immune cells within the kidney may be an under-appreciated target for intervention in the prevention of IRI and acute rejection. Whilst white cell filtration provides a non-discriminatory method of removing PLs from recirculating into the kidney, it opens the door to more sophisticated methods of ex vivo immune modulation; an evolving field of interest.

7 Chapter 7: Conclusion

7.1 Findings and implications of the thesis

7.1.1 Outlying patient groups and the need for improved deceased donor assessment

Outcomes for deceased donor kidney transplant recipients demonstrated in this thesis are excellent for the overwhelming majority of patients. This corroborates registry data showing that 94% of deceased donor kidney recipients have a working transplant at one year, with a risk-adjusted mean eGFR of 49 mL/min/1.73^2 (168). However, this thesis has also demonstrated that there are under-recognised groups of patients that have inferior outcomes. These outlying groups of transplant recipients have appeared due to the increased reliance on sub-optimal deceased donor kidneys in response to the disparities in organ transplantation and donation. Further expansion of the donor pool may see more patients with less-than-optimal graft function and survival. Accurate assessment of deceased donor organs is therefore becoming increasingly important to the effective utilisation of deceased donor organs. Where possible, amelioration of graft function is becoming necessary to gain full potential of sub-optimal organs, as a means of maintaining national outcome standards.

Immediate graft function is only achieved in 60.1% and 79.6% of circulatory death donor and brain death donor kidney transplants, respectively (Chapter 3, Sections 3.4.1 and 3.4.7). The implications of this are that a significant proportion of recipients of deceased donor kidney transplants require dialysis in the first week of transplantation due to insufficient early graft function. This thesis has reaffirmed the understanding that in DBD donor kidney transplantation, DGF of any duration is associated with inferior patient and graft survival. A novel finding is that in DCD donor kidneys appear to be more tolerant of DGF. Only prolonged DGF translated to inferior longer-term outcomes in DCD donor kidney transplantation. DGF lasting greater than 14 days almost doubles the risk of graft loss and patient death, relative to DCD donor kidneys that function immediately. These data have shown that DGF as a spectrum, based on its duration, allows a better understanding of its longer-term implications for patients.

A common theme across the chapters is that increasing donor age is associated with inferior transplant outcomes. In unadjusted analyses, donor age was associated with inferior graft function and

survival in both DBD and DCD donor kidneys. There was also a positive correlation between donor age and apparent chronic kidney histological damage. In DCD donor kidney transplantation, the use of donors over the age 70 years was predictive of prolonged DGF in DCD donor kidneys, with almost triple the risk of graft loss.

Another common finding across the chapters was that prolonged CIT is a risk factor for inferior outcomes. CIT was associated with increased rate of DGF in DCD kidney transplantation and was an independent predictor of prolonged DGF. CIT exceeding twelve hours was predictive of inferior graft survival. Intrinsically linked to cold ischaemia time is preservation method. Static cold storage was also associated with prolonged DGF in DCD donor kidneys, which in turn was linked to inferior outcomes. This thesis has therefore highlighted cold storage as a critical opportunity for intervention in potential amelioration of graft function.

What do we do with this information? It is clear from the literature that the answer is not necessarily to avoid the use of these organs altogether (60, 69, 153, 154, 221). As a finite resource that may provide improved quality of life for a period of time for an appropriately matched patient, deceased donor kidneys are a precious commodity. The decision to decline and discard should be taken with the gravity that it deserves. The use of organs that are identified as 'higher risk' of graft loss should be weighed against the risks of remaining on the waiting list or indeed the realistic probability of receiving a better offer within an acceptable timeframe. Therefore, if organ discard should be avoided, if possible, what can be done to mitigate the risk of adverse outcomes? Risk factors which are not modifiable (e.g., donor age) can be considered during recipient selection. Modifiable risk factors (e.g., CIT and preservation method) should be identified and undergo necessary conditioning to improve graft function. However, the initial step in the process of improving outcome is identifying organs at risk of inferior outcomes.

7.1.2 Preimplantation kidney biopsy

This thesis investigated the contentious subject of pre-implantation kidney biopsy as a means of quantifying donor risk and directing organ usage (Chapter 2). This study was performed in an advantageous setting within a unit that recorded Karpinski scores but (almost always) did not know the result at the time of transplantation. This analysis was therefore minimally affected by the bias it may have had on subsequent organ utilisation. This study demonstrated that there was no difference in the incidence of DGF between kidneys with high and low Karpinski scores, in both DCD and DBD donor kidneys. Likewise, there was no difference in estimated GFR after 5 years. Karpinski scores did not predict graft or patient survival, even when limited to older donors, or donors identified as high risk according to the UKKDRI. When considering whether organ utilisation would have changed if the Karpinski scores had been known prior to transplantation, it was estimated that organ usage would have fallen if the unit had followed the organ usage strategy advocated by Remuzzi and PITHIA (76, 172). This study demonstrates that simplistic histological scoring systems may not have sufficient positive predictive ability to direct organ usage and should not be depended on in isolation. However, it must be noted that biopsies may predict PNF rates and also had an ability to detect 1-year graft function in a multivariable analysis, detecting an approximate 3mL eGFR difference.

7.1.3 Ex vivo normothermic perfusion

A core aspect of this thesis was the investigation of ex vivo normothermic perfusion technology in the prediction and amelioration of early graft dysfunction. This was done in three overlapping settings. Firstly, in the immediate post-reperfusion period. Secondly in the first two hours of transplantation, and finally in the first week of transplantation.

The use of EVNP prior to transplantation as a means of ameliorating post-perfusion hypotension and reducing inotrope, fluid and blood product usage was investigated as a sub-group analysis of a RCT (Chapter 5). In doing so, the definition of PRS was tested and variations of the definition were

interrogated in sensitivity analyses. PRS did not occur in any kidney randomised to EVNP, but occurred in 16.7% of kidneys undergoing SCS, though this did not reach statistical significance.

Likewise, amelioration of IRI was not demonstrated despite the depletion of PLs from the donor kidney (Chapter 6). None-the-less, this study confirmed that large numbers of donor-derived immune cells are released into circulation during EVNP. Many of these cell types have been associated with IRI and acute rejection. However, donor T regulator cells were also demonstrated, though in low numbers, which have been shown to be protective against inflammation and rejection. Indiscriminate depletion of both pro- and anti-inflammatory cells by a white cell filter may not achieve improved graft function, and selective cell depletion may not be achievable with EVNP. Regardless, an important finding of this study was that the cellular makeup of the kidney can be manipulated during machine perfusion prior to transplantation. Much of the research to-date has focused on the delivery of cellular therapies to the organ during EVNP, rather than removal of cells. This study demonstrates a feasibility for immunodepletion. Finally, this thesis considered the use of EVNP in the reduction of the incidence of DGF in a human clinical trial (Chapter 4). The study made use of the fact that often the contralateral kidney undergoes an alternative preservation method. However, EVNP did not show statistical superiority over static cold storage.

7.2 Limitations

The limitations of each study design are addressed in each chapter. However, some broader limitations and concepts across the chapters are explored in further detail.

7.2.1 Comparator groups

An important limitation that should be recognised in this thesis, and which is also present in many observational studies in transplantation, is the choice of comparator group. Patients remaining on the waiting list were not included as a competing risk in these analyses. In the analysis of the effect of DGF

duration on transplant outcomes (Chapter 3), it was found that DCD donor kidneys with prolonged DGF and DBD donor kidneys with DGF of any duration are at risk of morbidity and mortality relative to organs that function immediately, *not* relative to patients on the waiting list. The latter comparator group provides a more meaningful comparison when considering the decision-making process that faces transplant clinicians. After the initial surgical risk during the first 100 days of transplantation, there is a strong survival and QOL benefit of transplantation compared to those still on the waiting list (30, 37, 39-41). Another consideration should be those *suspended* from the waiting list, as this is also a competing group that is a possible eventuality if an organ offer is declined. Patients suspended from the deceased donor waiting list have a significantly higher chance of death compared to those on the waiting list (aHR 1.79, 95% CI 1.64-1.95). This risk of death is carried through even if the patient returns to the waiting list or is later transplanted (309).

Another important comparator group, in addition to patients on the waiting list, is living donor kidney transplant patients. Indeed, some patients have a living donor option available at the time of a deceased donor kidney offer. Therefore, at least three options are available to patients: deceased donor kidney transplant, living donor kidney transplant or remaining on the waiting list in hope for a better offer. Structuring studies with these appropriate comparator groups may better aid decision-making in the peri-transplant period.

Inclusion of a control of patients on the deceased donor kidney transplant waiting list requires age and comorbidity matching to mitigate and adjust for the intrinsic differences seen in patients that proceed to transplantation, relative to those who continue waiting.

7.2.2 Sample size and statistical significance

Small sample size was a common weakness of the studies examining EVNP. This was true for the examination of PRS and DGF. Since EVNP is in its early stages in clinical transplantation, the numbers undergoing EVNP are relatively small. The study design of the DGF analysis allowed paired kidneys undergoing SCS elsewhere to be a comparator group. However, if the contralateral organ was discarded,

it could not be included in the analysis; this design further limited the sample size. Sample size was also small in the basic science studies examining passenger leukocytes. The reason for the small sample size was partly due to the decision to use human organs over animal organs. Declined human organs are available in much smaller numbers than in animal studies but have the advantages of relevance and translatability. Studying human organs through the national transplant programme is also pragmatic, with these organs being exposed to 'real world' conditions. In order to improve the number of declined human kidneys, study inclusion criteria need to be broad, however this has the drawback of greater heterogeneity in the type of organs included.

Small sample sizes meant some aspects of the work were underpowered to detect statistical differences. However, a paradox encountered during the execution of this thesis was the concept of apparent statistical significance versus clinical significance. Indeed, statistical significance is simply an indicator of the reliability of the study results, whilst clinical significance describes its impact in clinical practice. For example, the rate of PRS following the use of EVNP was 0%, whilst the rate of PRS was 16% following static cold storage but failed to reach clinical significance.

7.2.3 Other methods of assessing or ameliorating donor risk

This thesis was not exhaustive in its characterisation of current and future assessment of donor risk in deceased donor kidney transplantation. The thesis did not investigate serum and urinary biomarkers, or radiological assessment of donor and organ. These should be considered in future work.

Likewise, in situ machine perfusion techniques, namely NRP, were not considered in the course of this work. NRP allows organs to be perfused with oxygenated donor blood following circulatory death prior to organ retrieval but may improve early kidney graft function (187). The combined effect of NRP and EVNP on subsequent early and late graft function and survival needs to be investigated, possibly through registry analyses.

7.3 Future work

7.3.1 Assessing donor kidneys

Interrogating transplant registries

The transplant registry allows population-based analysis of characteristics and events to find factors that are predictive of organ function and survival. Developments in mathematics and artificial intelligence may provide better yield from registry data. This thesis used regression models to find predictive factors for transplant outcomes, however novel methods have been developed under the broad term of 'machine learning'. Machine learning is a breed of methods, allied to computational statistics, in which computers build models based on training data to make predictions. Machine learning methods were constructed in order to improve predictive value over conventional statistics, because of computers' ability to find hidden associations in complex data (310). This may be particularly useful in transplantation where donors and transplant recipients are highly heterogenous, with multiple clinically relevant characteristics, in combination with complex immune responses post-transplantation. The conventional statistical models used in this thesis, such as Cox proportional hazards model and logistic regression, assume that covariates are independent of each other, and are less suited to handling complex non-linear interactions. Never-the-less, the comparative performance of machine learning over these traditional methods in time-to-event data, which is so fundamental in transplant and patient survival, is unclear.

In a systematic review of machine learning methods, the most commonly used strategies among 18 studies in transplantation included decision trees, artificial neural networks, and Bayesian belief networks (311). Seven studies also allowed comparison of predictive performance with conventional statistical methods. Although one study found logistic regression to be superior to decision tree modelling, the other studies found machine learning techniques to have better predictive ability (311). As the transplant registry continues to grow in size and complexity, adoption of machine learning techniques will become increasingly important. In particular, machine learning may be used to tie in registry data into other forms of donor risk assessment such as donor kidney histology and organ

performance during machine perfusion. Further work is therefore needed to revise and validate the UKKDRI to incorporate more donor information available prior to transplantation.

Finally, quality of registry data is fundamental in accurate prediction of outcomes, regardless of the statistical method used. Data from the UK's EVNP centres should also be accurately captured by the registry in order for us to learn from and adapt to novel organ preservation practices. Collaboration between national transplant registries provides highly valuable information on how transplant outcomes vary according to differing practices and policies.

Clinico-histological prediction models

This thesis, and other published literature, have found that histological scoring systems prior to transplantation have not enabled accurate organ viability information. This thesis only considered the Remuzzi/Karpinski/PITHIA style of scoring, which is based on factors described in Table 12. However, more recent work has demonstrated that there are other histological features that are more promising. Avigan et al performed high-dimensional histological analysis of PIKBs and mass cytometry. They found that features not seen on conventional PIKB histology techniques were strongly associated with DGF, namely a reduction in tubular cells and increased macrophage infiltration. Molecular and genetic biopsy diagnostics has also become possible (312). At the 2019 Banff meeting, molecular profiling using a panel of 770 genes were identified by experts. Expression profiling from a biopsy is made possible using real-time polymerase chain reaction. Small amounts of mRNA within FFPE biopsies can be obtained using NanoString, a technology in which molecular 'barcodes' detect and quantify gene expression. Although these were not applied to PIKBs, this demonstrates that there is potentially a large amount of information available from kidney biopsies which may be informative to clinicians prior to transplantation.

Future work in donor histological analysis should also focus on combining these data with registry data. Loupy et al combined the use of donor and recipient characteristics (found to be individually associated with graft survival) with donor histological data in order to generate an algorithm to predict graft failure, which they termed the 'iBox' (313). However, biopsies were taken after

implantation meaning that these could not necessarily be used to inform utilisation decisions. Histological parameters that were found to be associated with graft failure were also not exclusively donor-related changes, and included C4d deposition in the graft, evidence of rejection and nephropathy recurrence. Trailin et al found that combining a donor kidney damage index in PIKBs with other demographic information, such as CIT, improved its DCGS predictive accuracy (from C-statistic 0.75 to 0.81) (314). These works demonstrate the potential value of composite risk assessment tools which consider histological data in combination with other information that may be available in the peri-transplant period. Composite risk assessment tools need to be validated against a test population to ensure accuracy is achieved before application to clinical practice.

Finally, if the predictive ability of PIKBs are to be improved and employed more widely in the UK, there is a further challenge that needs to be overcome: reproducibility. A recent study examined the consistency of PIKBs among 1000 kidneys. The initial PIKB demonstrated poor consistency with finding found on subsequent biopsies. Indeed PIKBs demonstrated no association with graft survival on both unadjusted and adjusted analyses (315). With the advent of whole-slide digital scanning, it is possible for a single centre, or small group of specialist renal histopathologists, to provide reporting for a region. This is likely to improve inter-pathologist and inter-centre variation in interpretation (316).

Ex vivo normothermic perfusion for the assessment of donor kidneys

Small studies have demonstrated that kidneys thought to be untransplantable may indeed be successfully implanted after evaluation during EVNP (196, 198). These studies based their decision of transplantability on three main characteristics, namely kidney appearance, urine output and renal blood flow index, forming an EVNP score (see Table 8) (197). Hosgood and Nicholson found that these characteristics were associated with superior organ function based on a series of only 74 human kidneys. Future work is needed to determine the predictive ability of the EVNP quality assessment tool and validate it against known outcomes. However, there may be further parameters measurable during EVNP that provide useful information regarding the quality of an organ, its likelihood of immediate graft

function and potentially its longevity. These may be creatinine clearance, oxygen consumption, potassium excretion, serum or urinary biomarkers and novel visual / radiological assessment of the organ during perfusion.

The use of creatinine clearance to measure graft renal function during EVNP has been demonstrated in a number of animal studies (194, 317, 318). Since there is no endogenous creatinine in the EVNP circuit, measurement of creatinine clearance required exogenous creatinine 1000μmol/L to be added to the perfusate (194). There is currently no clinical-grade exogenous creatinine available, meaning that creatinine clearance cannot yet be safely measured in human kidneys undergoing transplantation. The ability to provide an estimated glomerular filtration rate of a deceased donor organ during ex vivo perfusion prior to implantation may be helpful when assessing organ quality and would be an output that is familiar to nephrologists. Alternative biomarkers markers of renal excretion could also be used to measure graft function during EVNP, such as inulin clearance (319).

Further work is also needed to determine whether biomarkers within the perfusate, or excreted into urine, during EVNP may act as indicators of graft quality. Urinary biomarkers have been successfully measured in porcine kidneys undergoing EVNP (199). These have included IL-6, TNF-a, endothelin-1 and neutrophil gelatinase-associated lipocalin (NGAL). In liver normothermic perfusion, higher levels of antioxidants within the perfusate have correlated with better early graft function (320). Further work is needed to determine whether biomarkers detected within perfusate and urine during EVNP correlate with transplant outcomes.

Novel radiological methods may be used to assess organ viability during normothermic machine perfusion. Schutter et al have recently demonstrated that EVNP may be combined with magnetic resonance imaging (MRI) to assess organ quality (321). MRI during EVNP enabled information regarding kidneys' intrarenal perfusion distribution to be monitored over time. In livers undergoing normothermic machine perfusion, gadolinium injected into the perfusate, and its excretion into bile, has been demonstrated using magnetic resonance imaging (322). This has yet to be applied to donor kidneys but could act as a more sophisticated method of measuring a kidney's exocrine function, rather than simply

measuring urine output volume. However, techniques combining EVNP with cross-sectional imaging has logistical limitations as the organ is moved to a fixed scanner. Novel bedside imaging technologies, such as infra-red and hyper-spectral imaging, have also been used to monitor perfusion of living tissues in other settings (323-325). Further work is therefore needed to determine whether combining EVNP with novel imaging techniques provides useful clinical information, and whether these data enable more accurate predictions of organ function. These data would be obtainable through an observational study in parallel with an existing machine perfusion service or trial.

Donor risk indices and matchability scores

Donor risk indices have evolved over the years, beginning with simplistic SCD/ECD categorisation, to more complex equations that consider multiple donor variables. Currently, the UKKDRI (2019 modification) considers seven donor variables: age, sex, height, hypertension, CMV seropositivity, donor eGFR and days in hospital. The UKKDRI was formulated by determining whether donor variables in the transplant registry were independently predictive of graft survival using Cox proportional hazards modelling, before formulating the risk index and validating it against a comparable donor population. As donor assessment becomes more complex, inclusion of organ performance during machine perfusion and/or histological findings may need to be considered in future donor risk indices. However, beyond this foreseeable development, mathematical developments have taken place since the development of Cox regression in 1972 (326). Although Cox proportional hazards remains the most commonly used regression modelling framework to explore predictors of survival, there are now novel alternative statistical methods that may be employed. Cox regression must satisfy the proportional hazards assumption, which means that the hazard ratio must remain constant over time. Trinquart et al analysed 54 randomised controlled trials and found evidence of nonproportionality of hazards in 13 (24%) of studies, which may have led to misleading conclusions (327). If more complex donor variables need to be considered in a single donor risk index, increasingly sophisticate and robust statistical methods need to be employed. Alternative statistical methods that could be considered in the development of future

indices include the restricted mean survival time (RMST), which has gained significant interest because it does not rely on the assumption of proportional hazards (328).

Communicating risk to patients and clinicians

This thesis has explored means of improving donor risk assessment. However, this information needs to be effectively communicated to, and understood by, patients and clinicians for it be of benefit. Communicating risk is challenging due to the variation in people's understanding of risk (329). Indeed, it is the duty of the transplantation team to effectively counsel the patient prior to accepting or declining organ offers. As donor organ risk assessment becomes more complex, incorporating more tests of organ function, which in itself requires further understanding of processes in transplantation (e.g., organ perfusion technologies) communicating donor risk will become even more challenging. Graphical presentation of risk is being increasingly used to in medicine and surgery as an aid during treatment decisions with patients (330). The utility of individualised graphical risk profiles should be considered by more widely NHSBT, potentially as an output of donor risk indices, for patients and their clinicians.

The first publicly accessible risk tool for patients was developed in 2019 and is available at www.odt.nhs.uk/transplantation/tools-policies-and-guidance/risk-communication-tools/. This is an online personalised calculator for kidney recipients, as well as other solid organ recipients. It aims to enable patient to visualise the possible outcomes for patients from the point of listing.

Further expansion of the donor pool

If greater predictive accuracy of potential donors prior to transplantation is achievable, with machine-learning built algorithms that incorporate 'big data', histological and machine perfusion information gained prior to implantation, further expansion of the donor pool may be possible. This may enable greater confidence utilising the highest risk donor types, including uncontrolled DCD donor

kidneys (Maastricht type I, II), kidneys from elderly donors, and kidneys with severe AKI (including donors on haemofiltration). The use of simplistic criteria used, such as ECD and other DRIs need to be superseded by more sophisticated methods of assessing donor risk.

Improved donor assessment may also improve prospects for paediatric recipients. The risk appetite in paediatrics differs from adult transplantation. This is due to a number of reasons including limitations of dialysis in children, the likelihood retransplantation within the lifetime of a child, the need to minimise sensitising events, recurrence of primary renal disease and the availability of living-donor transplant options. In the UK, DCD donor kidneys are underutilised for paediatric recipients. DCD donor kidneys were implanted into paediatric recipients in only 6% of deceased donor kidney transplants in the year 2018/2019. Although this figure increased to 24% in 2020/2021, this is significantly lower than the DCD donor kidney implantation rates in adults in the same year (37%) (331). Reluctance to use DCD donor kidneys in children may, in part, be due to concerns regarding the high rate of DGF. If DGF can be predicted prior to transplantation, this may help to improve DCD donor acceptance rates. If DGF can be ameliorated using methods like EVNP, this may furthermore improve early and late outcomes for children.

Finally, expansion of living donation should remain an important focus. Currently, numbers of living donor kidney transplants remains static (45). Since the COVID-19 pandemic, early reports from NHSBT indicate a possible reduction in living donor transplantation. Increasing donation from directed and non-directed donors is an important strategy in maintaining, and possibly inceasing donation from this important pool. Increasing awareness and access to the potential donors should remain an important public message, both through NHSBT as well as transplant charities.

Expansion of the living donor criteria should also be revised regularly, based on data fed back through NHSBT and other international registries/studies. Careful expansion of permittable comorbidities may help to increase the number of living donor transplants, such as previous malignancy, viral illness and renal tract calculi.

Some of the strategies aimed at assessing and ameliorating graft function may be applied to living donor kidneys. The presence of DGF doubles the risk of graft loss in living donor kidney transplantation (151), and therefore should be reduced as much as possible using the tools and treatments available in deceased donor transplantation. This should include both perfusion technologies, kidney biopsies and medications.

7.3.2 Ex vivo normothermic perfusion for the amelioration of graft function

Beyond the ability of EVNP to assess organ quality, EVNP has the potential to resuscitate and recondition organs prior to transplantation (332). The potential for EVNP to ameliorate graft function can be divided into two modes of action. Firstly, the ability for EVNP provide a favourable physiological to undergo processes such as ATP repletion. Secondly the ability for *additional* active ingredients, such as cellular or pharmacological agents, to be delivered via EVNP.

Perfecting the physiological environment

When considering the first mode of action, there are certainly opportunities to improve EVNP. EVNP in its current form is far from the finished product. There are many potential ways in which the perfusate ingredients, temperature and pressure may be further optimised to meet the complex metabolic needs of the kidney. During short periods of EVNP, changes to the various elements of EVNP (e.g., pressures, oxygenation, perfusate composition etc) are only likely to make subtle improvements in organ preservation. Indeed, the studies considered in this thesis have looked at EVNP for only 60 minutes, which may not be enough for the organ to gain full benefit. Providing a near-perfect physiological environment becomes more important in prolonged (>24 hours) normothermic preservation (333). Whilst EVNP was initially found to replete ATP within only two hours of perfusion, prolonged EVNP has

additional benefits of supporting an organ during IRI (lasting hours to days) and during organ recovery and regeneration (days to weeks). Prolonged EVNP may also facilitate treatment of donor-related infections that would otherwise preclude transplantation. Antimicrobial or antiviral treatment of the organ may be delivered until clearance of the pathogen has been demonstrated or to prevent graft reinfection (334). Other benefits of prolonged EVNP include avoidance of transplantation during the night or during periods of limited staffing and bed capacity.

Perfusate ingredients play an important role in maintaining an ideal electrolyte and pH balance. Although the kidney itself possesses mechanisms to maintain electrolyte homeostasis, acidosis and hypokalaemia can lead to impaired renal function (335). However, urine output from the organ will cause fluid and electrolyte loss from circulation. This can be managed either by replacement with crystalloids (298) (such as Ringer's lactate, Williams Medium E) or by recirculating urine output into the perfusate (298, 299). In a comparison of perfusate ingredients, Pool et al found that the latter approach resulted in significantly better electrolyte and pH balance over a seven hour period (298). The addition of albumin in the perfusate resulting in reduced urine output. However, the absence of oncotic pressure within the perfusate accentuating urine output through ultrafiltration, rather than improving graft function (336). A common concerning finding across EVNP studies is that kidneys gain weight during perfusion, which is hypothesised to be oedema. Perfusate that did not contain mannitol 10% gained the most weight. However, Pool et al did not demonstrate definitive areas of tissue oedema histologically.

Further optimisation of EVNP may be gained by reducing the oxygen content of the perfusate. Within the studies and experiments in this thesis, 95% oxygen was delivered at 0.1L/min, resulting in non-physiological partial pressures of oxygen within the perfusate (60-80kPa). Theoretically hyperoxygenation may lead to ROS overproduction and oxidative damage (337, 338). Maintenance of lower partial pressures of oxygen (13.5-34kPa) should be considered in future studies if prolonged EVNP is to be considered.

Packed red blood cells are the most commonly used oxygen carrying/delivering substance. Although logistical challenges exist for the safe use of third-party blood products in EVNP (reviewed in

Callaghan et al (339)), red blood cells promote physiological endothelial cell function (340). Packed red blood cells are also readily available in a clinical setting and relatively inexpensive. However, there are several drawbacks of packed red blood cells, including iron deposition and haemolysis (341, 342) and their ability to act as a culture medium for organisms (considered in Section 4.4.3). Alternative artificial oxygen carrying substances that may be considered in future studies include Hemopure (haemoglobin glutamer-250; Hemoglobin Oxygen Therapeutics LLC, Pennsylvania, USA) and pyridoxylated bovine haemoglobin (343). Entirely acellular oxygen carrying substances include STEEN solution (Xvivo Inc., Goteborg, Sweden) (344) and Aqix RS-I (Aqix Ltd., London UK) (345).

Perfusate temperature is another variable worth considering in future studies aiming to enhance EVNP. Lowering the temperature of the perfusate to a 'subnormothermic' range (21-32^{o}C) has the theoretical benefit of supporting ATP production whilst curtailing the organ's metabolic rate to ensure an ATP positive equity. Whilst this failed to demonstrate superior graft function when in a study using packed red blood cells (346), a subnormothermic perfusion may be better achieved by other oxygen-carrying substances that are optimised for this temperature, such as Hemopure.

Finally, abridging NRP and EVNP technologies may be possible in order to achieve ischaemia-free retrieval. This has been demonstrated in one case in Guangzhou, China, in which arterial cannula was passed through intrarenal abdominal aorta whilst the supra-renal aorta was clamped, in order to provide in-flow of warm oxygenated blood during retrieval. Outflow from the kidney was established by cannulating the infra-renal inferior vena cava with downstream occlusion. This essentially allowed the kidney to be removed without cessation of perfusion and straight into an EVNP circuit (347). Consistent and safe replication of this technique needs to be demonstrated.

Delivering novel therapies

In addition to providing a physiological environment for kidneys, EVNP may provide a means of delivering active therapies to the organ in isolation. A number of potential therapies exist when scanning the horizon for promising treatments. Their delivery through normothermic machine perfusion may act synergistically.

One of the most researched cellular therapies in transplantation is mesenchymal stem cells (MSC). MSCs are multipotent, cultured from bone marrow, the umbilical cord, adipose tissue, and blood. MSCs are fibroblast-like cells that have the ability to differentiate into osteoblasts, adipocytes, and chondrocytes, which is why their first application was in the regeneration of connective tissue.

MSCs are capable of self-renewal and multilineage differentiation and have anti-inflammatory and pro-tolerogenic effects. Multiple effector mechanisms have been proposed, including suppression of allogeneic T cell responses and promotion of tissue regeneration (348, 349). MSCs appear able to inhibit IRI, ameliorate acute kidney injury, reduce fibrosis and enable lower doses of immunosuppression post-transplantation(350). The ideal timing, route of administration, and dose of MSCs is yet to be determined in renal transplantation(351), but is likely to be in the order of 10^4-10^7 cells for these to be demonstratable within glomeruli of kidneys (213, 352).

EVNP provides a model whereby live MSCs can be administered directly into the target organ, avoiding trapping in capillary beds elsewhere if given systemically, and the need for MSC homing (353). Given that MSCs appear to be procoagulant, the administration of MSCs via a fully heparinised EVNP circuit is also likely to be advantageous (354). In addition, direct administration may enable a lower dose of MSCs that may reduce the risk of potential systemic adverse effects such as the potential for developing malignancy (355). MSC therapy may act to promote organ regeneration, through physical and paracrine interactions, to achieve improved graft function (352). The delivery of MSCs via EVNP has been demonstrated as being viable (214, 356). Future trials are therefore necessary to evaluate MSC/EVNP therapy in clinical trials (352). In a porcine model of transplantation, MSCs delivered during EVNP demonstrated reduced lactate dehydrogenase and other markers of injury (357, 358). Human clinical trials combining MSC/EVNP treatment are needed to evaluate this further.

A novel angle that should also be considered is that the action of MSCs may be enhanced if they are 'licenced' by a proinflammatory environment. EVNP perfusate, which contains high concentrations of cytokines, including IFNγ (295), may provide a medium for MSC licensing. EVNP may therefore provide a means of licencing and delivering MSCs to enhance organ recovery and function.

Other novel therapies in transplantation that are deliverable via EVNP include gene therapies, nanoparticles, biological therapies and antibodies (reviewed by Hosgood et al (359)).

National perfusion service

This thesis has discussed the potential for improved organ assessment, with the amelioration of graft function with novel perfusion technologies, with and without additional therapeutics. However, for this to be effectively upscaled in the UK there are logistical and economic challenges. The development of an EVNP service requires significant investment in equipment and training, as well as the development of governance, clinical pathways, and guidance (339, 360). It may therefore be more effective to develop regional organ perfusion hubs, that provide an EVNP service to local transplant units. In the US, a lung perfusion hub has been established and provides regional lung transplant centres with normothermic machine perfusion (361). If EVNP is shown to provide meaningful information that significantly influences organ utilisation rates or demonstrates amelioration of graft function with expansion of the donor pool, then regional perfusion hubs should be considered to allow equal access for patients to this technology.

To summarise, there are a number of promising avenues of research in which the composition, delivery, and national implementation of EVNP may be optimised.

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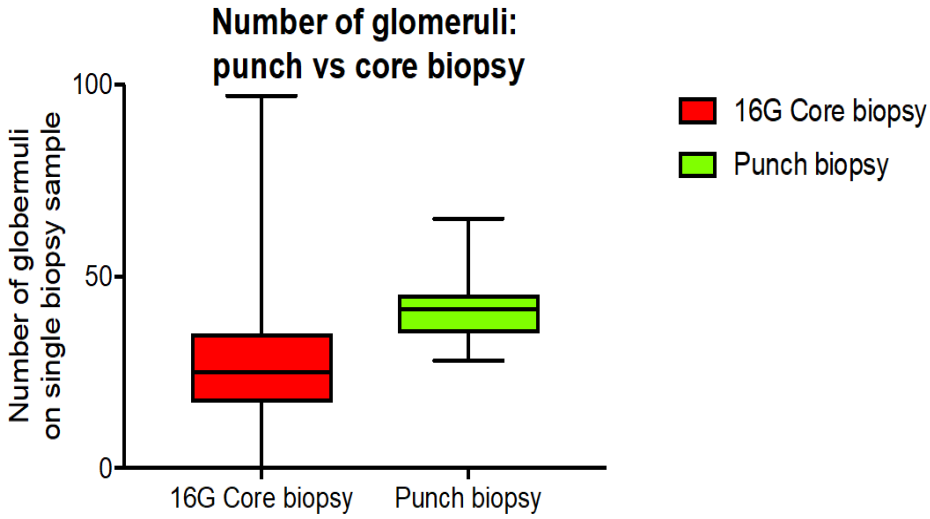
Appendices

Appendix 1: Difference in yield of glomeruli between 16-gauge core biopsy versus 3mm punch biopsy



	Punch biopsy	16G Core biopsy
Number of values	16	576
Minimum	28	0
25% Percentile	35.25	17
Median	42	25
75% Percentile	45.25	35
Maximum	65	97
Shipiro-Wilk test of normality	Non-parametric	
Mann-Whitney test comparing both medians	p = <0.001	
Fisher's exact test comparing adequacy	p = 0.005	

Appendix 2: List of the author's publications directly related to the thesis

1. Callaghan CJ, **Phillips BL**, Foukaneli T, Robinson S, Watson CJE. The use of third-party packed red blood cells during ex situ normothermic machine perfusion of organs for transplantation: Underappreciated complexities? *American Journal of Transplantation*. 2021 Apr;21(4):1376-1381. doi: 10.1111/ajt.16355. Epub 2020 Nov 1. PMID: 33048419
2. **Phillips BL**, Ibrahim M, Greenhall GHB, Mumford L, Dorling A, Callaghan CJ. Effect of delayed graft function on longer-term outcomes after kidney transplantation from donation after circulatory death donors in the United Kingdom: A national cohort study. *American Journal of Transplantation*. 2021 Oct;21(10):3346-3355. doi: 10.1111/ajt.16574. Epub 2021 May 6. PMID: 33756062
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