QUALITY IN ORGAN DONATION AND TRANSPLANTATION

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QUALITY IN ORGAN DONATION AND TRANSPLANTATION

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ABSTRACT

Kidney transplantation is the treatment of choice for most patients with End-Stage-Renal-Failure (ESRF). Transplantation offers improved quality of life as well as reducing the mortality rate of patients with ESRF when compared with being on dialysis. As the prevalence of chronic kidney disease, and therefore ESRF, is increasing, there is a growing demand for transplants. This means that transplant clinicians have to expand the kidney donor pool by utilising kidneys from older donors with potentially multiple co-morbidities. The on-going challenges in donation and transplantation now are to be able to accept and transplant these higher risk donor organs, without compromising outcomes.

My doctoral research has evaluated the effects that each stage of the donation process has on the organs to evaluate whether we can still get good outcomes from these organs.
I have used data from the National Transplant Registry to explore whether the duration of Brain Death, thought to be a period of pro-inflammation, affects the outcomes of transplantation, and this has shown that there is no detrimental effect when durations of brain death are longer. This raises the possibility that this time can be used to observe, investigate and potentially deliver therapeutic interventions to improve organ quality.
I also used data from the National Transplant Registry to evaluate the effect that acute kidney injury (AKI) has on outcomes following kidney transplantation. This showed that although there is a statistical difference in outcomes between kidneys with and without and AKI (1-year graft survival 91% versus 93%), this is unlikely to be of clinical significance and therefore kidneys with an AKI should be considered for transplantation and could further expand the donor pool.
I used an animal model for Donation after Circulatory Death to investigate whether the current preservation solutions used for retrievals are effective at cooling the organs and washing them out. I looked at histology as well as specific cellular markers to look for signs of injury to see whether any solution is inferior to the others (as has previously been suggested). This study found that the preservation solution used in the UK for retrievals is not inferior to the other solutions, and could perhaps even cool the kidneys more effectively.
Finally I did a systematic review of studies that have evaluated whether donor biopsies correlate with clinical outcomes, and therefore can be used to aid clinicians in their organ utilisation decision-making. The review found that there is no consensus as to whether biopsy findings predict outcomes.

Based on the findings in this thesis, future work should concentrate on identifying markers of injury or repair in kidneys from donors with different durations of brain death or with AKI. Interventions that could be used in donors during brain death and retrieval techniques are also studies that could be carried out.

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My family have shown me a lot of support and provided me with encouragement and many meals when needed.

This thesis is dedicated to my mum who, I know, would have been so proud to know I completed it.

DECLARATION

All the work presenting in this DPhil Thesis is my own research except where otherwise stated.

I have undertaken the research with the support and guidance of my Supervisors.

Statisticians from NHSBT helped me run the statistical analysis of the transplant registry data, they spent time talking me through the software SAS, explaining the coding for analysis and helping me interpret the results.

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PUBLICATIONS AND PRESENTATIONS

Publications from this Thesis

Transplantation of kidneys from donors with acute kidney injury; friend or foe?
Boffa C, van de Leemkolk F, Curnow E, Homan van der Heide J, Gilbert J, Sharples E, Ploeg RJ
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Past, present and future of dynamic kidney and liver preservation and resuscitation
I. Jochmans, M. Z. Akhtar, D. Nasralla ,P. Kocabayoglu, C. Boffa, M. Kaisar, A. Brat, J. O'Callaghan, L. H. M. Pengel, S. Knight and R. J. Ploeg
American Journal of Transplantation, 2016 Sept;16(9):2545-2555

New classification of donation after circulatory death donors definitions and terminology.
Thuong M, Ruiz A, Evard P, Kuiper M, Boffa C, Akhtar MZ, Neuberger J, Ploeg RJ
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Ethical, legal and societal issues and recommendations for controlled and uncontrolled DCD
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Transplant International 2016 Jul;29(7):771-9

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Murphy P, Boffa C, Manara A, Ysebaert D and de Jongh W
Transplant International. 2016 Jul;29(7):760-70

Utilization of DCD organ donors for vascularized pancreas and islet of Langerhans transplantation: Recommendations from an expert group
Thierry Berney, Catherine Boffa, Titus Augustine, Lionel Badet, Eelco de Koning, Johann Pratschke, Carlo Socci, Peter Friend
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Participations in conferences with oral presentations

1. *Does the Duration of Brain Death Affect Outcomes following transplantation?*
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2. *Does the Duration of Brain Death Affect Outcomes following transplantation?*
ATC Annual Conference, Philadelphia, May 2015 (Young Investigator Award)

3. *Does the Duration of Brain Death Affect Outcomes following transplantation?*
ESOT Conference, Brussels September 2015

4. *Kidneys from Donors with Acute Kidney Injury: Friend or Foe?*
ESOT Conference, Brussels, September 2015 (Beldonor Award)

5. *The use of kidneys with Acute Kidney Injury in Transplantation: The good, the bad and the ugly*
Medawar Presentation BTS Annual conference, Glasgow, February 2016

6. Viscous or not: Does solution viscosity really affect quality of wash-out at time of organ retrieval and preservation
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3. Kidney Research UK Educational Bursary Award, The Transplantation Society Congress 2018
Efficacy and Quality of Flush-out prior to cold storage of Liver and Kidney in Donation after Circulatory Death

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
ADH	Antidiuretic Hormone
ADP	Adenosine di-phosphate
ADQI	Acute Dialysis Quality Initiative
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ATP	Adenosine Tri-Phosphate
ATI	Acute Tubular Injury
ATN	Acute Tubular Necrosis
AST	Aspartate Aminotransferase
BD	Brain Death
BCA	Bicinchoninic Acid
BMI	Body Mass Index
BPAR	Biopsy Proven Acute Rejection
BSA	Bovine Serum Albumin
CADI	Chronic Allograft Damage Index
CDS	Chronic Damage Score
CEUS	Contrast Enhanced Ultrasonography
CI	Confidence Interval
CIT	Cold Ischaemic Time
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease-Epidemiology Collaboration
CNS	Central Nervous System

CrCl	Creatinine Clearance
CVA	Cerebrovascular Accident
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DD	Deceased Donor
DDS	Deceased Donor Score
DGF	Delayed Graft Function
DKT	Dual Kidney Transplant
DI	Diabetes Insipidus
ECD	Extended Criteria Donor
EDTA	Ethylenediaminetetraacetic Acid
eGFR	Estimated Glomerular Filtration Rate
ESRD	End Stage Renal Disease
ESRF	End Stage Renal Failure
ET	Eurotransplant
GMC	General Medical Council
HCV	Hepatitis C Virus
HES	Hydroxyethyl Starch
HIF	Hypoxia Inducible Factor
HLA	Human Leucocyte Antigen
HO-1	Haem-Oxygenase-1
HRP	Horse Radish Peroxidase
HSP	Heat Shock Protein
HTK	Histidine-tryptophan-ketoglutarate

ICP	Intracranial Pressure
IF	Immediate Function
IgG	Immunoglobulin
IGL-1	Institute of George Lopez Solution
IHC	Immunohistochemistry
IL	Interleukin
INR	International Normalised Ratio
IQR	Interquartile Range
IRI	Ischaemia Reperfusion Injury
ITBL	Ischaemic Type Biliary Stricture
KBAS	Kidney Biopsy Audit Score
KDIGO	Kidney Disease: Improving Global Outcomes
KDPI	Kidney Donor Profile Index
KDRI	Kidney Donor Recipient Index
K-Score	Karpinski Score
LDH	Lactate Dehydrogenase
MAPI	Maryland Aggregate Pathology Index
MDRD	Modification of Diet in Renal Disease
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
NHS	National Health Service
NHSBT	NHS Blood and Transplant
NORS	National Organ Retrieval System
NOS	Newcastle Ottawa Score

OPTN	Organ Procurement and Transplantation Network
OR	Odds Ratio
PEG	Polyethylene Glycol
PHD	Prolyl-hydroxylase Domain
PNF	Primary Non Function
PTA	Pancreas Transplant Alone
PVDF	Polyvinyldifluoride
QUOD	Quality in Organ Donation
RBC	Red Blood Cells
RCT	Randomised Controlled Trail
RIFLE	Risk, Injury, Failure; Loss and End-stage renal Disease
ROS	Reactive Oxygen Species
RRT	Renal Replacement Therapy
SCD	Standard Criteria Donor
SCr	Serum Creatinine
sCR1	soluble Complement Receptor 1
SCS	Static Cold Storage
SKT	Single Kidney Transplant
SPK	Simultaneous Pancreas and Kidney Transplant
sPSGL	soluble P-Selectin Glycoprotein Ligand
SRTR	Scientific Registry of Transplant Recipients
SST	Serum Separating Tube
T3	Triiodothyroxine
TNF	Tumour Necrosis Factor

UK	United Kingdom
UNOS	United Network for Organ Sharing
UO	Urine Output
US	United States
USA	United States of America
USS	Ultrasonography
UW	University of Wisconsin Solution
UW-CSS	University of Wisconsin- Cold Storage Solution
UW-MPS	University of Wisconsin- Machine Preservation Solution
WI	Warm Ischaemia
WIT	Warm Ischaemic time
4-HNE	4-hydroxynonenal

CHAPTER 1 INTRODUCTION

Kidney transplantation is the treatment of choice for most patients with End-Stage-Renal-Failure (ESRF). Transplantation offers improved quality of life as well as reducing the mortality rate of patients with ESRF when compared with being on dialysis (1, 2). The long-term mortality rate of patients who receive a deceased donor kidney transplant is 68% lower than that of patients on the waiting list, and this improvement includes patients who are older (60-74 years of age) at the time of transplantation (1).

The first successful transplantation between humans was done in 1954. John Merrill, Joseph Murray and Hartwell Harrison in Boston in the United States of America (USA), led a team who transplanted a kidney from one identical twin to his brother who was dying of renal disease, and the patient survived for eight years (3). This achievement came 50 years after the initial experimental transplants done in dogs by Ullmann in Vienna in 1902, and followed on from a series of kidney transplantations from deceased and living donors where all recipients eventually succumbed to their primary disease with the longest survival being five and a half months (4, 5).

Following on from the success in Boston, many more transplants between twins were carried out, but it wasn't until 1962 when Azathioprine became available for use in humans that transplantation really took off. Prior to the introduction of Azathioprine, patients were treated with irradiation to induce tolerance, as Peter Medawar had found this had a profound and long-lasting effect on graft acceptance. This regimen was difficult to control, and most patients developed graft versus host disease, and so alternatives to irradiation were looked at (6). 6-mercaptopurine, an antimetabolite used for the treatment of cancer was found to have immunosuppressive properties useful for transplant recipients. Roy Calne, then a surgical trainee in London, used 6-

mercaptopurine for transplants in dogs and in his experiments none of the dogs had rejection of the transplanted kidneys. New derivatives of 6-mercaptopurine were developed, including Azathioprine, and these were less toxic than 6-mercaptopurine whilst still being successful in preventing the development of rejection (6, 7).

The addition of prednisolone to the maintenance immunosuppression regimen improved 1-year patient survival to over 90% by the 1980s, and the introduction of the calcineurin inhibitor ciclosporin in the early 1980s improved graft survival too. Transplantation was now a growing field, and from the 1990s the development of new immunosuppressive agents continued. Tacrolimus and mycophenolate mofetil, as well as the use of newer induction agents, now means that loss of kidney transplants due to acute rejection has been reduced and 1-year kidney transplant survival rates are now over 95% (5, 6). As loss of kidneys from acute rejection is much less common, it might be expected that graft survival rates would increase, however, this hasn't been the case and may in part be due to the increasing use of more older and higher risk donor organs (8).

As the general population is living longer, rates of chronic kidney disease (CKD) are also increasing. In 2011, 2.6 million people aged 16 and over had CKD stage 3-5 (defined as an estimated glomerular filtration rate (eGFR) of <60ml/min/1.73m2 using the modification of diet in renal disease (MDRD) equation), which is 6.1% of the population in this age group (95% CI 5.3%-7.0%). This is predicted to increase, so that 4.2 million people (8.3% of the population) will have CKD stage 3-5 by 2036 (9). The introduction of the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) formula for eGFR in 2011 showed an improvement in the concordance of eGFR and true GFR. When this

definition is used, the number of people with CKD stage 2 and 3 reduce, whereas the number of people with CKD stages 4 and 5 will increase by 32,000 (10). This increase is seen mainly in men over the age of 70, and clearly as the population is aging, these results are significant.

Patients with CKD are at an increased risk of developing ESRF and therefore of needing renal replacement therapy (RRT) in the form of dialysis or transplantation. By the end of 2014, 115 people per million population were on RRT, with 7411 patients being started on RRT in 2014, and this number is only going to increase (11).

As of February 29th 2020, there were 4,618 patients active on the adult kidney only transplant waiting list in the United Kingdom (UK) (12), and between 1st April 2019 to 31st March 2020, in total 3,190 kidney transplants were performed (Figure 1.1). In an attempt to reduce this disparity, transplant centres are increasing the number of living donor transplants. This increase in activity has seen the number of living donors more than double over the last 15 years from 427 in 2005 to 949 in 2020 (Figure 1.2) (12, 13). Although this increase in living donation is a fantastic effort, it alone is not enough to equal the number of patients needing a kidney transplant.

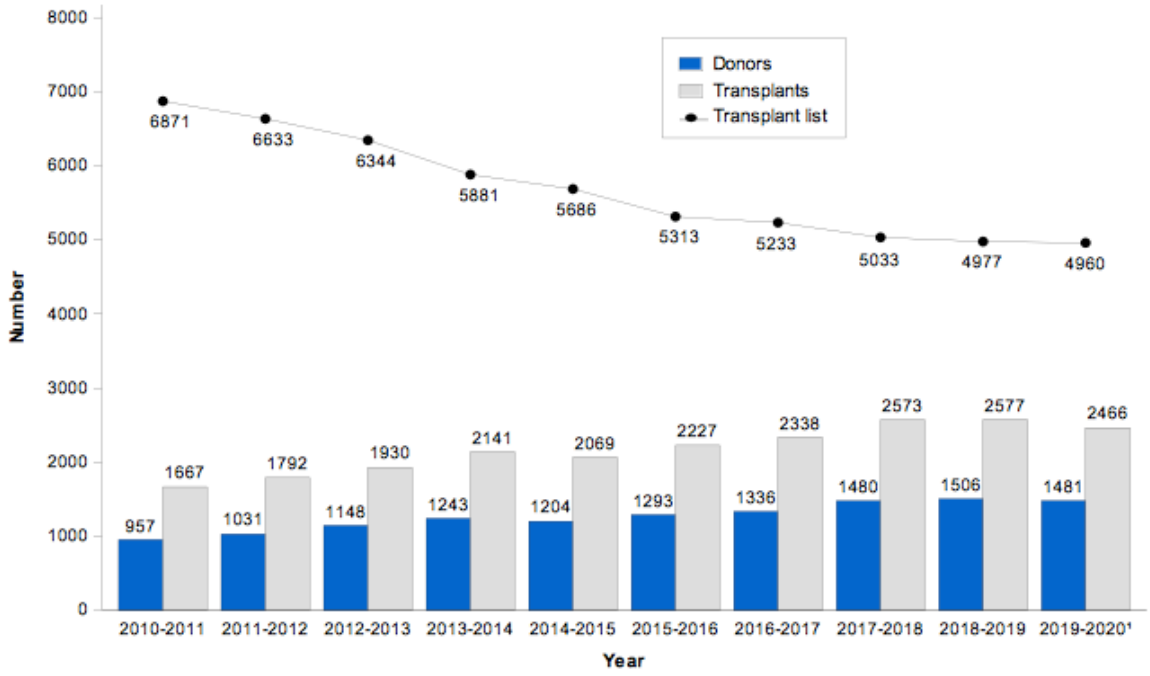


Figure 1.1 Number of patients on the kidney transplant waiting list (line) compared to the number of deceased donors (blue bar) and number of transplant performed (grey bar) in the UK in the period April 2010 to March 2020 (from NHS Blood and Transplant 'Organ Donation and Transplantation Activity Report 2019-2020'). ([1] waiting list as at 29th February 2020).

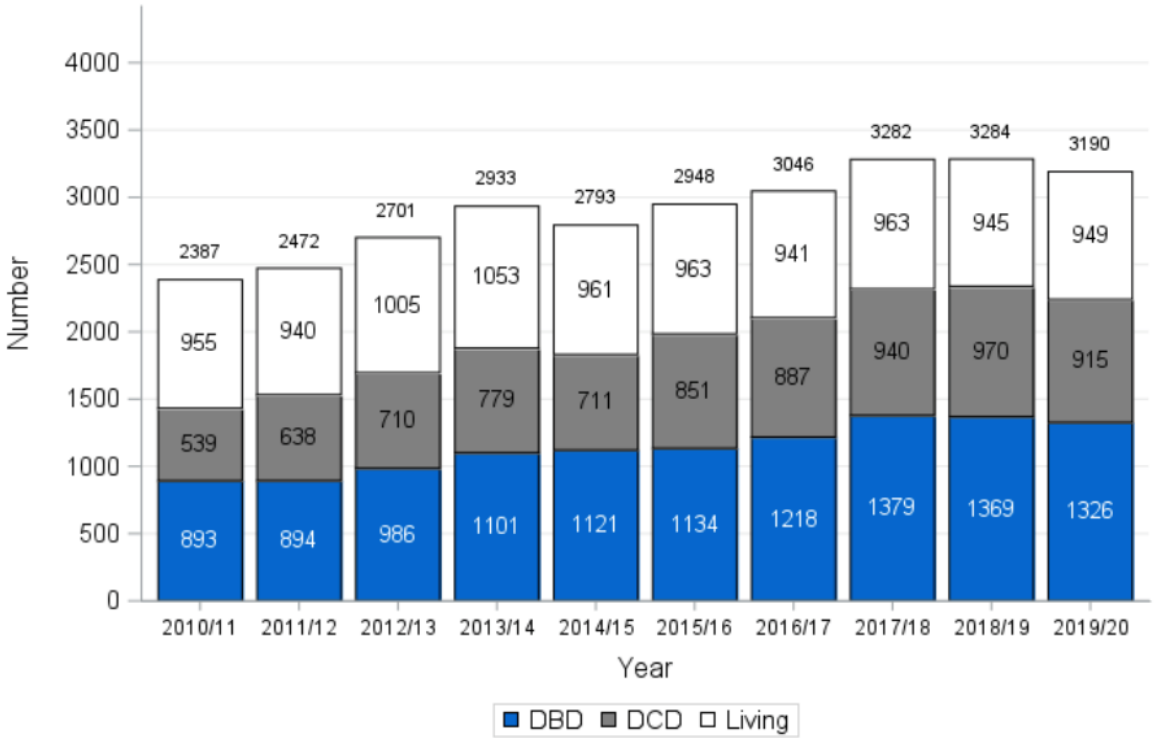


Figure 1.2 The number and type of kidney donor per year from April 2010 to March 2020 (from NHS Blood and Transplant 'Annual Report on Kidney Transplantation 2019-2020')

1.1 Deceased Organ Donation

Today, the challenge in kidney transplantation is finding enough kidneys to meet the demands of the waiting list. Although living donors provide the gold standard in renal transplantation with 1 and 5- year graft survival rates of 98% and 93%, the majority of kidneys for transplantation are procured from deceased donors (70% of kidneys transplanted between April 2019 and March 2020). These organs provide excellent outcomes with patient survival at 1 year being 97% and this remains high at 5- and 10-years, with 88% and 75% of patients being alive respectively. Kidney graft survival is also good with 94% of transplant working at 1 year, and 86% working at 5 years (12).

To help bridge the gap between supply and demand, as well as using kidneys from donors after brain death (DBD) the donor pool has been expanded with the utilisation of donors after circulatory death (DCD) and extended criteria donors (ECD) (Figure 1.2, 1.3 and 1.4) (12, 14).

Up until around 20 years ago, the deceased donor was younger than 50 years of age, and had most likely died due to trauma (15-17); today donors are older and more likely to have co-morbidities.

Graft survival and function following DCD kidney transplantation is similar to that following DBD kidney transplantation in the UK with 5-year graft survival rates of 85% (DCD) vs 83.2% (DBD). This is also the case in the United States (US) with 5-year survival rates of 81.8% (DCD) vs 81.3% (DBD) (18-20). Although transplantation with ECD donors has reduced graft survival versus standard criteria donors (SCD) (18, 21-23), this donor source still offers a survival benefit when compared to staying on dialysis or the waiting list (24, 25).

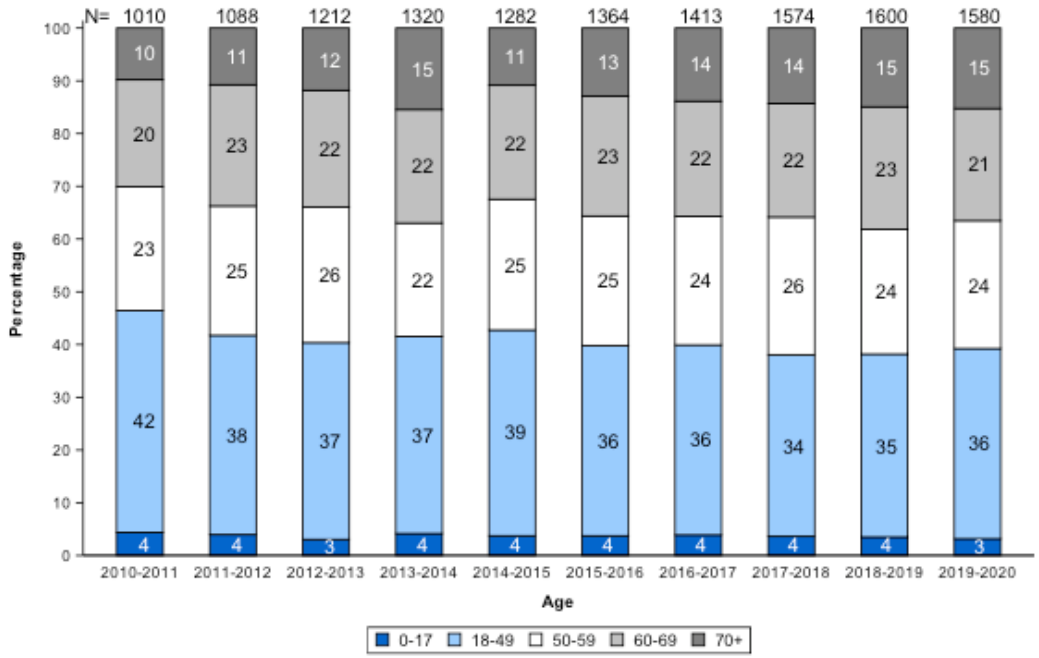


Figure 1.3 The age of deceased donors that had organs accepted and transplanted from April 2010 to March 2020 (from NHS Blood and Transplant 'Organ Donation and Transplantation Activity Report 2019-2020')

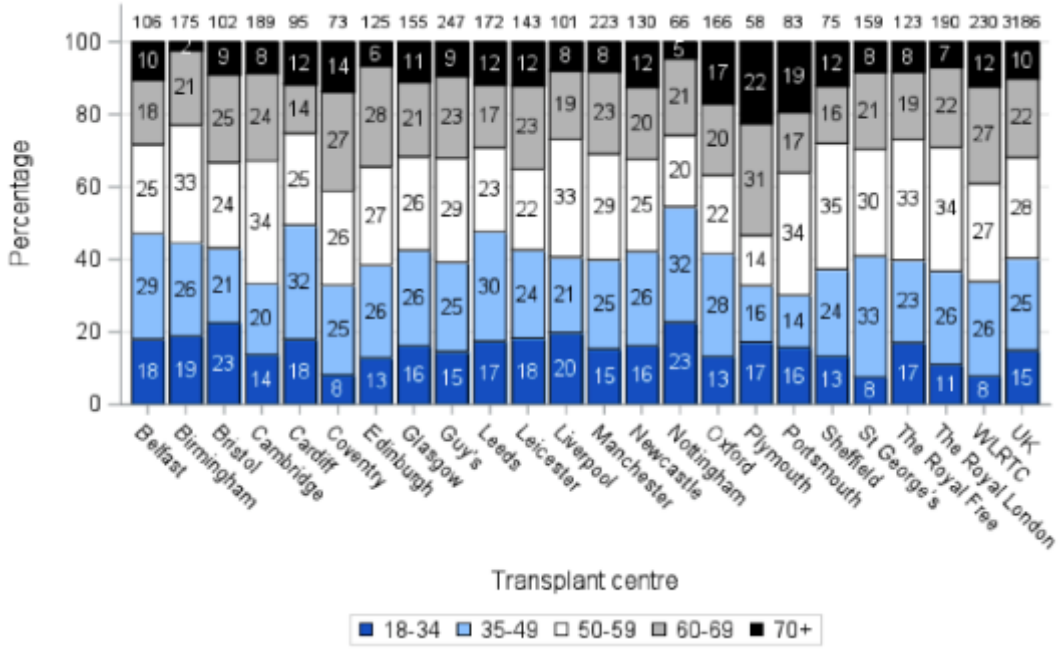


Figure 1.4 The number of kidney transplants done per transplant unit by age in 2019-2020 (from NHS Blood and Transplant 'Kidney Transplantation Activity Report 2019-2020')

1.1.1 Donation after Brain Death

Donors after brain death provide a major source of kidneys for transplantation, accounting for 42% of all donors in the UK (and 59% of deceased donors) (12).

Brain death (BD) is defined as 'the irreversible loss of the capacity for consciousness, combined with irreversible loss of the capacity to breathe' (26). For brainstem death to be tested, the cause of the brain damage needs to be known, reversible causes of coma need to have been excluded and the patient must be apnoeic and ventilated (27). When this is established, two General Medical Council (GMC) certified doctors perform brain stem death testing. The brain stem death tests are to confirm the absence of brain stem reflexes and the absence of respiratory effort when the ventilator is switched off. The tests are repeated to remove observer error (27). Brain death is the terminal event following cerebral trauma, cerebrovascular haemorrhage, infection or extended hypoxia and once brain stem death has been confirmed, and the family have consented for organ donation the process of offering organs and arranging the retrieval can occur.

Brain death results in a chain of systemic haemodynamic, inflammatory and hormonal changes (28-30). It is associated with a profound and often progressive systemic pro-inflammatory and pro-coagulatory response. Raised intracranial pressure (ICP) leads to compression of cerebral arterioles, which causes cerebral ischaemia. This activates the sympathetic nervous system, and causes a release of catecholamines (the so called 'catecholamine storm'). There is systemic vasoconstriction that leads to hypertension, and this activates the parasympathetic nervous system through the baroreceptors, which causes bradycardia, known as the Cushing's Reflex (31). This severe hypertension is an attempt to maintain cerebral perfusion by overcoming the raised intracranial

pressure. The escalation in sympathetic activity results in widespread systemic vasoconstriction leading to micro thrombi formation, which impairs the perfusion to potential donor organs. As the ICP remains higher than the mean arterial pressure, cerebral perfusion continues to be impaired, and so ischaemia extends down the brain stem. Eventually the sympathetic centres become necrotic, and this alongside herniation of the brain stem causing ischaemia of the spinal cord leads to progressive hypotension as vascular and cardiac sympathetic stimulation reduces. This further reduces perfusion of the organs (29, 30, 32, 33).

As well as the increased levels of catecholamines found as soon as 15 minutes after brain death, other hormonal changes occur. Levels of antidiuretic hormone (ADH) reduce within 15 minutes and are un-recordable after six hours of brain death. There are also reduced levels of adrenocorticotrophic hormone (ACTH) and free triiodothyronine (T3) levels are less than 50% of normal within 24 hours of brain death (29). The reduction in ADH levels results in excessive diuresis, which leads to hypovolaemia, hypernatraemia and hyperosmolarity, also known as Diabetes Insipidus (DI). Vasopressin (ADH) therapy may be required to reduce pulmonary oedema seen with only crystalloid replacement of urine losses in DI (29).

Alongside these haemodynamic and endocrine changes, inflammatory responses are also initiated. Cerebral oedema, elevated ICP, global brain ischaemia, cellular death, metabolic derangements and haemodynamic instability all lead to disruption of the blood-brain barrier and this allows inflammatory cells to flow both ways across the blood-brain barrier therefore permitting central nervous system (CNS) cytokines (IL-1β

and TNF-α) to be released into the systemic circulation where they can find their way to tissues and cause local inflammation (30, 34).

Infiltration of granulocytes into kidney cortex tissue has been seen as early as 30 minutes after brain death in a rat model (35), and histology has confirmed the presence of organ damage after brain death in rodents, large animals and humans with kidney biopsies showing some evidence of distal tubule necrosis. Livers and hearts from these animals have also shown mitochondrial injury and cell necrosis (36, 37).

In both clinical work and experimental models, organ damage also induces an inflammatory response, which includes the production and release of cytokines, the activation of surface molecules and infiltration of leukocytes, macrophages and T cells into peripheral organs following brain death (35, 38-42). It has been shown that the levels of mRNA for the cytokines TNF-α, IFN-γ, and IL-10 are higher in the tissues of kidneys from deceased donors, and that the gene expression for these cytokines in deceased donor kidney tissues is similar to that found in tissue of kidney allografts with acute rejection (43). Koo et al found that the activation of the surface adhesion molecule E-Selectin is found in brain dead donor kidneys, but not in living donor (LD) kidneys (44). Proximal tubular ICAM-1 and VCAM-1 were also detected in brain dead donor kidneys and not in living donor kidneys (44, 45), which may trigger an immune response in the recipient and accelerate the process of rejection. Kim et al (43) found that acute rejection occurred more frequently within six months post transplant in deceased donor kidney recipients when compared to living donor recipients.

In an attempt to ameliorate some of these effects seen during brain death, donor care pathways have been instigated in intensive care units across the world. These donor-care bundle protocols have been introduced to try to alleviate the haemodynamic and hormonal instability in brain dead donors. The goals of these management pathways are to maintain temperature, normovolaemia, blood pressure and optimise cardiac output to allow adequate perfusion of organs to improve function (46, 47).

1.1.2 Donation after Circulatory Death

As can be seen in figure 1.2, the number of donors following circulatory death is increasing. With 915 DCD donors in the UK in 2019/20, this donor source now accounts for 41% of the deceased donor kidneys transplanted (12).

Death following cardiorespiratory arrest is identified by the irreversible onset of apnoea, unconsciousness and the absence of circulation. However, unlike brain stem death, there are currently no standardised criteria for the confirmation of death following cardiorespiratory arrest (48). The Medical Royal Colleges Working group has issued guidelines that death can be confirmed when apnoea and unconsciousness in the absence of circulation occurs when all contributing factors (body temperature, endocrine, metabolic and biochemical abnormalities) have been corrected. The individual should be observed by the doctor confirming death for at least five minutes to ensure that irreversible cardiorespiratory arrest has happened, and that spontaneous return of cardiac or respiratory function does not occur. As brain function ceases quickly following the loss of circulation and respiration after these five minutes, the pupillary

response to light, the corneal reflex and any motor responses to supra-orbital pressure should be checked, and if these are absent, death can be confirmed (48, 49).

DCD donors are characterised into different categories depending on the particular circumstances that result in the patient's death. The Maastricht classification, originally devised in 1995, has been modified in a meeting in Paris in 2013 and is still used (Table 1.1) (50). In the UK, the majority of DCD donors are Maastricht category III donors and the decision to withdraw life support is made with the family (51).

In the case of donation after circulatory death, the medical team making the decision that further treatment of a patient is futile must be made independently from the transplant team (52). Once the withdrawal of life supporting treatment has been discussed with the family of the patient, organ donation can then be introduced. If the family consent, the retrieval teams will be mobilised and the planning of end of life care will begin.

Category I. *Uncontrolled*	**Found Dead** *IA. Out of hospital* *IB. In of Hospital*	Sudden-unexpected cardiac arrest without any attempt of resuscitation by a medical team, warm ischaemic time to be considered according national life-recommendations in place, in- or out-of-hospital (IH-OH) setting.
Category II. *Uncontrolled*	**Witnessed Cardiac Arrest** *IIA. Out of hospital* *IIB. In of Hospital*	Sudden-unexpected-irreversible cardiac arrest with **unsuccessful resuscitation by a medical team**, reference to in- or out-of-hospital (IH-OH) setting**.**
Category III. *Controlled*	**Withdrawal of life-sustaining therapy**	Planned withdrawal of life-sustaining therapy, expected cardiac arrest
Category IV. *Uncontrolled*	**Cardiac arrest while brain dead**	Sudden cardiac arrest after brain death diagnosis during donor management but prior to the retrieval.

Table 1.1 The Modified Maastricht Classification of DCD (50)

Following controlled withdrawal of life support, the patient's blood pressure will decrease, at varying rates, until cardiac activity ceases and circulation stops. The time taken from withdrawal of life support to cardiac arrest and asystole is called the agonal period. The length of this period will determine whether donation is possible. Most DCD protocols try and limit the agonal period to minimise the time that the organs are relatively poorly perfused or oxygenated due to reduced cardiac and respiratory function (53, 54). In a study done by Suntharalingam et al, the time from withdrawal of life support to death (the agonal period) varied from five minutes to three days, however, 70% of patients died within three hours (55). This study found that 1-year graft survival was not significantly different if the patient died within one hour after withdrawal of life support compared to if they died after one hour.

If the donor becomes haemodynamically unstable during the agonal period, there could be a period of functional warm ischaemia, which is defined as the time from when the systolic blood pressure drops below 50mmHg until the start of in-situ cold perfusion. This time period may affect organ quality and post-transplant outcomes. This time along with the primary warm ischaemic time (WIT), the time from asystole to start of in-situ cold perfusion, needs to be taken into account when deciding whether the quality of the organs has been negatively affected. Warm ischaemia is detrimental to organs because metabolic processes are still continuing, but due to hypoxic conditions, the cells within the tissue switch from aerobic to anaerobic metabolism, which depletes Adenosine Tri-Phosphate (ATP) stores and results in the generation of oxygen free radicals (51, 56). When these kidneys have their blood flow restored at reperfusion during the transplant operation, there is further injury and this is known as the ischaemia reperfusion injury (IRI). During IRI, an inflammatory response results in endothelial injury and cell swelling

leading to blood vessel lumen narrowing which causes further tubular injury. Increased levels of cytokines and reactive oxygen species (ROS) are seen in the early postoperative period following transplantation using kidneys from DCD donors when compared to living donors meaning there is a pro-inflammatory environment (57-59).

Experimental models suggest that warm ischaemic times of 45 minutes or greater lead to increased cellular injury, reduced urine output and reduced rate of inulin clearance (60). Clinical studies have suggested that when the warm ischaemic time is less than 30 minutes, there is no detrimental effect on transplant outcomes, except to increase the rates of delayed graft function (DGF), and this is particularly evident when there is prolonged haemodynamic instability (61, 62). WIT of greater than 40 minutes leads to reduced graft survival at 1- and 3-years (63). Experimental studies have also suggested that it is the combination of warm ischaemia and an extended period of cold ischaemia that leads to poorer outcomes (64).

Despite these concerns, DCD donors have provided transplant clinicians and their patients with a valuable source of donor kidneys with survival rates that are as good as those following transplantation with DBD kidneys (20), and therefore this would suggest that careful selection of these donors is key (62, 63).

1.1.3 Extended Criteria Donors

Data from the US Scientific Registry of Transplant Recipients (SRTR) (from the Organ Procurement and Transplantation Network (OPTN)) have been used to describe the term extended criteria donors (ECD). ECDs are defined as kidneys from donors after brain death who are either 60 years and older, or aged 50-59 years of age with at least

two of the following three criteria; death due to a cerebrovascular accident (CVA), a past medical history of hypertension or a terminal serum creatinine of >133µmol/L (22).

Transplantation using kidneys from ECDs may lead to more primary non-function in recipients; increased rates of delayed graft function and reduced longer-term renal function (65). These older donors with more co-morbidities often have a higher UK kidney donor risk index, and make up nearly 40% of the current kidney donors in the UK (figure 1.5). The UK kidney donor risk index uses donor factors to help predict outcomes following transplantation, and is discussed more in 1.5.1 (23). With these 'marginal donors' becoming the norm, rather than the exception, clinicians need as much information as possible about what to expect when using these kidneys so they can chose an appropriate recipient.

In the USA, any recipient being listed on the transplant waiting list is given the option as to whether they would be willing to receive a kidney from an ECD donor. They are given written information, and must sign a consent form to go on a separate waiting list for ECD kidneys (these recipients are also on the waiting list for standard criteria donor (SCD) kidneys) (18). In the UK, there is not a separate waiting list for ECD and SCD kidneys. Recipients are on one list, and clinicians make the decision as to whether the kidney offered is suitable for a given patient.

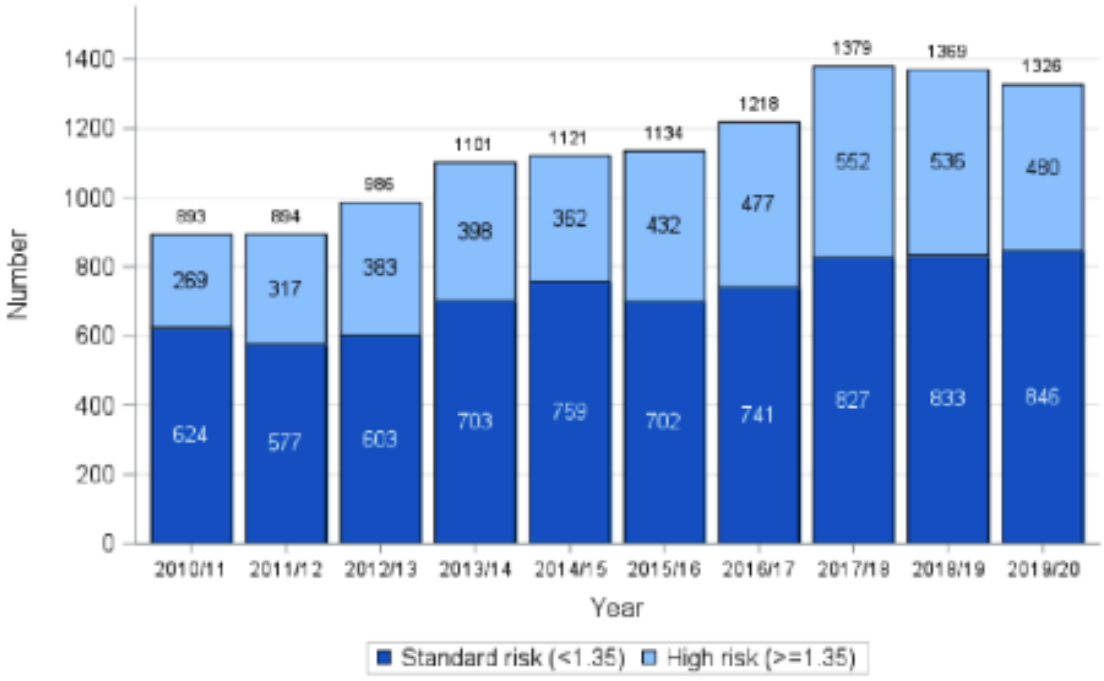


Figure 1.5 UK kidney donor risk index of transplanted DBD donor kidneys from 1st April 2010- 31st March 2020 (from NHS Blood and Transplant 'Annual Report on Kidney Transplantation 2019-2020')

Age

Age has been identified as an independent predictor of graft survival post transplantation (23).

Aging affects the kidney both structurally and functionally. With increasing age, there is loss of nephrons and renal mass, which stimulates podocyte hypertrophy. This seems to compensate for a long time, however this persistent stress becomes overwhelming, leading to podocyte detachment, vasoconstriction, capillary network collapse, epithelial cell activation, periglomerular fibrosis and glomerular sclerosis (66). This process starts after the age of 30, and from then on the number of glomeruli in the kidney decreases by about 6,500 per year. With increasing age, there is also impairment of the mechanisms required to maintain organ function, and organs lose the capacity to repair themselves. As renal tubules use high energy-consuming processes to perform their function of solute resorption these cells are at increased risk of accumulative oxidative damage over time, and this leads to a reduction in eGFR. The filtration rate reduces by approximately 5-10% per decade after the age of 35 years, and by the time a healthy individual reaches 90 years of age, their eGFR will be 50% what it was when they were young (66-68).

In an early study by Miura et al, following 45 minutes of ischaemia to the kidneys in old and young rats, old rats had higher concentrations of blood urea nitrogen when compared to young rats and the concentrations remained elevated for longer. There was also more severe cortical tubular necrosis seen in the kidneys of the older rats (69). This study shows that with increasing age, the kidney is more vulnerable to ischaemic damage, and therefore kidneys from older donors that may already have impaired

function, may not be able to recover as well from the ischaemic periods they are exposed to throughout the donation and transplantation process.

This has been confirmed by clinical studies that have found that the rates of DGF are increased when using donors over the age of 55, and graft survival is reduced when using donors over the age of 65 when compared to those younger than 65 (70, 71). Summers et al analysed the UK Transplant Registry data and found that kidneys from older donors (≥60 years old) had reduced graft survival when compared to kidneys from younger donors, but this graft survival was sufficiently acceptable to allow transplantation (72).

Hypertension

Donors with pre-existing hypertension are increasingly being used as kidney donors. In the USA, 38% of donors have a history of hypertension and this seems to be increasing (73).

Long-term hypertension leads to hypertrophy of the arteriolar muscular media with an increase in smooth muscle cells as well as fibrous thickening of the intima. Together these cause narrowing of the arterioles, specifically the afferent arteriole, which reduces the blood flow and causes glomerulosclerosis (74). As already described this can lead to reduced renal function, and therefore hypertension in an organ donor may lead to worse outcomes. Analysis of both UK and US data identified that a history of hypertension in a kidney donor increases the risk of graft failure post transplantation, and is one of the components of both the UK and US kidney donor risk index (23, 75).

Acute kidney injury

Acute kidney injury (AKI), defined as an abrupt reduction in kidney function (76), occurs in many hospitalised patients, including up to 25% of critically unwell patients (77). Therefore it is likely that many potential organ donors may have some degree of renal impairment and is why it has been included in the criteria to define ECDs.

A common cause of AKI is ischaemia, when poor renal blood flow leads to reduced oxygen and nutrient delivery to the cells, and there is diminished cellular waste product clearance. Together this causes injury to the tubular epithelial cells, and if this is for long periods of time, or the insult is severe, this leads to apoptosis, necrosis and eventual organ dysfunction (78).

While this injury is occurring, repair mechanisms are also taking place; viable epithelial cells proliferate and differentiate to cover the denuded area of the basement membrane, and help restore the nephron to a working unit (78). This intrinsic capacity of the kidney to repair itself and recover from an ischaemic or toxic insult means the majority of patients with AKI may fully recover their renal function (79, 80).

However, this repair is frequently faulty with incomplete repair of tubules, persistent inflammation, fibroblast proliferation and extracellular matrix production leading to fibrosis and potentially chronic kidney disease (78).

Kidneys with AKI that may be in various stages of injury or repair may be offered for transplantation, and it is unclear how the donation process with varying degrees of warm and cold ischaemic injury will affect their recovery. So the challenge for transplant clinicians is knowing whether kidneys with AKI will recover and give acceptable post transplant function, or whether they will sustain more injury and have impaired or even no function.

Some centres have reported their experience with using kidneys from donors with AKI, and mostly they report favourable outcomes, with comparable graft survival to kidneys without AKI (81-83). These are single centre reports with small numbers, using different definitions to classify AKI, making it difficult to draw definite conclusions, however, it is apparent that these kidneys may be a valuable source to expand the donor pool.

1.2 Preservation of Organs

Organ preservation is an important determinant of outcome and success in solid organ transplantation. The period of preservation starts during the retrieval operation with the initial 'cold flush' using a hypothermic (4°C) preservation solution. This intends to both wash out the blood from the organ as well as rapidly cooling the organ to reduce its metabolism.

The loss of circulation for an organ initiates a cascade of events that would eventually lead to the death of the organ. Most organs can only tolerate a couple of hours of circulatory arrest before severe metabolic derangements take place. Therefore, when organ transplantation is being considered, the objective of the retrieval is to quickly and effectively start this 'cold flush' and preservation.

1.2.1 Hypothermia

The metabolic rate of the organ halves with every 10°C drop in temperature, meaning the metabolic rate is 10-12% of normal metabolism at 4°C (84). Enzymes are also deactivated at these temperatures, and this protects the cell by lowering the energy demands by reducing the use of adenosine tri-phosphate (ATP). This decreases the

accumulation of lactate and hydrogen ions, and reduces the continual catabolism of metabolites and the destruction of the cellular structure (85).

Cell Swelling

Hypothermia may induce cell swelling because it supresses ionic pumps found in the cell membrane, which leads to loss of the membrane potential. The energy dependent sodium/potassium-ATPase pumps cannot excrete sodium so passive diffusion of sodium across the membrane leads to accumulation of sodium within the cell. This increased osmolarity causes an influx of water into the cell by osmosis, and therefore cell swelling (84, 86, 87). In an attempt to reduce this phenomenon, so-called impermeants and colloids are added to the solution used for the cold flush and subsequent preservation.

Acidosis

Although the metabolic rate is supressed, it is not stopped completely and so there are still some on-going enzymatic reactions, which lead to an ischaemic cascade. Ischaemia stimulates the use of anaerobic glycolysis, which depletes the cells' ATP content (anaerobic glycolysis only produces 2 molecules of ATP compared to 38 that are produced in aerobic glycolysis). Anaerobic glycolysis also produces lactic acid that activates phospholipases and proteases that induce lysosomal lysis and this leads to cell death (85). It has been suggested that mild acidosis (pH of 6.9-7.0) has a protective effect on the cell by inhibiting phosphofructokinase, which limits glycolysis. Therefore it is important that preservation solutions have adequate buffering capacity to reduce acidosis as much as possible (84).

Reactive Oxygen Species

During ischaemia, xanthine dehydrogenase is converted to xanthine oxidase by proteolytic enzymes. Xanthine oxidase is a potent reactive oxygen species (ROS) generator, producing hydrogen peroxide and superoxide anions upon reperfusion. These ROS react rapidly with other molecules, resulting in damage to proteins, lipids and nucleic acids (84, 85).

1.2.2 Wash out of blood

The perfusion of a donor organ also needs to wash blood out of the organ. Red blood cells (RBC) tend to cause aggregates (clumping of RBCs) under low shear forces or at stasis, which is what would happen when the circulation stops in an organ donor. If RBC aggregates form in the small vessels of an organ, such as the capillaries in the peripheral areas of the liver and kidney, the aggregates may lead to inadequate micro vascular perfusion during preservation, meaning the preservation solution cannot penetrate all of the organ leaving some cells unprotected from the effects of ischaemia. This can reduce the structural and functional integrity of the organ.

Having donor RBCs present in the capillaries along with lysed erythrocytes and endothelial cells (due to the cold ischaemia and inadequate micro vascular perfusion of the preservation solution) could lead to a hyper-coagulable state on reperfusion. This can cause intravascular thrombus formation (88). Therefore it is important that the preservation solution used at time of retrieval achieves good cortical perfusion, and that it does so quickly before these RBC aggregates can form.

1.2.3 Properties of a preservation solution

As already described in sections 1.2.1 and 1.2.2, perfusion solutions need to cool the organs, counteract the effects of ischaemia and hypothermia and effectively flush out the blood from the organs. The composition of the preservation solution is thought to be an important factor to prepare an organ so it can tolerate hypothermic storage (89).

Initially, solutions were made to match intracellular conditions with high potassium and low sodium concentrations. This creates equilibrium between the intracellular component and the solution. However, there is now evidence that solutions that match extracellular conditions are equally effective, and that these solutions may improve the wash out of blood due to reduced vasospasm as is seen in the solutions with high potassium concentrations (85, 89).

The preservation solution also needs to have a good buffering capacity to prevent cellular acidosis. Both phosphate and histidine are effective buffers and are therefore used in preservation solutions. Histidine has the added benefit of also being an impermeant (84, 85).

Impermeants are required in a preservation solution to generate an osmotic force outside the cell to reduce the cell swelling induced by hypothermia. In preservation solutions, saccharides and negatively charged anions are used as impermeants. Raffinose is the most effective due to its large size as a saccharide. Sucrose and mannitol are also used. Glucose is a much smaller molecule and no longer used as it will eventually cross the cell membrane and there it can be used as a substrate for anaerobic glycolysis, which will produce lactate. Although mannitol can also cross the cell membrane, it also acts as an oxygen radical scavenger, which is a beneficial effect.

Non-saccharide impermeants are anions, such as citrate, gluconate and lactobionic acid, that reduce cell swelling by producing a negative electrochemical force (85).

Having ROS scavengers, such as Glutathione, in a preservation solution also appears to be desirable. Substances that inhibit ROS producing enzymes are used; such as Allopurinol, which is a xanthine oxidase inhibitor.

The use of colloids in a preservation solution has been debated. Initially they were not used in static cold storage solutions, but were used in solutions intended for machine preservation as the colloid counteracts the extravasation of fluids due to hydrostatic pressure. Colloids were also seen to reduce interstitial oedema formation, and therefore are now added to some preservation solutions, such as University of Wisconsin (UW) solution (hydroxyethyl starch (HES)) and Institute of George Lopez (IGL-1) solution (polyethylene glycol (PEG)), with significant beneficial effects on outcomes (85, 89).

Many components of the preservation solution actually have dual actions, which help prevent injury and to maintain the organ viability. For example, histidine is not only an effective buffer; it also acts as an impermeant, and chelates calcium. Lactobionic acid, which is primarily used as an impermeant, also chelates calcium. Calcium chelators are beneficial as they decrease the uptake of calcium into the cell therefore reducing activation of hydrolytic enzymes (85).

Current preservation solutions contain different combinations and concentrations of the components above, and therefore there may be advantages of one over the other for certain aspects of the preservation period. Choosing the correct solution is important to ensure the best preservation of the organs that are to be transplanted so the outcomes of transplantation are not negatively affected.

1.3 Outcomes following transplantation

Kidney transplants from living donors give the best outcomes in terms of patient and graft survival when compared to outcomes following deceased donor kidney transplantation. This is despite poorer human leucocyte antigen (HLA) matching between the donor and recipient in the living donor situation (90). Therefore it is evident that there are other factors that influence the outcome. Previous studies investigating the outcomes of kidneys from deceased donors have found that donor age, history of hypertension, cerebrovascular cause of death and cold ischaemic time significantly affect both short and long-term outcomes (22, 23). Donor age and the cold ischaemic time (CIT) also affect the initial graft function with older donors and longer CIT being predictive of delayed graft function.

1.3.1 Delayed graft function

Delayed graft function, defined as the requirement of dialysis within the first week following transplantation, is a common complication following kidney transplantation from deceased donors and can affect longer-term outcomes of the kidney especially in DBD kidneys (91, 92).

US data suggests it occurs in 20-29% of recipients following deceased donor kidney transplantation, and although UK data confirms this in recipients of DBD kidneys (with DGF rates of 24%), the rate is much higher in DCD kidneys with up to 50% of recipients experiencing DGF (72, 93). As expected the rates are much lower following living donor kidney transplantation with rates reported to be 3.6% (94). DGF is not only a significant cause of morbidity following transplantation, it also increases the length of hospital stay for the recipient, and also increases the health care costs associated with

transplantation due to the requirement of longer dialysis treatment and more hospital visits (95).

As the rates of DGF differ significantly for the different donor types, it is likely that there are different processes causing the dysfunction. In brain dead donors, as already described, there is an inflammatory and pro-coagulatory response, which initiates up-regulation of cell adhesion molecules. Koo et al looked at biopsies taken 20-40 minutes after reperfusion in kidneys from DBD and living donors and found that there was infiltration of neutrophils and increased P-selectin expression in the kidneys from DBD donors. 31% of these kidneys had DGF, and they also had increased serum creatinine levels at 3 and 6 months post transplant. This would suggest that there is an early inflammatory response against the graft (96).

For kidneys from DCD donors, the pathophysiology behind DGF has been related to the warm ischaemic time, as well as the effects of reperfusion and the ensuing IRI (as described in section 1.2.2).

As well as the pathophysiology of DGF being different for the different donor types, the effects of DGF on longer-term function are also different. In kidneys from DBD donors, DGF is strongly predictive of reduced graft survival and it is a risk factor for reduced graft function at 1 year (16, 20). However, for recipients of kidneys from DCD donors, DGF is not associated with graft survival (20, 91). DGF is a risk factor for both acute and chronic rejection (16, 96). In a study by Wu et al, DGF increased the risk of biopsy proven acute rejection (BPAR) rates at 1-, 3- and 5-years post transplantation (rates were 16%, 21.8% and 22.6% in the DGF group compared to 10.1%, 12.4% and 15.7% in the non-DGF group p=0.01). This study also found that DGF increased the risk for both acute antibody

mediated rejection (highest risk in the first month post transplant) as well as for T-cell mediated rejection (longer term) (97).

1.3.2 Primary non-function

Primary non-function (PNF) is defined as the absence of kidney function immediately following transplantation and that does not recover. In the analysis of UK NHS Blood and Transplant (NHSBT) data, Summers et al found the rates of PNF were the same for kidneys from both DBD and DCD donors at 3% (20). Dahmane et al found that rates were higher when using ECD kidneys when compared to SCD kidneys (7.7% vs. 1.8%) (65). PNF is obviously a very important complication as it leads to graft failure with significant morbidity for the recipient and can increase their sensitisation making future transplantations more problematic.

1.4 Discard of Organs

Despite the organ shortage, deceased donor kidneys are still discarded and this is likely to be due to the increasing number of offers of more older and higher-risk organs and the concerns of how they may function post transplant. With rates of PNF significantly higher with ECD kidneys this decision may be particularly difficult given the current era of more marginal kidneys being offered for transplantation.

The rates of discard are increasing, and this is perhaps not surprising, as ECD donors are becoming more standard practice. In the UK, the rates of deceased donor kidney discard have increased from 5% in 2002/2003 to 12% in 2011/2012. A higher proportion of those discarded are DCD kidneys, with 18% of these kidneys being discarded compared

to 8% of DBD kidneys (98). These rates are similar to European and US rates where 11% of kidneys are discarded across Eurotransplant (ET) and 10% of standard criteria kidneys are discarded in the US. In the US the number of discards increase when using ECD donors with 50% of kidneys from donors over the age of 60 being discarded, 32.5% of kidneys from donors with AKI and 35.2% of kidneys from hypertensive donors being discarded (73, 99, 100).

In the UK the Discarded Kidney Scheme was introduced in 2006 in an attempt to minimise the number of kidneys discarded. Between 2006 and 2012, 200 kidneys were offered through this scheme (which was only 13% of discarded kidneys over this time period) and 77 (39%) of them went on to be transplanted in another centre. The 3-year transplant survival rates of DBD kidneys transplanted after offering through the discard scheme was similar to those that were offered through the standard offering system (98). Callaghan et al then went on to further assess a selection of 20 kidneys that were discarded. The authors only agreed with the decision to discard the kidney in seven of these 20 (35%) cases, and found that at least 20% of the kidneys would have been deemed suitable for transplantation in their centre (98). This study shows the importance of offering kidneys to further centres when one or even more have declined the organ, as other transplant units may have a suitable recipient. As a result of this study NHSBT together with the UK kidney transplant community introduced the new Fast Track Scheme to try and improve transplantation of hard-to-place kidneys. In this scheme, if a certain number of transplant centres (five for DBD kidneys or three for DCD) decline the kidney for either kidney or donor issues, or the organ has accrued six hours of CIT and has not been accepted for transplantation, the kidney is simultaneously offered to centres that have agreed to participate in the Fast Track Scheme. These

centres have 45 minutes to respond to the offer, and the centre with the highest priority patient is allocated the kidney.

NHSBT have emphasised the importance of increasing transplantation rates from deceased donors in their strategy document *'Taking Organ Transplantation to 2020'.* This strategy stresses the importance of developing measures to aid surgeons in making the assessment of the risk-benefit of using an organ for their patient (101).

1.5 Current Strategies to predict outcome

With more kidney donors being classified as higher-risk, the transplant clinician faces the difficult decision of whether to accept the kidney or not for transplantation. As the quality of the donor kidney is an important determinant of the post-transplant outcomes, being able to predict the most likely outcome is important as it allows the clinician to better weigh up the benefit risk ratio for a particular patient, and to then be able to discuss this with patient so they can make an informed decision.

The kidney can currently be assessed using visual assessment of the macroscopic appearance, along with clinical parameters of the donor, although this may identify obviously unsuitable kidneys, it is not enough to determine the organ quality or to predict the outcomes.

Currently, there are donor risk indexes that are based on donor factors available and some centres will also use histological analysis and scoring on pre-implantation biopsies to support the decision-making.

1.5.1 Kidney donor risk index

There are several donor risk indexes published in the literature that clinicians can use to help them in their decision making.

The Deceased Donor Score (DDS) is a score based on five donor factors (age, history of hypertension, creatinine clearance (CrCl), HLA mismatch and cause of death), with each being given a score to give a total result out of 39. The kidney is then allocated to a 'grade' of A-D with kidneys graded as 'A' (score 0-9) having a 53% chance of having an excellent CrCl (defined as CrCl>60ml/min) whereas those graded 'D' (score 30-39) only have an 8% chance of having an excellent CrCl. This study suggests that a cut off score of 20 be used to define marginal grafts with ECD kidneys having a score of greater than 20 (102).

The Donor Risk Score (DRS) grades donors according to the summation of all significant donor risk factors. The Grades I – V are associated with predicted graft survival half lives of 10.7 years for those kidneys graded I and this gradually decreases over the grades to 4.5 years for Grade V kidneys (103).

The most commonly used index in the USA is the Kidney Donor Risk Index (KDRI) (75). This index is based on 10 donor factors (age, ethnicity, serum creatinine, history of hypertension, history of diabetes, cause of death (CVA), height, weight, DCD status or Hepatitis C serology) and provides an estimate of the relative risk of graft failure post transplant compared to a reference donor.

The median graft survivals for the quintiles of the KDRI are as follows:

0.45 < 0.79	*13.6 years*
0.79 < 0.96	*12.6 years*
0.96 < 1.15	*10.6 years*
1.15 < 1.45	*9.2 years*
≥ 1.45	*7.5 years*

The KDRI has been modified by the United Network for Organ Sharing (UNOS) and OPTN to give a kidney donor profile index (KDPI). The KDPI maps the KDRI to a cumulative percentage scale with reference to the population of donors (all US kidney donors from the previous year) to rank the quality of a donor. A donor with a KDPI of 75% means that kidney has a relative risk of graft failure of greater than 75% of the population. Every kidney is given a KDPI, and is thought to be superior to just labelling kidneys as SCD or ECD as it takes into account more donor factors, it is a continuous score and factors in that not all ECDs are alike (104).

Massie et al looked at the outcomes of all patients on the UNOS kidney transplant waiting list between 2002 and 2011, and found that although the relative risk of mortality was increased in the first 30 days following transplantation with a kidney with a KDPI of 71-80, the relative risk of mortality after then reduced compared to those waiting for a kidney from a donor with a KDPI <70. Recipients of kidneys with a KDPI of over 80 have a longer period of increased mortality post transplantation, but this reduces in the long term (105).

Watson et al has analysed outcomes of UK transplant recipients, and has postulated a UK KDRI based on a few donor risk factors including donor age, history of hypertension, donor weight, number of days in hospital and use of adrenaline (23). Again the KDRI is associated with graft survival as follows

KDRI	***1yr survival***	***3yr survival***	***5yr survival***
≤0.87	*92.8%*	*87.6%*	*83%*
0.88-1.02	*89.3%*	*84.7%*	*77.2%*
1.03-1.34	*88.7%*	*83%*	*76.1%*
≥1.35	*88.6%*	*79.7%*	*70%*

Although these indexes can provide some information at the time of organ offer, they do not take other transplant and recipient factors into consideration, and so some clinicians also like to have additional histological information from a donor pre-implantation biopsy.

1.5.2 Donor biopsies

Donor biopsies can give information about the quality of the organ, as well as finding pre-existing changes in the donor kidney. Pre-implantation biopsies can also be used as a baseline to which future biopsies can be compared. This is a tool that is frequently used in the US, with 50% of all deceased donor kidneys having a biopsy taken, and this increases to 85% in ECD kidneys (106). However, in the UK and other countries across Europe, access to histopathology reporting is not as readily available as in the US, and so not nearly as many kidneys are biopsied. In these countries the percentage of

transplants done using ECD kidneys is greater, without a detrimental affect on graft outcomes following transplantation, so perhaps biopsy findings increase the number of organs discarded. In the US 34% of kidneys are discarded based on the biopsy findings (107).

The main findings in the biopsy that are reported are the amount of glomerulosclerosis, tubular atrophy and interstitial fibrosis. Vascular changes are also reported with arterial and arteriolar hyalinosis forming part of the biopsy report.

There have been concerns about using biopsy findings to help in the decision of whether to use a kidney or not for a plethora of reasons.

There is inter-observer differences in the grading of the findings, especially of interstitial fibrosis, and if a non-renal pathologist is reviewing the biopsy the results may not be as accurate as when an experienced renal pathologist reviews it. People also disagree as to whether the size or site of the biopsy matters. Glomerulosclerosis is more prevalent just beneath the capsule and so wedge biopsies may overcall the extent of this in the biopsy. Most importantly, there are conflicting findings in the published literature regarding which histological parameters are most useful in predicting outcomes (108-110).

As most pathologists agree that formalin fixed specimens give the best quality for accurate assessment, this can take 3-5 hours to be processed. Therefore this needs to be taken into account as it may increase the CIT for a kidney, which could have further detrimental effects on an organ where the quality of which is already being questioned.

With less than perfect methods to assess the quality of donor kidneys, it is not surprising that many kidneys are discarded despite the need for them, and it is likely to explain the international and even interregional variations in what kidneys are being used or discarded (107).

Clinicians need as much information as possible about the outcomes of kidneys from varying donor types so that they can make informed decisions to ensure they are accepting a graft of good quality for their patient.

In summary, although there have been many achievements in kidney transplantation leading to better outcomes including lower graft failure and prolonged survival, we are now in an era of transplantation with older and more higher risk donors. The on-going challenges in donation and transplantation now are to be able to accept and transplant these higher risk donor organs, without compromising outcomes. This thesis will concentrate on several important factors that may impact on the viability of the donor organ, including AKI in the donor, the duration of brain death, preservation of organs during the retrieval process and whether pre-implantation biopsies can help predict the outcomes following transplantation.

CHAPTER 2 RATIONALE FOR THIS THESIS

At each stage of the deceased donation process donor organs are subjected to injury. This starts with characteristics of the donor, who may be older or have co-morbidities, and continues through the donor management process of brain death or circulatory death and throughout the actual retrieval procedure. In **Chapter 1** I have outlined the clinical challenges of organ donation and transplantation facing clinicians today. This process of donation, and the injury it induces in donor organs, together with the increasing use of higher risk donor organs leads to uncertainty as to the outcomes following transplantation.

Currently, the tools used to evaluate donor kidneys and to try and predict outcomes are limited with somewhat conflicting evidence for and against them. These strategies may lead to more discards of valuable organs. Therefore, giving clinicians as much information as possible regarding the outcomes of transplantation from these commonly used donors (both standard criteria and more marginal) will allow them to formulate their own risk calculation for the specific organ offer they have, and allow them to give their patients enough information so that together they can make an informed decision about whether to accept the offered organ for transplantation. Once an organ has been accepted for transplantation, the donor moves down the donation pathway to the retrieval process, where the aim is to cool the organs and preserve them to minimise damage to the organs and to maintain their viability.

Therefore, the aim of my DPhil is to evaluate effects that the different stages of the donation process have on the organs whilst ensuring that we can still get good outcomes from the organs that are currently available to us.

As the process of donation is taking longer now than a decade ago, it is important to know whether the hostile environment following brain death, which may induce a

significant inflammatory process, is affecting the outcomes following transplantation. Should organs therefore be removed early from this environment to avoid possible further damage or can we use the period of donor management after brain death to optimise conditions to ameliorate any damage that has been initiated. This is explored in **Chapter 3**. In this chapter the UK transplant registry data is used to investigate whether the duration of brain death, and the systemic effect produced by this, affects transplant survival and kidney function after abdominal organ transplantation.

As already stated, the shortage of donor organs has meant increasing utilisation of more marginal organs kidneys are being offered, and this includes kidneys with acute kidney injury. Much of the current evidence on the outcomes of using these kidneys is mixed, and a lot of the evidence comes from smaller centres outside of the UK, and so the interpretation of these outcomes is limited leading to potentially unjustified discard of these organs. In **Chapter 4** I look at the outcomes of transplantation using kidneys from donors with acute kidney injury in the UK in a retrospective analysis of the UK transplant registry.

Once the organs from a potential donor have been accepted by transplant centres, the donor then moves along the donation pathway. Organ preservation is a crucial part of this process. There is debate about whether adequate flushing and preservation of the entire parenchyma of organs, which is needed for optimal preservation, is achieved with particular preservation solutions.

As the numbers of DCD kidneys being utilised are increasing, it is important to ensure that the organ preservation solutions that are used are sufficient to maintain the

viability of the organs. There is a perception that colloid containing preservation solutions may not penetrate the micro vascular circulation and therefore not adequately flush the blood out of the organ, but there is also evidence that using these solutions give excellent outcomes following transplantation. In **Chapter 5** I describe the DCD large animal model that I have developed and my comparison of several solutions used in clinical practice concerning their perfusion properties. The aim of this model is to assess whether the preservation solution used during the retrieval procedure affects the quality of the organs by looking at specific physiological parameters of the organs during the flush-out procedure, as well as markers of injury and the histological findings.

To try to reduce uncertainty when accepting organs, many transplant centres use donor kidney biopsies to help their clinical decision making about whether to accept or decline a kidney. However, this 'aid' can lead to an increased discard of up to 40%. Biopsies can show chronic changes, acute on chronic changes within the kidney, and therefore in **Chapter 6**, I have performed a systematic review of the literature looking into whether pre-implantation biopsies are actually useful in helping to predict the outcomes following renal transplantation.

In **Chapter 7** I have summarised the main findings of my thesis, how this may affect transplantation from now on, and the future work that may emerge form the work presented here.

In summary the aims of my thesis are as follows:

Aims:

- To investigate whether the duration of brain death affects outcomes following abdominal organ transplantation;

- To look at the use and outcomes of kidneys from donors with acute kidney injury in transplantation;
- To assess the efficacy and quality of flush-out of organs prior to cold storage using commonly used preservation solutions in a large animal DCD model;
- To review the literature as to whether pre-implantation biopsies can predict clinical outcomes following renal transplantation.

CHAPTER 3 DOES THE DURATION OF BRAIN DEATH AFFECT THE OUTCOMES FOLLOWING ABDOMINAL ORGAN TRANSPLANTATION?

3.1 Introduction

Cerebral injury and brain death (BD) initiate in a profound and progressive systemic inflammatory and pro-coagulatory response that leads to a hostile environment in a potential organ donor.

At the same time, however, the stress of BD also induces up-regulation of hypoxia responsive genes that may be able to stimulate protective cellular mechanisms and initiate repair (111), including hypoxia inducible factor (HIF)-dependent up-regulation of genes that code for heat-shock proteins (HSP) and haemoxygenase-1 (HO-1) (112).

Heat shock proteins have been shown to play a protective role in ischaemia-reperfusion injury by repairing damaged proteins, and removing the stimulus for apoptosis. Haemoxygenase-1 has similar properties and also induces vasodilation during the degradation of haem, reducing the effects of vasoconstriction and thus alleviating the pathway for initiating inflammation (113). Van Dullemen et al showed that brain dead rats had increased renal expression of genes coding for HO-1 and HSP-70 when compared to living controls after 4 hours of brain death.

Therefore, it is likely that although injury in donor organs becomes evident during brain death, up-regulation of defence mechanisms also occurs, initiating repair pathways. The consequences of this creates a clinical dilemma of whether to 'rush and retrieve' donor organs as soon as possible after consent, to minimise the effects of the hostile environment, or whether to 'relax' and wait for the up-regulation of in-situ enhancing repair mechanisms, or even to actively optimise the donor with improved donor management and targeted interventions.

There are only limited data available with regards to this issue, which is surprising as the time before retrieval appears to be crucial for the quality of organs on the verge of the

biological challenges of preservation and reperfusion at time of transplantation (114, 115). Nieboer et al from the Groningen group analysed US OPTN data on kidney transplant outcomes, and Kunzendorf et al analysed the outcomes of kidney transplants from Berlin. In these studies the median duration of brain death was 23.8 hours in the US, and only 7.8 hours in Germany, however, both studies found that longer durations of brain death reduced the risk of DGF and improved kidney graft survival. UK transplant outcomes in relation to the duration of brain death have not been analysed before.

3.2 Aims

The aim of this study was to investigate the effect that the duration of brain death has on outcomes following transplantation in the UK. For this, I have looked at immediate and 1-year kidney function, and 1- and 3-year graft survival following kidney, liver and pancreas transplantation in the UK using data from the National Transplant Registry hosted by NHS Blood and Transplant (NHSBT).

3.3 Methods

3.3.1 Study Population

Data for all UK adult donors after brain death who donated at least one transplanted abdominal organ (kidney, liver or pancreas) between 1 January 2008 and 31 December 2012 was requested from NHSBT. The variables requested included donor variables, recipient variables and transplant variables as well as outcome measures.

Donor Variables:

- Sex
- Age

- Body Mass Index (BMI)
- Ethnicity
- Cause of death
- History of hypertension
- History of diabetes
- Duration of brain death
- Use of adrenaline in the donor
- Donor cardiac arrest
- Year of donation
- Whether donor was a cardiothoracic organ donor

Transplant Variables:

- Cold ischaemic time
- HLA Match (for kidney and pancreas dataset)
- ABO match (for liver dataset)
- Transplant type (single or dual kidney, simultaneous pancreas and kidney (SPK) or pancreas alone, whole or split liver)
- Organ appearance (liver dataset)

Recipient Variables:

- Sex
- Age
- Ethnicity
- Cause of organ dysfunction
- Time spent on waiting list

- Recipient in-patient status at time of transplant (liver dataset)
- Clinical ascites in recipient (liver dataset)
- Recipient encephalopathy grade prior to transplant (liver dataset)
- HCV status (liver dataset)
- Oesophageal varices prior to transplant (liver dataset)
- Serum bilirubin (liver dataset)
- Serum creatinine (liver dataset)
- Serum Sodium (liver dataset)
- Serum potassium (liver dataset)
- INR (liver dataset)
- Lifestyle activity score (liver dataset)

Outcomes:

- Transplant survival
- Initial graft function (immediate, delayed, primary non-function)
- Serum creatinine at 3 and 12 months

Recipients were followed up until the earliest of death, graft failure or 16 June 2014.

Brain death duration was defined as the time from BD to retrieval, and was calculated as the difference between the time when fixed and dilated pupils were first noted and the in-situ cold perfusion start time. The time when fixed and dilated pupils was chosen as the time of declaration of brain stem death is known to be later (sometimes much later), than the actual event, and therefore is an unreliable time marker. In the UK, the time of coning, and time that fixed and dilated pupils are noted is also recorded. The time that fixed and dilated pupils was chosen because it was recorded much more often (83%)

than time of coning and it is believed that time of fixed dilated pupils is the closest reliable time against which everything subsequent can be appraised.

3.3.2 Post-transplant endpoints

Kidney

Initial graft function following kidney transplantation was categorised as immediate function (IF), delayed graft function (DGF) or primary non-function (PNF). DGF was defined as at least one dialysis treatment required in the first week after transplantation and PNF is defined as failure of the graft to ever function. Short and long-term kidney function post-transplant was measured using estimated Glomerular Filtration Rate (eGFR), calculated using the 4-variable MDRD equation from serum creatinine values reported at approximately 3-months and 1-year post-transplant. This was then subdivided into four categories (≥ 60, 45-<60, 30-<45 and <30 ml/min/1.73m^2). In order to reduce bias in the distribution of eGFR post-transplant, recipients whose graft had failed by each end-point were included in a further category: 'returned to dialysis'.

Transplant survival at 1- and 3- years was used to measure long-term outcome following kidney transplantation. Transplant survival was defined as time from transplant to graft failure or patient death, censoring for patients who were alive with functioning grafts at the end-point of the analysis.

Liver and Pancreas

Transplant survival at 1- and 3-years was used to measure long-term outcome following liver and pancreas transplantation. Pancreas transplant survival data include, in the

majority of cases, simultaneous pancreas and kidney transplants (SPK), but also a smaller number of pancreas transplants alone (PTA).

Initial graft function and clinical outcomes used to assess longer-term graft function after liver or pancreas transplant are not granular enough when reported to the UK Transplant Registry. Hence, liver and pancreas function post-transplant are not considered in this study.

3.3.3 Statistical analysis

Distribution of BD duration

The distribution of BD duration across donors was described using histogram, median and inter-quartile range. The Kruskal-Wallis test was used to assess any difference in median BD duration across year of donation. Donor characteristics were also summarised for the kidney cohort (as this cohort had the largest number of donors) for four categories (quartiles) of BD duration (0-27.6, 27.6 – 35.7, 35.7 – 48.7 and $\geq$ 48.7 hours). Differences in donor characteristics across BD duration categories were tested univariably using the likelihood ratio test for an ordered logistic regression model, to allow for the ordered nature of the BD duration categories.

Transplant outcome and function

Kaplan-Meier estimates of transplant survival were compared for each type of abdominal organ for four categories of BD duration (categories as before) using the log-rank test.

Regression models were used to investigate the relationship between BD duration and long-term kidney, liver and pancreas transplant outcome (Cox regression), initial kidney

function (logistic regression) and short and long-term kidney function (eGFR as a categorical variable) (multinomial regression). Normal regression analysis was used to further describe the relationship between BD duration and long-term kidney function (eGFR as a continuous variable) for the subset of recipients for whom eGFR was reported. All analyses were adjusted for risk factors pertinent to kidney, liver and pancreas transplantation (23, 116-118), (Table 4).

All analyses were also adjusted for risk factors related to BD duration, namely, whether the donor suffered a cardiac arrest at the time of donation (as a proxy for donor instability) and year of donation.

All statistical analyses were undertaken using SAS/STAT software version 9.4 (SAS Institute Inc., Cary, NC, USA).

3.4 Results

Table 3.1 shows the number of donors, the number of transplants resulting from these donors and full criteria for inclusion in the study, by type of abdominal organ.

Distribution of BD duration

Figure 3.1 shows the distribution of BD duration by year of donation for kidney, liver and pancreas donors.

The median BD duration increases significantly over the study period. Median BD duration was 33 hours (IQR: 25-48 hours) in 2008, steadily increasing to 36 hours (IQR: 27-51 hours) in 2012 ($p=0.03$).

Table 3.2 shows the donor characteristics for the kidney cohort by BD duration category. Donor characteristics were different ($p<0.05$) across the BD duration categories, except for donor sex, BMI, history of diabetes and CIT. In particular, donors with longer BD duration are younger, less likely to have died from intracranial injury or to have a history of hypertension, be white and are more likely to have suffered a cardiac arrest at the time of donation.

Organ	No. of donors[1]	No. of transplants	Criteria for inclusion
Kidney	1881	2815	Adult donor and recipient (aged 18 years or over); first kidney only
Liver	1555	1555	Adult donor (aged 16 years or over) and recipient (aged 17 years or over); elective; first liver only
Pancreas	588	588	Adult donor and recipient (aged 18 years or over); first pancreas only or first simultaneous pancreas and kidney (SPK)
[1] The same donors may be included in the study cohort for kidney, liver *and* pancreas Note: Paediatric definition varies for different organs due to current practice and clinical guidance			

Table 3.1 Criteria for inclusion in the study, by type of abdominal organ

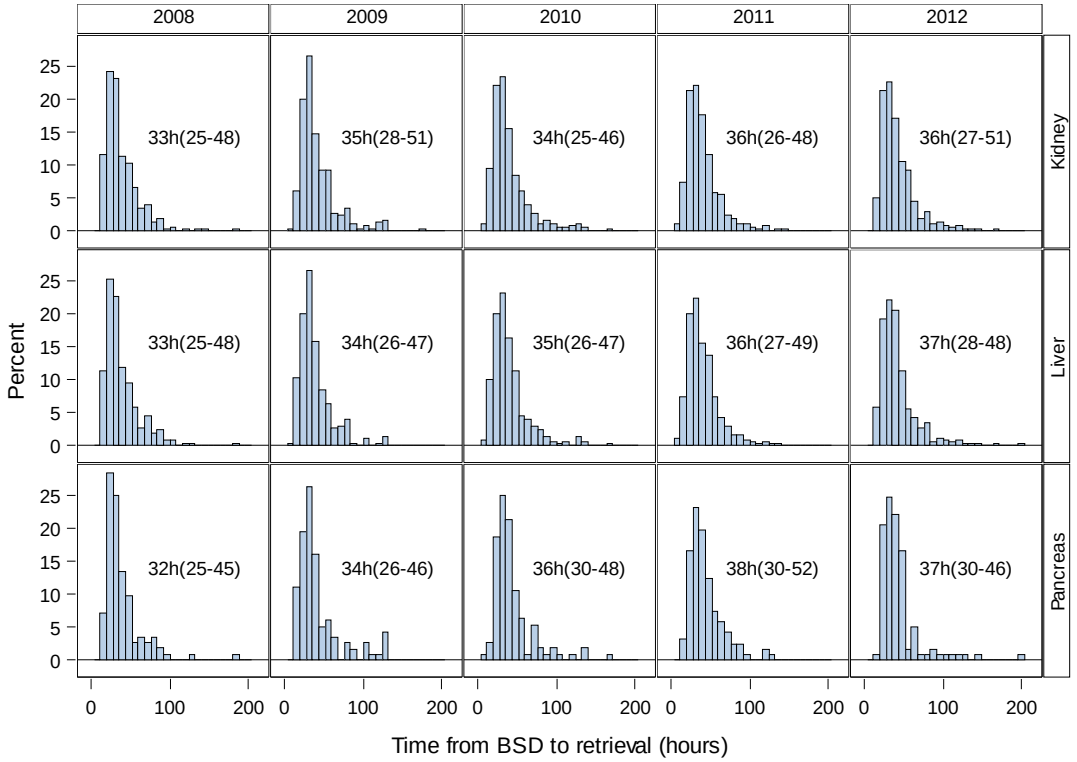


Figure 3.1 Distribution of time from brain death (BD) to retrieval across donors, by year of donation. Median time from BD to retrieval (inter-quartile range) also shown. Kidney: N=1881. Liver: N=1555. Pancreas: N=588.

Factor (not reported)	Level	BD to retrieval (hrs) 0.0-<27.6	27.6-<35.7	35.7-<48.7	≥48.7	P value
Year of donation	2008	103 (19)	71 (16)	58 (14)	70 (15)	
	2009	87 (16)	93 (21)	70 (16)	98 (21)	
	2010	132 (24)	100 (22)	95 (22)	91 (20)	0.03
	2011	112 (21)	91 (20)	102 (24)	94 (20)	
	2012	106 (20)	95 (21)	102 (24)	111 (24)	
Age		53 (14)	49 (14)	48 (14)	47 (15)	<0.0001
Sex	Male	245 (45)	219 (49)	217 (51)	237 (51)	0.05
	Female	295 (55)	231 (51)	210 (49)	227 (49)	
Ethnicity	White	527 (98)	431 (96)	403 (94)	428 (92)	
	Asian	5 (1)	6 (1)	5 (1)	8 (2)	<0.0001
	Black	2 (0)	5 (1)	10 (2)	15 (3)	
	Other	6 (1)	8 (2)	9 (2)	13 (3)	
BMI (4)		27 (5)	27 (5)	27 (5)	27 (5)	0.26
Cause of death	Intracranial injury	429 (79)	328 (73)	304 (71)	258 (56)	
	Trauma	17 (3)	9 (2)	5 (1)	16 (3)	<0.0001
	Other	94 (17)	113 (25)	118 (28)	190 (41)	
Hx hypertension (38)	Yes	177 (33)	100 (23)	120 (29)	104 (23)	<0.0001
Hx diabetes (15)	Yes	32 (6)	20 (4)	30 (7)	35 (8)	0.15
Use of adrenaline (6)	Yes	40 (7)	23 (5)	39 (9)	47 (10)	0.03
Cardiac arrest (30)	Yes	115 (22)	105 (24)	107 (26)	148 (32)	<0.0001
CIT (64)		16 (5)	16 (6)	16 (6)	16 (6)	0.32

Table 3.2 Characteristics of kidney donors by time from brain death to retrieval (quartiles). Presented per donor (N=1881), except CIT, which is presented per transplant (N=2815). Data reported as Number (%) or Mean (SD)

Transplant outcome

The results show that there is evidence that transplant survival of first adult kidney-only transplant increases as BD duration increases (p=0.01), but we found no significant difference in transplant survival across BD duration groups for liver or pancreas (log-rank p=0.47 and p=0.11, respectively) (figures 3.2, 3.3 and 3.4).

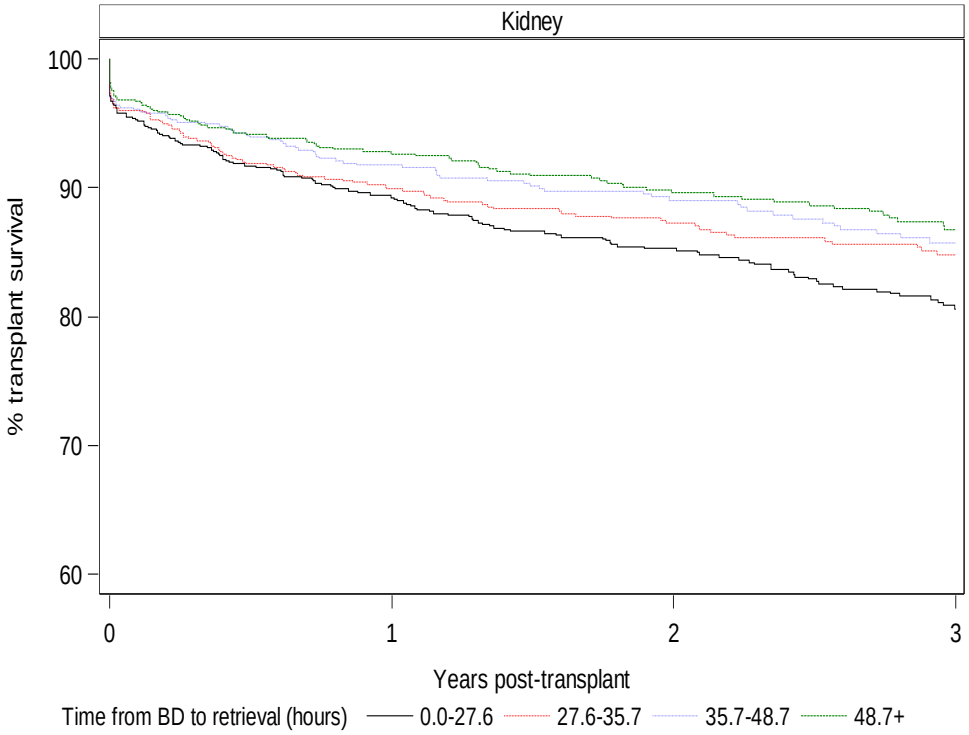


Figure 3.2 Transplant survival after first adult kidney-only transplant by time from BD to retrieval (quartiles)

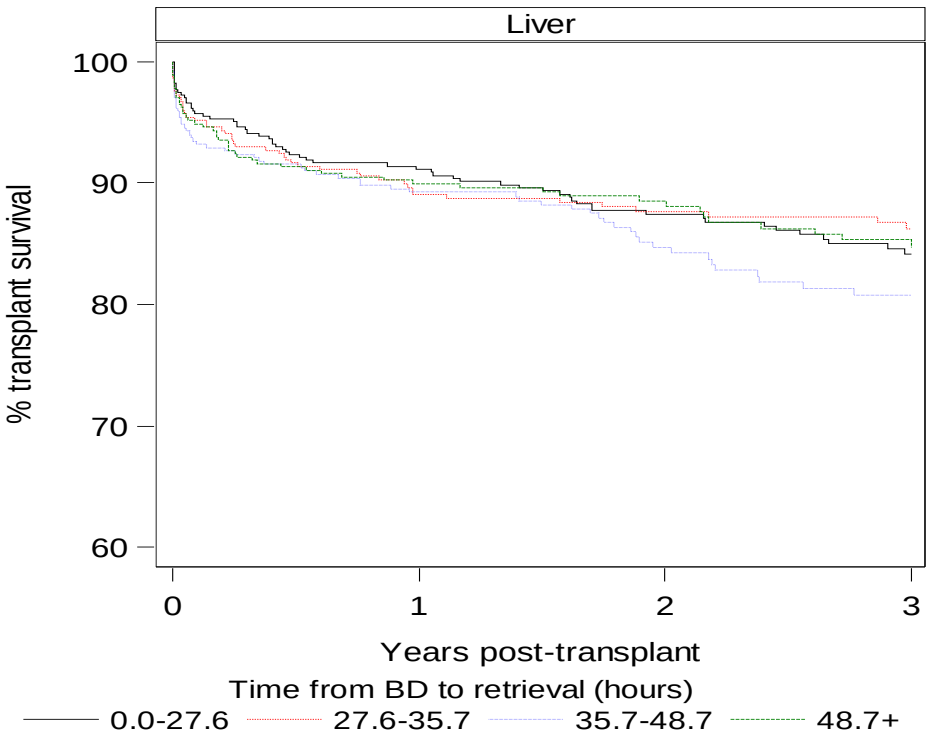


Figure 3.3 Transplant survival after first adult liver-only transplant by time from BD to retrieval (quartiles)

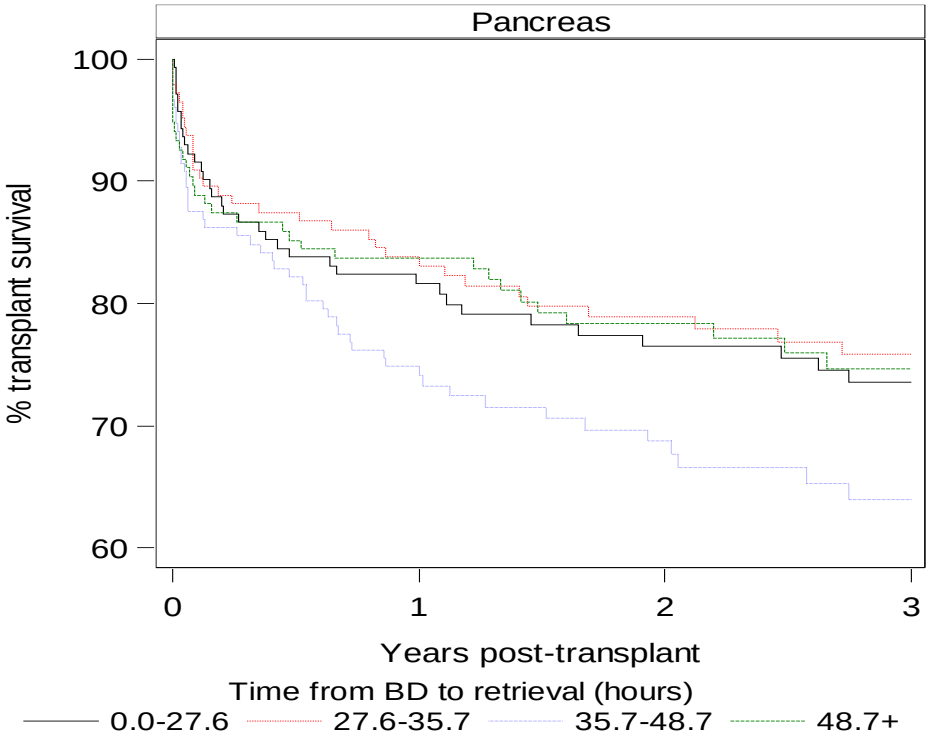


Figure 3.4 Transplant survival after first adult pancreas-only transplant by time from BD to retrieval (quartiles)

Kidney transplantation

Kidneys from 1794 donors were used in the 2655 transplants included in the kidney analysis. Of these donors, 861 (48%) donated kidneys to two different recipients in the cohort. Of the 933 donors who only donated to one recipient in the kidney cohort, 11 were dual kidney transplant donors. The majority (N=802) donated their other kidney for use in an SPK or other multi-organ transplant, for re-grafts or to paediatric recipients. In 120 cases, the other kidney was not available or not used.

Risk adjusted analysis of transplant survival at 1- and 3-year post-kidney transplant suggests evidence of an interaction between BD duration and CIT (p=0.02 and 0.09 respectively), as shown in Table 3.3. The risk of graft survival increases as BD duration increases. This effect is particularly noticeable when CIT is between 18 and 24 hours (hazard ratio for graft failure/death at 1-year post-transplant, for a 12-hour increase in BD duration (HR): 0.73, 95% confidence interval (CI): 0.59 - 0.92). No evidence of any other significant interactions in the kidney models were found.

The risk of DGF increases as BD duration increases (p<0.005), table 3.4. For each 12-hour increase in BD duration, the odds of DGF are 9% greater. There was no evidence of interactions.

There was no evidence of an association between PNF and BD duration (p=0.62) (Table 3.4). Interaction terms were not included in the PNF model due to the very small number of cases of PNF.

Parameter	Interaction	End-point	Transplants (N)	Events (N)	Hazard ratio (95% CI) (12-hour increase)	P value
BD to retrieval (hrs)	CIT < 12 (hrs)	1 year	557	42	1.09 (0.94 - 1.27)	
	12 ≤ CIT < 18 (hrs)		1289	110	0.99 (0.89 - 1.11)	
	18 ≤ CIT < 24 (hrs)		561	55	0.73 (0.59 - 0.92)	0.02
	CIT ≥ 24 (hrs)		248	29	0.91 (0.72 - 1.15)	
BD to retrieval (hrs)	CIT < 12 (hrs)	3 years	557	65	1.03 (0.89 - 1.18)	
	12 ≤ CIT < 18 (hrs)		1289	176	0.98 (0.89 - 1.07)	
	18 ≤ CIT < 24 (hrs)		561	81	0.81 (0.68 - 0.95)	0.09
	CIT ≥ 24 (hrs)		248	40	0.87 (0.71 - 1.06)	
Adjusted for: Recipient - age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity Donor - age, cause of death, CIT, hypertension, weight, use of adrenaline, cardiac arrest, year of donation						

Table 3.3 Cox regression analysis for transplant survival post kidney transplant showing the interaction between brain death duration and CIT (CIT was found to be a significant interaction in the risk adjusted analysis)

Parameter	Immediate function (N)	DGF/PNF (N)	Odds ratio (95% CI) (12-hour increase)	P value
DGF vs. immediate function				
BD to retrieval (hrs)	1883	585	1.09 (1.04-1.15)	<0.005
PNF vs. immediate function				
BD to retrieval (hrs)	1883	57	0.96 (0.81-1.14)	0.62
Adjusted for: Recipient - age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity Donor - age, cause of death, cold ischaemic time, hypertension, weight, use of adrenaline, cardiac arrest, year of donation, whether cardiothoracic organs were donated				

Table 3.4 Logistic regression analysis for chance of DGF/PNF post kidney transplant

Univariable multinomial linear regression analysis of the association between BD duration and recipient kidney function at 90 days and 1 year post-transplant suggested that the odds of being in a better (higher) eGFR category increased as BD duration increased ($p<0.005$ in each case). However, after risk-adjustment, multinomial linear regression analysis suggested no association between BD duration and short- and long-term kidney function ($p=0.84$ and 0.64, respectively) (Table 3.5).

Risk-adjusted normal regression analyses of recipient eGFR (continuous measure) also suggest no association with BD duration ($p=0.74$ and 0.93, at 90 days and 1- year post-transplant respectively) (Table 3.6).

In both categorical and continuous models, risk-adjusted analysis of long-term kidney function suggests evidence of an interaction between BD duration and donor age ($p=0.01$ and 0.03 for categorical and continuous models, respectively). Specifically, for donors aged between 18 and 29 years, for each 12-hour increase in BD duration, the odds of better kidney function (higher eGFR category) were 11% smaller (OR: 0.89, 95% CI: 0.80 – 0.99). This corresponds to a small absolute reduction in eGFR of 0.95 ml/min/1.73m^2 (95% CI: 0.07-1.83). For donors aged between 30 and 39 years, for each 12-hour increase in BD duration, the odds of better kidney function (higher eGFR category) were 14% greater (OR: 1.14, 95% CI: 1.02 – 1.28). This corresponds to a small absolute increase in eGFR of 1.04 ml/min/1.73m^2 (95% CI: 0.05-2.02) (Tables 3.7 and 3.8). No other interactions in the kidney function models were found.

	End-point	eGFR (ml/min/1.73m^2) (N, %)					Odds ratio (95% CI) (12-hour increase)	P value
		≥60	45-60	30-45	<30	Return to Dialysis		
BD to retrieval (hrs)	90 days	648 (27)	686 (28)	674 (28)	314 (13)	109 (5)	1.00 (0.96-1.04)	0.84
BD to retrieval (hrs)	1 year	746 (30)	711 (28)	621 (25)	253 (10)	165 (7)	1.01 (0.97-1.05)	0.64
eGFR: estimated glomerular filtration rate calculated using the 4-variable MDRD equation, in ml/min/1.73m^2. Adjusted for: Recipient - age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity Donor - age, cause of death, cold ischaemic time, hypertension, weight, use of adrenaline, cardiac arrest, year of donation								

Table 3.5 Multinomial regression analysis for eGFR category at 90 days and 1-year post transplant

BD Duration (Hours)	eGFR (ml/min/1.73m^2)	P Value
0 - 27.7	49.35 ± 18.95	0.93
27.7 – 35.7	52.64 ± 19.68	
35.7 – 48.7	53.83 ± 19.76	
≥48.7	53.11 ± 20.52	
Adjusted for: Recipient - age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity Donor - age, cause of death, cold ischaemic time, hypertension, weight, use of adrenaline, cardiac arrest, year of donation		

Table 3.6 Mean eGFR at 1-year post transplant for each brain death duration quartile

Donor age (years)	eGFR (ml/min/1.73m^2)			
	BD duration (hours)			
	0 - 27.7	**27.7 – 35.7**	**35.7 – 48.7**	**≥48.7**
18 - 30	72 (21)	76 (18)	74 (20)	67 (22)
30 - 40	56 (23)	56 (18)	62 (23)	63 (19)
40 - 50	54 (17)	55 (20)	54 (18)	57 (20)
50 - 60	49 (17)	50 (16)	52 (17)	47 (17)
60 or more	42 (16)	41(15)	42 (12)	41 (16)

eGFR: estimated glomerular filtration rate calculated using the 4-variable MDRD equation.

[1] Adjusted for:

Recipient - age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity

Donor - age, cause of death, CIT, hypertension, weight, use of adrenaline, cardiac arrest, year of donation

Table 3.7 Kidney function at 1-year post transplant by BD duration and donor age. Mean, standard deviation (s.d.) shown. N=2336

Parameter	Interaction	Odds ratio (95% CI) (12-hour increase)	P value
BD to retrieval (hrs)	18 ≤ donor age < 30 yrs	0.89 (0.80-0.99)	
	30 ≤ donor age < 40 yrs	1.14 (1.02-1.28)	
	40 ≤ donor age < 50 yrs	1.06 (0.98-1.16)	0.01
	50 ≤ donor age < 60 yrs	0.94 (0.87-1.03)	
	Donor age ≥ 60 yrs	1.04 (0.96-1.13)	

Table 3.8 Multinomial regression analysis of chance of better kidney function at 1-year post transplant for complete kidney case cohort. N=2496

Liver transplantation

For liver transplantation, no evidence of a relationship between 1- and 3-year transplant survival after first liver-only transplant and BD duration was found (p=0.89 and 0.93 respectively), as shown in Table 3.9. No significant interactions in the liver model were found.

Parameter	Interaction	End-point	Transplants (N)	Events (N)	Hazard ratio (95% CI) (12-hour increase)	P value
BD to retrieval (hrs)	N/A	1 year	1439	143	0.99 (0.90 - 1.10)	0.89
BD to retrieval (hrs)	N/A	3 years	1439	203	1.00 (0.92 - 1.08)	0.93
Adjusted for: Recipient - gender, ethnicity, aetiology, HCV status, in patient status, ascites, encephalopathy, renal support, previous abdominal surgery, varices and shunt, life style activity, age, BMI, serum bilirubin, serum creatinine, serum sodium, serum potassium, INR, serum albumin, time on transplant list Donor - graft appearance, CIT, gender, ethnicity, cause of death, history of diabetes, ABO match, graft type, age, BMI, cardiac arrest, year of donation						

Table 3.8 Cox regression analysis for transplant survival post liver transplant (no significant interactions)

Pancreas transplantation

Risk-adjusted analysis of 1- and 3-year transplant survival post-pancreas transplant (*i.e.* not on exogenous insulin), suggests an interaction between BD duration and year of donation ($p<0.005$ and 0.04 respectively) (Table 3.10).

In the later years of the study (2010-2012) the risk of graft failure/recipient death decreases as BD duration increases, whilst in the early years of the study (2008-2009) the risk of graft failure/recipient death increases as BD duration increases. No other significant interactions in the pancreas model were found.

Parameter	Interaction	End-point	Transplants (N)	Events (N)	Hazard ratio (95% CI) (12-hour increase)	P value
BD to retrieval (hrs)	Donation year=2008	1 year	96	17	1.26 (1.10 - 1.45)	<0.005
	Donation year=2009		106	22	1.13 (0.98 - 1.31)	
	Donation year=2010		103	13	0.99 (0.74 - 1.33)	
	Donation year=2011		106	26	0.63 (0.44 - 0.90)	
	Donation year=2012		108	24	1.02 (0.87 - 1.21)	
BD to retrieval (hrs)	Donation year=2008	3 years	96	28	1.21 (1.06 - 1.38)	0.04
	Donation year=2009		106	31	1.07 (0.94 - 1.22)	
	Donation year=2010		103	22	0.93 (0.73 - 1.19)	
	Donation year=2011		106	32	0.79 (0.61 - 1.02)	
	Donation year=2012		108	25	1.01 (0.86 - 1.19)	

[3] Adjusted for:
Recipient - SPK transplant, time on transplant list, age
Donor - age, BMI, CIT, cardiac arrest, year of donation

Table 3.9 Cox regression analysis for transplant survival post pancreas transplant showing interaction between brain death duration and year of donation/transplant (year of donation was identified as significant interaction factor in the risk adjusted analysis)

Discussion

This retrospective analysis of the UK National Transplant Registry shows that increasing durations of BD are not detrimental to graft outcome following abdominal organ transplantation and, in a Cox regression analysis, prolonged brain death duration may be associated with improved graft survival following adult, primary kidney transplantation.

When multivariable analysis was performed using risk factors that are known to have an independent effect on outcome (23, 119-122), CIT was the only variable found to have a significant interaction with BD duration in kidney transplant outcomes. There is some evidence that the chance of transplant graft survival increases as BD duration increases, and this effect is particularly noticeable when CIT is between 18 and 24 hours. This effect is not seen when the CIT is less than 12 hours, and this is likely to be because the beneficial effects of longer BD duration are less important than the known positive effects of a very short CIT.

This finding is consistent with previous analyses, which have shown that longer durations of BD may improve graft survival (114, 115). Conversely to what has been found in this study, both these previous studies demonstrated that as duration of BD increased, the rate of DGF reduced. Although this study has found that as duration of BD increased, the rate of DGF increased; the effect of longer BD durations did not affect the rate of PNF, and the increase in DGF rates did not affect graft function at 1-year.

Although we found an association between BD duration, donor age and kidney function at 1-year, the difference in eGFR was small (0.95ml/min and 1.04ml/min) and unlikely to be of clinical significance. The contradictory results that kidney function is improved

when donors are 30-39, whilst it is reduced when donors are younger than 29 are difficult to explain, and may be due to statistical power, rather than being a true effect. There was no association between the duration of BD and 1- or 3-year survival in both univariate and multivariate analyses for transplant survival following liver transplantation. Although we did not find benefit for survival following liver transplantation, we found no evidence that prolonged BD duration is associated with any adverse outcomes for the liver.

There is no evidence that prolonged BD duration was associated with worse outcomes following pancreas transplantation. Changes in clinical practice and patient mix due to the introduction of the National Organ Retrieval System (NORS) and the Pancreas Allocation Scheme occurred during this period, and multivariable Cox regression analysis showed a significant interaction between year of donation and BD duration. Between 2008-2010 there is some evidence of worse outcomes associated with longer BD duration. However, there is some evidence in recent years (2010-2012) of a protective effect of longer BD duration following pancreas transplantation. Given the continuation of NORS and the Pancreas Allocation Scheme, it is likely that future pancreas transplant outcomes will most closely match the results found in the latter part of the study.

These data are encouraging, as the time for the donation procedure, from donor identification through to the organ retrieval procedure is increasing (Figure 3.1), and the results from this study are reassuring that these longer durations of brain death are not detrimental to outcomes, allowing optimisation of donor management and possibly a beneficial effect of cytoprotective mechanisms responding to the initial systemic

inflammatory response.

It has previously been stated that the unphysiologic state of brain death creates a hostile, pro-inflammatory environment that is detrimental to function and graft survival following transplantation. Prolonged periods of brain death may lead to more unstable donors, and hence donor-care bundle protocols have been introduced to try to alleviate the haemodynamic, metabolic and hormonal instability in BD donors. The goals of these management pathways are to maintain temperature, normovolaemia, and optimise cardiac output to allow adequate perfusion of organs to improve function (46, 47). The introduction of a donor pathway by UNOS lead to a significant increase in the number of organs retrieved and transplanted per donor (123). This pathway included the use of hormone replacement therapy that consisted of cortisol or methylprednisolone, tri-iodothyronine or thyroxine, insulin and antidiuretic hormone.

Echocardiography is useful to assess cardiac function as well as to assess the volume status of a patient. Blood and blood products should be used as necessary, and monitoring serum electrolytes should guide the use of crystalloids with or without bicarbonate. Starch based colloids should be avoided as it can induce renal tubular epithelial injury which can lead to impaired graft function (46, 47). Diabetes insipidus can be managed by administering vasopressin to avoid excessive fluid loss and hypotension. Although vasopressin has not been conclusively shown to improve graft function following transplantation, it may improve graft survival (124, 125).

Targeted suppression of harmful inflammatory pathways has been the goal of many experimental and clinical studies. Pratschke et al administered brain dead rats with either intravenous steroids or a soluble P-selectin glycoprotein ligand (sPSGL) versus controls. The animals that received organs from the treated donors had improved

survival. They postulated that sPSGL inhibits the infiltration of leucocytes and expression of major histocompatibility complex (MHC) class 2 antigens in the kidney reducing cell-mediated damage (41). Clinical studies have also shown a benefit following liver transplantation of methylprednisolone therapy in the donor (126).

As complement activation has an important role in ischaemia reperfusion injury (IRI), Damman et al treated brain dead rats with the complement regulator protein; soluble complement receptor 1 (sCR1) before or after the induction of brain death. They demonstrated that complement activation was significantly decreased with both pre-BD and post BD treatment with sCR1. This targeted intervention lead to improved renal function in rats by protecting the graft from IRI following transplantation (127).

It is likely that careful management of the brain-dead donor in the intensive care is contributing to the lack of a detrimental effect to outcomes following abdominal organ transplantation. The introduction of a cardiothoracic 'Scout' program, which sees members from the cardiothoracic retrieval/transplantation team attend the donor and perform investigations to guide the management of the patient, is also likely to contribute to the overall outcomes following transplantation. This analysis shows that using this period of brain death to manage the donor in this way is not causing a detrimental effect to the abdominal organs, and may actually allow targeted intervention to optimise the quality of donor organs.

More recently there has also been evidence of up-regulation of genes that initiate protective pathways and induce repair mechanisms. Nijboer et al (128) collected kidney biopsy specimens from brain dead donors just prior to organ retrieval and preservation and found that HO-1 and HSP-70 gene expression was increased when compared to

living donor kidney biopsy specimens ($p<0.05$ and $p<0.01$). Kusaka et al also found up-regulation of HO-1, as well as the anti-apoptotic gene Birc2 in kidneys from BD donor rats before and 1 hour after transplantation (129). Heat shock proteins prevent protein denaturation and therefore protect cells from damage, whilst HO-1 catalyses the oxidation of heme molecules thus reducing oxidative stress (128, 130). Redaelli et al found that rat kidney transplants from BD donors with higher HSP and HO-1 expression had improved graft function (131).

Hypoxia inducible factor (HIF) enables aerobic organisms to adapt to hypoxia through the action of transcription of up to 200 genes (including genes that code for HSP and HO-1) that are important for cell metabolism and survival (132). Prolyl-hydroxylase domain (PHD) containing enzymes hydroxylates HIF in normoxic conditions, leading to its ubiquitination and breakdown, but hypoxia inhibits PHD. Therefore targeted therapeutic interventions to inhibit PHDs or stabilise HIF under normoxic conditions may provide strategies for protecting organs against IRI (132).

This study suggests that optimisation of the donor status, and thereby of donor organs, is important and relevant for transplant outcomes. It is in the interest of the donor and donor families that have consented to retrieval for transplantation to provide the best possible donor organs. Collaboration between transplant clinicians and intensive care physicians to determine which targeted interventions in the DBD donor could further enhance the condition and repair of the grafts-to-be is needed, also requiring support from ethics committees and competent authorities.

This study has its limitations. As a retrospective registry review only analysis of the

results of organs that were accepted and transplanted can be done, and are limited by the data collected and recorded. Although it is possible that some donors have short BD durations because they are unstable and need to be rushed to theatre, the analysis did not show a significant effect when taking into account the effect of donors suffering cardiac arrest or requiring inotropic support. Conversely it is possible that donors with longer brain death durations may intentionally be managed for longer to allow donation of cardiothoracic organs. To investigate this, the analyses were adjusted for whether the donor was a cardiothoracic donor and again no association was found with being a cardiothoracic donor and any of the outcomes.

In conclusion, this analysis shows that longer brain death duration does not exacerbate brain death related injury, and may actually have a beneficial effect for kidney graft survival, especially after prolonged CIT, whilst having no detrimental effect on liver or pancreas graft survival. This finding supports that the period of brain death followed by intense donor management in the intensive care unit may be used to adequately optimise organ donors (abdominal and cardiothoracic) and suggests a window of opportunity for in-situ organ conditioning with targeted intervention. This may particularly be of value as more older and higher-risk donors are being accepted for transplantation to enhance organ utilisation. The extra time may also be used to ensure that organs are allocated to appropriate recipients. Our analysis renounces the need for a 'rush and retrieve' policy and suggests that the DBD environment may be less 'hostile' and more 'cytoprotective' than previously perceived.

CHAPTER 4 THE USE OF KIDNEYS FROM DONORS WITH ACUTE KIDNEY INJURY IN TRANSPLANTATION

Introduction

Despite the persistent donor shortage and gap between supply and demand in solid organ transplantation, most transplant centres in many countries will decline donor kidneys from donors with an acute kidney injury (AKI) at time of offering. The perception is that the condition of AKI is not compatible with successful transplantation as long-term function and survival will be significantly inferior rendering the recipient at risk. As a result, this uncertainty leads to the frequent discard of many potentially viable donor kidneys (77).

In the UK, DBD kidneys are offered via a National Allocation System and DCD kidneys from donors younger than 50 years of age are offered regionally with one kidney being offered locally, and if the donor is over 50 years old both kidneys are offered locally (133). This allocation policy is not altered when ECD kidneys, or kidneys with AKI are offered.

To date, several classifications for AKI have been identified and validated. In 2004, the Acute Dialysis Quality Initiative (ADQI) group developed the "RIFLE" criteria, referring to Risk, Injury and Failure with the two outcome classes, Loss and End-Stage Renal Disease (ESRD). The three severity grades Risk, Injury and Failure are defined on the basis of changes in serum creatinine (SCr) or urine output (UO) where the worst of each criterion is used. The two outcome criteria, Loss and ESRD, are defined by duration of loss of kidney function (134). In 2007, a multidisciplinary collaborative network, the Acute Kidney Injury Network (AKIN) endorsed the RIFLE criteria with a small modification to include small changes in Serum Creatinine (SCr) (≥ 0.3mg/dl or ≥26.4µmol/l) when they

occur within a 48-hour period (Table 4.1) (76), and this classification was confirmed in 2011 by KDIGO ("Kidney Disease: Improving Global Outcomes") in its Clinical Practice Guideline for AKI (135).

As it has been suggested by some authors that kidneys from donors with AKI may recover (81-83), the question is clinically highly relevant whether discard of these kidneys is justified against the background of the persistent donor kidney shortage.

AKI Stage	Serum Creatinine	Urine Output
Stage 1	Increase of ≥0.3mg/dL (26.4µmol/L) or increase to ≥150%-200% (1.5 to 2.0 fold) from baseline	<0.5 ml/kg/hr for 6-12 hours
Stage 2	Increase to >200% to 300% (2.0 to 3.0 fold) from baseline	<0.5 ml/kg/hr for ≥12 hours
Stage 3	Increase of ≥4.0mg/dL (≥354µmol/L) or increase to >300% (3.0 fold) from baseline	<0.3 ml/kg/hr for ≥24 hours or anuria for ≥12 hours

Table 4.1 The AKIN criteria

Aims

The aim of this study was to investigate the use of kidneys from donors with AKI, and to investigate what effect donor AKI has on the outcomes following kidney transplantation in a large UK cohort using the National Transplant Registry hosted by NHS Blood and Transplant (NHSBT) and to see whether the current discard of these kidneys is justified.

Methods

Study population

Data for all UK adult deceased donors where at least one kidney was offered for transplantation between 1st January 2003 and 31st December 2013 was requested from NHSBT. The variables requested included donor variables, recipient variables and transplant variables as well as outcome measures.

Donor Variables:

- Sex
- Age
- BMI
- Ethnicity
- Cause of death
- History of hypertension
- History of diabetes
- Creatinine on admission
- Creatinine at retrieval
- Use of adrenaline in the donor

- Donor cardiac arrest

Transplant Variables:

- Cold ischaemic time
- HLA mis-match
- Transplant type (single or dual kidney)

Recipient Variables:

- Sex
- Age
- Time on transplant list
- Primary renal disease
- Ethnicity
- Sensitisation

Outcomes:

- Transplant survival
- Initial graft function (immediate, delayed, primary non-function)
- Serum creatinine at 3 and 12 months

Change in serum creatinine between admission and retrieval, and urine output were used to classify each donor as AKI stage 1, 2 or 3 (135). Donors that did not meet the AKIN criteria were classified as 'no AKI'. Donors without sufficient data to allow classification were excluded from all analyses. Offered kidneys used in first adult kidney-only transplants were included in post-transplant analyses.

Post-transplant endpoints

Transplant survival at 90-days and 1-year was used to measure short- and long-term outcome. This was defined as time from transplant to graft failure or patient death, censoring for patients who were alive with a functioning graft at the end-point of the analysis.

Initial graft function was categorised as immediate function (IF), delayed graft function (DGF) or primary non-function (PNF). DGF was defined as at least one dialysis treatment required in the first week after transplantation. Kidney function post transplantation was measured using four categories of estimated glomerular filtration rate (eGFR) (≥60, 45-60, 30-45 and <30 ml/min/1.73m^2). This was calculated using the 4-variable MDRD equation from serum creatinine values reported at approximately 90 days and 1-year post transplantation. In order to reduce bias in the distribution of eGFR post-transplant, recipients whose graft had failed by each endpoint were included in a further category: 'returned to dialysis'.

Statistical analysis

Donor demographic characteristics (cause of death, age, BMI, ethnicity, sex and DBD or DCD) were summarised by offering outcome for No AKI versus AKI. For kidneys which were not transplanted; the reason for non-use was summarised by offering outcome and AKI stage.

For kidneys that were transplanted, recipient demographic characteristics (age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity and sensitisation) were summarised for No AKI versus AKI. Differences in donor and recipient characteristics, for No AKI versus AKI were tested using the likelihood ratio test for a

logistic regression model. For donor characteristics, additional adjustments were made for offering outcome or within each offering stage if significant evidence of an interaction. Differences in the proportion of kidneys not used due to poor function versus any other reason, across AKI stages, adjusted for offering outcome as before, were tested using the likelihood ratio test for a logistic regression model.

Kaplan-Meier estimates of transplant survival were compared across AKI stages using the log-rank test. Regression analyses were used to investigate the relationship between AKI stage and; short- and long-term kidney transplant outcome (Cox model); initial graft function (logistic model); short- and long-term kidney function post-transplant (eGFR as a categorical variable: ordinal logistic model; eGFR as a continuous variable: normal model).

Analyses were adjusted for risk factors pertinent to kidney transplantation (23, 117). Donor risk factors included age, history of hypertension, BMI, length of hospital stay, use of adrenaline, donor type (DBD or DCD), sex, ethnicity, blood group and cause of death. Transplant and recipient risk factors included cold ischaemic time (CIT), age, time on transplant list, primary renal disease, HLA mis-match level, ethnicity, sensitisation and DGF. Sub-group analyses were considered in each model by including interactions between donor AKI stage and donor age, type, BMI, sex, history of hypertension, cause of death, CIT and recipient age. Interaction terms were only retained in each model if the likelihood ratio test was significant at the 5% level.

Analyses were undertaken using SAS/STAT, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The study cohort from the time period 2003 to 2013 consisted of 11,649 donors. The majority of donors (79%) could be categorised by AKI stage based on both serum creatinine and urine output. 15 % of donors were categorised based on change in serum creatinine or urine output and 686 (5.9%) donors were excluded for insufficient data. Demographic characteristics of all 11,649 donors, by offering outcome and No AKI versus AKI are shown in Table 4.2.

Factor (p-value)	Level	Offered, not accepted			Accepted, not transplanted			Transplanted		
		Total	AKI Stage		Total	AKI Stage		Total	AKI Stage	
			No AKI	AKI		No AKI	AKI		No AKI	AKI
TOTAL		**2361 (100)**	**1627 (69)**	**510 (22)**	**571 (100)**	**389 (68)**	**139 (24)**	**8717 (100)**	**6937 (80)**	**1361 (16)**
Cause of death (<0.005)	Intracr. Injury	**1154 (49)**	843 (52)	177 (35)	**326 (57)**	238 (61)	62 (45)	**5522 (63)**	4548 (66)	716 (53)
	Trauma	**21 (1)**	14 (1)	2 (0)	**3 (1)**	0 (0)	2 (1)	**339 (4)**	266 (4)	45 (3)
	Other	**1186 (50)**	770 (47)	331 (65)	**242 (42)**	151 (39)	75 (54)	**2856 (33)**	2123 (31)	600 (44)
Age (0.32)	18 to 29	**98 (4)**	69 (4)	19 (4)	**13 (2)**	7 (2)	5 (4)	**1108 (13)**	872 (13)	177 (13)
	30 to 39	**170 (7)**	118 (7)	35 (7)	**35 (6)**	25 (6)	8 (6)	**1178 (14)**	958 (14)	169 (12)
	40 to 49	**345 (15)**	229 (14)	91 (18)	**81 (14)**	58 (15)	14 (10)	**2009 (23)**	1588 (23)	329 (24)
	50 to 59	**535 (23)**	370 (23)	114 (22)	**129 (23)**	87 (22)	30 (22)	**2280 (26)**	1832 (26)	346 (25)
	60 or older	**1213 (51)**	841 (52)	251 (49)	**313 (55)**	212 (54)	82 (59)	**2142 (25)**	1687 (24)	340 (25)
BMI (<0.005)	<18.5	**67 (3)**	50 (3)	8 (2)	**6 (1)**	4 (1)	1 (1)	**179 (2)**	150 (2)	24 (2)
	18.5 to <25	**730 (33)**	552 (35)	119 ((24)	**155 (28)**	116 (30)	31 (23)	**3462 (41)**	2880 (42)	463 (35)
	25 to <30	**841 (37)**	602 (38)	172 (35)	**228 (42)**	161 (42)	53 (40)	**3237 (38)**	2579 (38)	522 (39)
	≥30	**608 (27)**	384 (24)	190 (39)	**155 (28)**	100 (26)	49 (37)	**1616 (19)**	1222 (18)	332 (25)
Ethnicity (0.34)	White	**2268 (96)**	1560 (96)	488 (96)	**543 (95)**	370 (95)	132 (95)	**8298 (95)**	6608 (95)	1282 (94)
	Asian	**40 (2)**	28 (2)	11 (2)	**11 (2)**	7 (2)	3 (2)	**135 (2)**	107 (2)	27 (2)
	Black	**19 (1)**	15 (1)	3 (1)	**7 (1)**	4 (1)	2 (1)	**89 (1)**	69 (1)	17 (1)
	Other	**34 1)**	24 (1)	8 (2)	**10 (2)**	8 (2)	2 (1)	**195 (2)**	153 (2)	35 (3)
Sex (<0.005)	Male	**1421 (60)**	947 (58)	339 (66)	**357 (63)**	242 (62)	89 (64)	**4571 (52)**	3456 (50)	870 (64)
	Female	**940 (40)**	680 (42)	171 (34)	**214 (37)**	147 (38)	50 (36)	**4142 (48)**	3477 (50)	491 (36)
Type (<0.005)	DBD	**384 (16)**	237 (15)	138 (27)	**199 (35)**	143 (37)	52 (37)	**6209 (71)**	5190 (75)	936 (69)
	DCD	**1977 (84)**	1390 (85)	272 (73)	**372 (65)**	246 (63)	87 (63)	**2508 (29)**	1747 (25)	425 (31)

Data are reported as N (%). Row percentage reported for total row; column percentage reported otherwise. Ordinal logistic regression used to test for differences in each donor characteristic across AKI Stage, adjusted for offering outcome, or within each offering outcome if significant evidence of interaction (donor type only).

Table 4.2 Donor characteristics by offering outcome and AKI stage where at least one kidney was offered for transplantation (N=11,649)

The proportion of donors with AKI was different across the three offering outcomes ($p<0.005$). Transplanted donors are more likely to be classified as no AKI than donors whose kidneys are offered but not accepted. There was a difference in the proportion of donors with AKI for all donor characteristics ($p<0.005$ in each case) except donor age ($p=0.34$) and ethnicity ($p=0.31$).

There was no evidence that the relationship between each donor characteristic and AKI was different for each offering outcome, except for donor type. Of donors whose kidneys were offered but not accepted, DBD donors are more likely to be classified as AKI than DCD donors, whilst of donors whose kidneys were transplanted, DCD donors are more likely to be classified as AKI than DBD donors.

Offering Outcomes

Overall, 16,735 (72%) of 23,204 offered kidneys from the 11,649 donors were transplanted and 6,469 (28%) kidneys from 3,563 donors were not used (*Figure 4.1*).

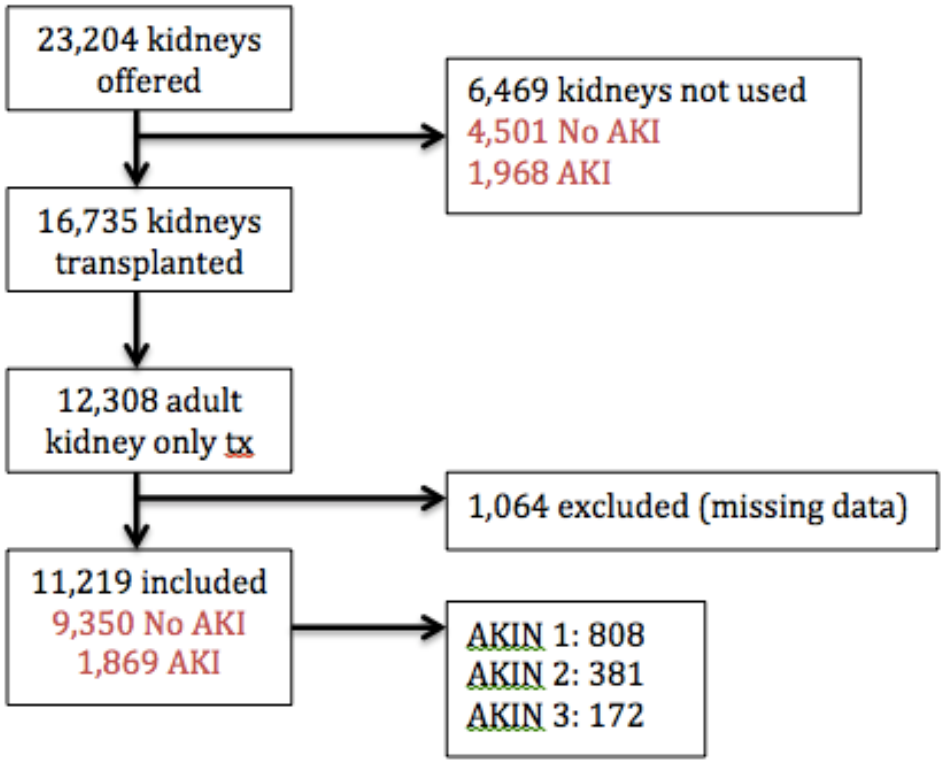


Figure 4.1 Flowchart of offering outcomes

Out of the whole cohort (11,649 donors), where both kidneys were offered, the offering outcome differed for each kidney in 653 (6%) of cases. In the majority (631, 97%) of these 653 cases one kidney was transplanted.

The proportion of kidneys not used due to poor function was significantly different for donors at the different AKI stages and this association was different at each stage of offer ($p<0.005$) (Table 4.3).

For kidneys that were not accepted, the odds of non-use due to poor function were 4 to 5 times as great for donors at AKIN stages 1 or 2, and more than 20 times as great for donors at stage 3, compared with donors with no AKI (Odds Ratio (OR): 5.01 (3.71-6.77); OR: 4.36 (3.19-5.97); OR: 20.53 (15.43-27.33) for stages 1, 2 and 3, respectively). For kidneys that were accepted but not transplanted, the odds of non-use due to poor function were about the same for donors at stage 1 (OR: 1.42 (0.70-2.86)) and about 2.5 times as great for donors at stages 2 or 3, compared with donors with no AKI (OR: 2.46 (1.29-4.68); OR: 2.52 (1.19-5.33), for stages 2 and 3, respectively).

Reason (p-value)	Offered, not accepted					Accepted, not transplanted				
	Total	AKI Stage				Total	AKI Stage			
		No AKI	1	2	3		No AKI	1	2	3
Poor function (<0.005)	456 (9)	152 (4)	74 (19)	64 (17)	135 (49)	92 (6)	50 (5)	10 (7)	13 (11)	9 (11)
Donor unsuitable – medical	576 (12)	384 (11)	74 (19)	47 (12)	23 (8)	283 (19)	185 (18)	34 (23)	31 (26)	21 (26)
Organ unsuitable – clinical	762 (15)	436 (13)	87 (22)	76 (20)	68 (24)	461 (31)	330 (32)	42 (28)	40 (34)	24 (30)
Donor unsuitable – non medical	2743 (55)	2198 (64)	125 (32)	168 (44)	33 (12)	95 (6)	88 (8)	0 (0)	3 (3)	0 (0)
Donor age	90 (2)	57 (2)	9 (2)	6 (2)	6 (2)	10 (1)	2 (0)	4 (3)	2 (2)	2 (3)
Other	83 (2)	45 (1)	2 (1)	4 (1)	2 (1)	381 (26)	279 (27)	40 (27)	25 (21)	16 (20)
Not reported	287 (6)	186 (5)	24 (6)	18 (5)	11 (4)	150 (10)	109 (10)	20 (13)	4 (3)	8 (10)

Data are reported as N (%), where % are column percentages. Logistic regression used to test for differences in proportion not used due to poor function vs. any other reason across AKI Stage, within each offering outcome.

Table 4.3 Reasons for non-use of kidney, by offering outcome and AKI stage (N= 6,469)

Transplanted kidneys

Of the 16,735 transplanted kidneys, 12,540 (75%) were used in 12,308 first adult kidney-only transplants (12,076 single and 232 dual kidney transplants). Of these 12,308 transplants, 1146 (9%) were pre-emptive transplants. Characteristics of the recipients of all 12,308 transplants are shown in Table 4.4.

There was no difference in recipient groups with and without AKI with respect to age ($p=0.39$), time on the transplant list ($p=0.14$), primary renal disease ($p=0.72$), ethnicity ($p=0.34$) and sensitisation ($p=0.12$), although level of HLA mis-match was higher for recipients of AKI donor transplants ($p<0.005$).

Of these 12,308 transplants, 1,064 (8.6%) were excluded from further analyses due to either missing data, 25 (0.2%) or inadequate follow-up data. Therefore, 11,219 transplants were considered for inclusion in the analyses of post-transplant outcome and kidney function. 897 (8%) had no initial graft function data and were excluded from the DGF/PNF analysis and 985 and 692 had no serum creatinine reported at 90 days and 1 year post-transplant and were excluded from kidney function analyses.

Factor	Level	AKI Stage		P Value
		No AKI	AKI	
Age	18 to 29	721 (7)	135 (7)	**0.39**
	30 to 39	1330 (14)	250 (13)	
	40 to 49	2294 (24)	443 (23)	
	50 to 59	2586 (27)	530 (27)	
	≥ 60	2773 (29)	593 (30)	
Wait Time	<6 months	1301 (13)	235 (12)	**0.14**
	6m - <2yr	3068 (32)	602 (31)	
	≥2yr	5335 (55)	1114 (57)	
Ethnicity	White	7591 (78)	1496 (77)	**0.34**
	Asian	1202 (12)	255 (13)	
	Black	656 (7)	151 (8)	
	Other	255 (3)	49 (3)	
HLA mm	1	1231 (13)	203 (10)	**0.003**
	2	3150 (32)	600 (31)	
	3	4191 (43)	893 (46)	
	4	1132 (12)	255 (13)	
Sensitisation	0 - 9	7829 (81)	1587 (81)	**0.12**
	10 - 39	669 (7)	149 (8)	
	40 - 84	872 (9)	146 (7)	
	≥ 85	334 (3)	69 (4)	

Data are reported as N (%).

Table 4.4 Recipient characteristics for kidneys with and without AKI

Initial graft function

The risk of DGF and PNF increase with donor AKI stage ($p<0.005$ and 0.04, respectively) (Table 4.5). The risk of DGF is similar when the donor is classified as AKI stage 1 or 2 (OR: 1.33 (95% CI: 1.15 – 1.54) and 1.67 (95% CI: 1.37 – 2.03), respectively) when compared with donors with no AKI. However, where the donor is classified as AKI stage 3, the risk of DGF is about three times greater, compared with donors with no AKI (OR: 2.98 (95% CI: 2.25 – 3.96)). Although there is no evidence of an increased chance of PNF for donors classified as stage 1 or 2, the rates of PNF are about three times greater for donors classified as stage 3, compared with donors with no AKI (OR: 3.09 (95% CI: 1.54 – 6.16)). There are no significant interaction terms.

Factor		Odds ratio (95% CI)	p-value
DGF vs. immediate function			
1. Donor AKI stage	%DGF		<0.005
No AKI	28	1.00(-)	
Stage 1	35	1.33 (1.15-1.54)	
Stage 2	43	1.67 (1.37-2.03)	
Stage 3	55	2.98 (2.25-3.96)	
2. AKI stage by donor type			0.52
3. AKI stage by donor age			0.32
4. AKI stage by donor BMI			0.30
5. AKI stage by recipient age			0.73
6. AKI stage by CIT			0.57
7. AKI by donor sex			0.29
8. AKI by donor hx hypertension			0.36
9. AKI by donor cause of death			0.70
PNF vs. immediate function			
1. Donor AKI stage	%PNF		0.04
No AKI	4	1.00 (-)	
Stage 1	4	0.92 (0.59-1.42)	
Stage 2	3	0.90 (0.46-1.73)	
Stage 3	9	3.09 (1.54-6.16)	

Odds ratio reported for models where effect/interaction significantly associated with outcome. Interaction models also include main effects. No interaction models for PNF outcome due to small number of cases of PNF in AKI donor group.

All models adjusted for the following risk factors:

Recipient – age, time on transplant list, primary renal disease, HLA mis-match, ethnicity, sensitisation, CIT

Donor – age, type, BMI, sex, history of hypertension, length of stay in hospital, use of adrenaline, ethnicity, blood group, cause of death

Table 4.5 Logistic regression models for chance of DGF/PNF after first adult kidney-only transplant

Transplant survival

Kaplan-Meier estimates of transplant survival after first adult kidney-only transplant by donor AKI stage suggests some evidence that the chance of graft failure/recipient death after first adult kidney-only transplant differs across AKI stage (p=0.07) (*Figure 4.2a*). Due to the small numbers of events (<30) for some AKI stages, donors with and without AKI were compared, which found that the chance of graft failure/recipient death after first adult kidney-only transplant is greater for donors with AKI than for those with no AKI (p=0.02) (*Figure 4.2b*).

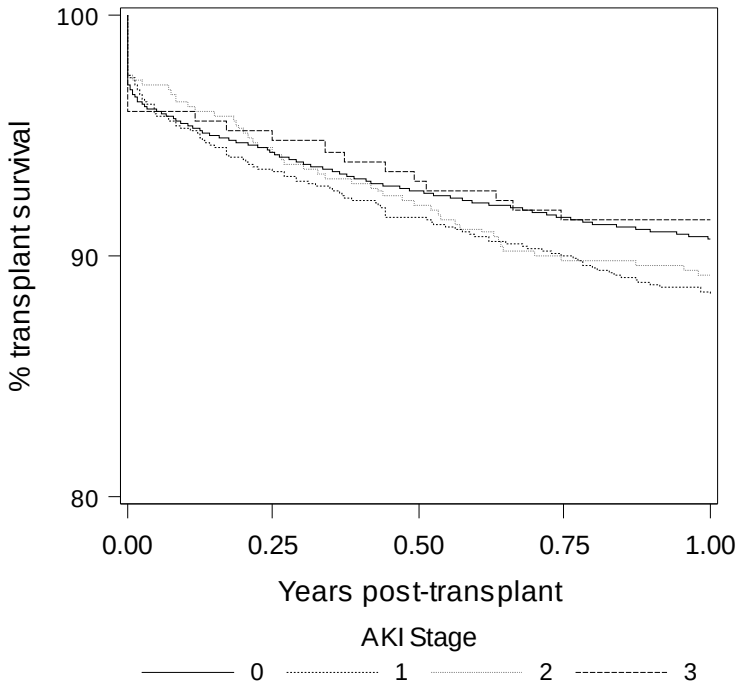


Figure 4.2a Transplant Survival after kidney-only transplant by donor AKI stage

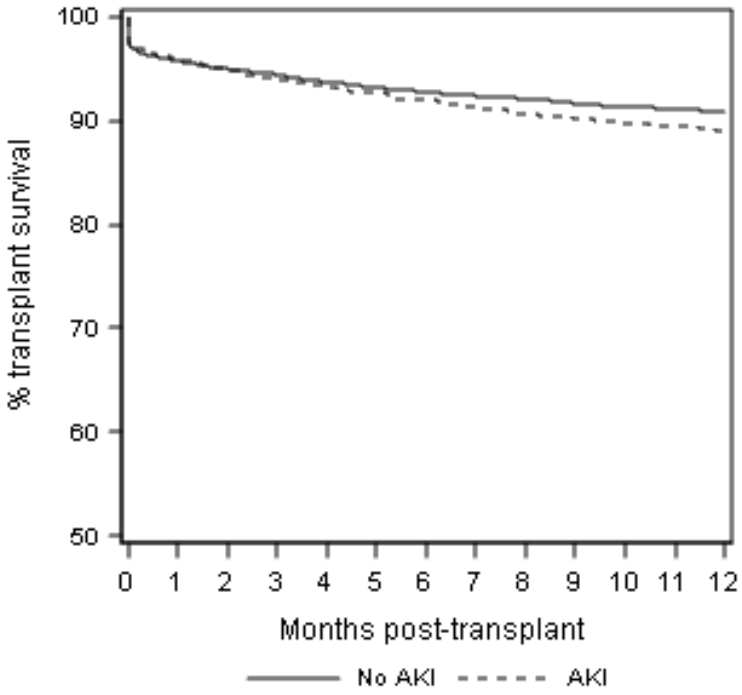


Figure 4.2b Transplant Survival after first adult kidney-only transplant compared for donors with and without AKI (N= 1869 and 9350 respectively). p=0.02

After risk-adjustment, there was no evidence of an association between transplant survival and donor AKI within 90-days post-transplant (p=0.24), but there was an association within 1-year post-transplant (p=0.02) (Table 4.6). The risk of graft failure/recipient death within 1-year is 20% greater (95% CI: 1.03-1.41) when the donor is classified as having an AKI (either AKIN stage 1, 2 or 3), compared with donors with no AKI. There are no significant interaction terms.

Model	Hazard ratio (95% CI)	p-value
90 days post-transplant (events/transplants: No AKI – 521/9350; AKI – 113/1869)		
1. Donor with/without AKI		0.24
2. AKI by donor type		0.76
3. AKI by donor age		0.58
4. AKI by donor BMI		0.58
5. AKI by recipient age		0.84
6. AKI by CIT		0.18
7. AKI by donor sex		0.20
8. AKI by donor hx hypertension		0.92
9. AKI by donor cause of death		0.59
1 year post-transplant (events/transplants: No AKI - 848/9350; AKI - 202/1869)		
1. Donor with/without AKI		0.02
No AKI	1.00 (-)	
AKI stage 1, 2 or 3	1.20 (1.03-1.41)	
2. AKI by donor type		0.42
3. AKI by donor age		0.20
4. AKI by donor BMI		0.11
5. AKI by recipient age		0.64
6. AKI by CIT		0.38
7. AKI by donor sex		0.54
8. AKI by donor hx hypertension		0.58
9. AKI by donor cause of death		0.71

Hazard ratio reported for models where effect/interaction significantly associated with outcome. Interaction models also include main effects.

All models adjusted for the following risk factors:

Recipient – age, time on transplant list, primary renal disease, HLA mis-match, ethnicity, sensitisation, CIT, DGF

Donor – age, type, BMI, sex, history of hypertension, length of stay in hospital, use of adrenaline, ethnicity, blood group, cause of death

Table 4.6 Cox regression model for chance of graft failure/recipient death within 90-days and 1-yer after adult first kidney-only transplant

Longer-term graft survival was also investigated, and although long-term follow-up is only available for about half the cohort, there remains a significant difference in long-term survival for AKI vs. no AKI with 3-year survival being 83% vs 85% for kidneys from donors with and without AKI, and 5 year survival being 76% vs 78% (Table 4.7).

	No AKI	AKI (stage 1-3)	p Value
1 year	91%	89%	0.02
3 year	85%	83%	0.02
5 year	78%	76%	0.009

Table 4.7 1, 3 and 5-year graft survival after adult first kidney-only transplant

Kidney Function

The chances of being in a lower eGFR category are between 20% and 30% greater when the donor is classified as AKI stage 1, 2 or 3, although odds ratios are not significantly greater than 1 for all of the AKI stages 1, 2 and 3. This may be due to the small numbers of transplants in the worse eGFR categories for some AKI stages. Therefore donors with and without AKI were compared and this found that the risk of being in a worse eGFR category are 19% (95% CI: 1.08 – 1.31) and 25% (95% CI: 1.14 – 1.38) greater at 90 days and 1-year post transplant, respectively for donors with AKI compared with donors with no AKI ($p<0.005$ in each case) (Table 4.8 and 4.9). There are no significant interaction terms. Similar results were obtained when eGFR was treated as a continuous variable. Donors classified as AKI stage 1, 2 and 3 have significantly lower eGFR at 90-days and 1-year than donors with no AKI ($p=0.01$ and $p<0.005$ at 90-days and 1-year, respectively), although the absolute reduction in eGFR is small (reduction of 1.1 mL/min/1.73m^2 (95% CI: 0.2-2.0) and 1.6 ml/min/1.73m^2 (95% CI: 0.7-2.5) at 90-days and 1-year, respectively).

End Point	eGFR mL/min/1.73m^2					p-value
	>60	45-60	30-45	<30	Return to Dialysis	
90 days						**<0.005**
No AKI	1953 (23%)	2465 (29%)	2542 (30%)	1171 (14%)	425 (5%)	
AKI	363 (21%)	447 (26%)	530 (31%)	279 (16%)	84 (5%)	
1 year						**<0.005**
No AKI	1951 (22%)	2447 (28%)	2915 (33%)	879 (10%)	618 (7%)	
AKI	342 (20%)	445 (26%)	614 (35%)	210 (12%)	131 (8%)	

Table 4.8 Recipient eGFR at 90-days and 1-year post adult first kidney-only transplant

Factor	Odds ratio (95% CI)	p-value
90 days post-transplant (eGFR ≥ 60, 45-<60, 30-<45, < 30, returned to dialysis)		
1. Donor with/without AKI		<0.005
No AKI	1.00 (-)	
AKI stage 1, 2 or 3	1.19 (1.08-1.31)	
2. AKI by donor type		0.31
3. AKI by donor age		0.88
4. AKI by donor BMI		0.77
5. AKI by recipient age		0.90
6. AKI by CIT		0.17
7. AKI by donor sex		0.64
8. AKI by donor hx hypertension		0.53
9. AKI by donor cause of death		0.46
1 year post-transplant (eGFR ≥ 60, 45-<60, 30-<45, < 30, returned to dialysis)		
1. Donor with/without AKI		<0.005
No AKI	1.00 (-)	
AKI stage 1, 2 or 3	1.25 (1.14-1.38)	
2. AKI by donor type		0.28
3. AKI by donor age		0.57
4. AKI by donor BMI		0.41
5. AKI by recipient age		0.25
6. AKI by CIT		0.47
7. AKI by donor sex		0.72
8. AKI by donor hx hypertension		0.34
9. AKI by donor cause of death		0.59

All models adjusted for the following risk factors:

Recipient – age, time on transplant list, primary renal disease, HLA mis-match, ethnicity, sensitisation, CIT, DGF

Donor – age, type, BMI, sex, history of hypertension, length of stay in hospital, use of adrenaline, ethnicity, blood group, cause of death

Table 4.9 Linear regression model for recipient kidney function at 90-days and 1-year after adult first kidney-only transplant

Discussion

The aim of this analysis was to assess the impact of donor AKI, as classified by the AKIN criteria, on outcomes in kidney transplantation. The question was whether discard of scarce donor kidneys is justified, if kidneys from donors with AKI are likely to result in a high percentage of graft failure and need for re-transplantation. This analysis has found that 17% of potential kidney donors have AKI (9% AKIN stage 1, 5% AKIN stage 2 and 3% AKIN stage 3), and so could contribute significantly to the donor pool.

Despite the expanding discrepancy between the number of patients waiting for a kidney transplant and the number of kidney donors, 28% of kidneys in this study are not used. As expected, kidneys are more likely to be declined due to higher AKIN stage, and kidneys with AKI stage 3 are 20 times more likely to be discarded than those with no AKI.

However, this analysis has shown that 1-, 3- and 5-year graft survival is only 2% lower when using kidneys from donors with AKI. Although this difference is statistically significant, this reduction in graft survival is unlikely to be clinically relevant. This reduced graft survival needs to be taken in the context of most patients remaining on the transplant waiting list and on dialysis with an annual death rate of 8.2% (25). The 20% increased risk of graft failure due to AKI in the donor is similar to the 17% increased risk of graft failure associated with dialysis vintage of 6 months when compared to pre-emptive transplantation, and is significantly lower than the 37% and 55% increased risk of graft failure when dialysing for longer than one or two years prior to kidney transplantation (136).

As might be expected, the rates of DGF increase as the AKI stage rises, and more importantly, the risk of PNF significantly increases when kidneys from donors with AKI

stage 3 are transplanted (9% versus 4% with no AKI). This is not necessarily an unexpected finding as Ali et al found that patients with severe AKI were less likely to recover their renal function (80).

Although there is a risk of an inferior eGFR at 1 year when using kidneys with AKI, this absolute reduction is only 1.6ml/min/1.73m^2 and is within the error of MDRD estimate and of no clinical significance.

To investigate whether there was a cumulative affect of AKI with many other risk factors known to be pertinent in kidney transplantation, a Cox-regression analysis was performed to examine the interaction of AKI with many of these risk factors, and surprisingly did not find any interactions. This was especially surprising for age, as previous studies have found that increasing age is a risk for the development of chronic kidney disease (CKD) in patients following AKI (137, 138).

These findings in the UK are consistent with Kayler's analysis of the Scientific Registry of Transplant Recipients (SRTR) in the US, between January 1995 and July 2007 (82,262 kidney transplant recipients). This study found that kidneys from SCDs with a terminal creatinine of over 2mg/dL had a graft survival that is as good as those from donors with a terminal serum creatinine of ≤1.5mg/dL. If the donor was classed as an ECD, then the survival was worse if they had renal dysfunction, but is equivalent to ECD donors with no AKI (139).

More recently, Hall, in New Haven, analysed the outcomes of 886 kidneys with AKI, comparing them to 2378 kidneys without AKI, using the AKIN criteria to define the severity of AKI. This study found that rates of DGF increased as the AKI stage increased, as this analysis has also shown, and this did not lead to worse survival, with there being no significant difference in 1-year graft survival between the groups (140). In this

analysis the 6-month eGFR was no different between the groups, but the 1-year data was not complete, and so I am unable to compare Hall's findings with the findings in this analysis. Heilman et al compared kidneys with no AKI to those with AKI, defined as a terminal creatinine of ≥2.0mg/dL. They also used the AKIN criteria to define the severity of AKI. As their control group includes many AKIN stage 1 kidneys it is difficult to draw comparisons between their findings and this analysis. They found no significant difference in 1-year graft survival between the AKI and control groups, and no difference in 1-year eGFR, which may be because the control group included kidneys that had a mild form of AKI (141).

Transplantation continues to be the treatment of choice for patients with end stage renal failure, and as more and more patients are listed, the need to expand the donor pool has inevitably lead to the acceptance of organs that are deemed to be 'marginal'. In many European national or international organ-sharing systems, it is current practice to not accept donor kidneys with acute kidney injury due to a perceived bad outcome after transplantation and need to return to dialysis. This data demonstrate that these policies appear to be unjustified.

As pointed out in a recent review by a collaboration of barristers and clinicians, the perceived risk should be discussed with the potential recipients of these organs so that they can make an informed decision (142). Despite many achievements in transplantation, uncertainty whether to accept donor organs for a particular recipient persists, to support better clinical decision making but also preventing unnecessary discard, more evidence is needed. In this study I have looked at the outcomes of a large

cohort of marginal kidneys in the UK, in an attempt to give clinicians as much information as possible to make an informed decision as to whether to use these kidneys, which they can then share with their patients to try and quantify this risk. From these results, it would seem that in the UK, transplant clinicians are correctly identifying kidneys to be used for transplantation, and that we are appropriately cautious with kidneys with AKIN stage 3. This caution comes from evidence that as the severity of AKI increases so does the risk of developing CKD (138, 143, 144), with Chawla et al demonstrating that as the RIFLE score increased, the odds of developing CKD Stage 4 increased significantly (138). The hypothesis behind this could be that the tubular injury, inflammation, endothelial cell dysfunction and vascular injury seen in AKI are exacerbated by the ischaemia reperfusion injury during transplantation, leading to renal fibrosis and impaired longer-term function (58, 145).

This study has its limitations. As a retrospective registry review, there has already been a selection bias with clinicians selecting the donors that are perceived to be at lower risk. Although the number of kidneys with AKI that were discarded and the reasons they were can be reviewed, I am unable to predict their outcome to see whether they were appropriately rejected. Unfortunately I am unable to analyse whether the results seen are due to an affect of differences in immunosuppression as the information about the immunosuppression used for each patient is poorly captured on the registry. Most UK centres will have individual immunosuppression protocols that will include a calcineurin inhibitor and an anti- proliferative, and most centres will not alter this for kidneys with an AKI. The results have been adjusted for centre and hopefully that minimises some of the uncertainty with this.

Other studies that have shown good outcomes with AKI kidneys have had a high proportion of kidneys with 'severe' injury being preserved using hypothermic machine preservation (140). Unfortunately this is one area of the UK registry that is not well populated, and I was unable to examine whether the kidneys in this study were preserved in this manner. As machine preservation is becoming more frequently used, this could be an important novel technology to assess and condition injured kidneys allowing them to be transplanted.

In conclusion, this large UK study has demonstrated that although a fair number of kidneys with AKI are still discarded, the ones that are actually transplanted give good outcomes. Over the 10-year period observed, in the UK over 1600 recipients received a kidney from a donor with AKI and still had a functioning graft at 1-year. I would suggest that kidneys from donors with AKI stage 1 and 2 should not be discarded as they give comparable outcomes to kidneys from donors with no AKI. Discard of these viable donor kidneys is not justified and transplantation will increase utilisation in many countries, significantly reducing waiting lists once the current 'decline policy' has been amended. Due to the size of the cohort in this study I would add that caution is advised for AKI stage 3 kidneys.

CHAPTER 5 EFFICACY AND QUALITY OF FLUSH-OUT PRIOR TO COLD STORAGE

5.1 Introduction

Good organ preservation that maintains optimum viability of the donor organ is a critical determinant of outcome and success in solid organ transplantation. The period of preservation starts during the retrieval operation with the initial 'cold flush' using a hypothermic preservation solution with the joint aims to wash out blood from the organ as well as rapidly cool the organ intravascularly. This should lower the temperature to preferably 5°C in order to significantly reduce its metabolism and prevent further ischaemic injury. The chemical composition of hypothermic preservation solutions is designed to maintain the viability of donor organs while counteracting unwanted effects of cold ischaemia related tissue injury such as oedema, acidosis and accumulation of precursors of reactive oxygen species (146-148).

This is of particular importance in the current day as more older and higher risk donors after circulatory death are providing organs for transplantation (figures 1.2, 1,3 and 1.4, Chapter 1), with grafts-to-be that are subjected to variable lengths of warm ischaemia.

During ischaemia, cells convert to using anaerobic respiration, which depletes cells adenosine tri-phosphate (ATP) stores. ATP-reliant transport pumps fail leading to a rise in the intracellular calcium concentrations. This triggers the release of free radicals and reactive oxygen species (ROS). The imbalance of free radicals and ROS is known as oxidative stress, which may lead to cell damage and eventual cell death through programmed cell death i.e. apoptosis and necrosis. Therefore, having an efficient preservation solution to flush out the organs and to reduce the effects of injury is invaluable.

To date, the most common static cold storage (SCS) preservation solutions predominantly used for all abdominal organs are University of Wisconsin cold storage

solution (UW-CSS) and Histidine-Trytophane-Ketoglutarate solution (HTK). A newly developed solution, which is a modification of the concept of the UW-CSS solution, is IGL-1. In parallel to those solutions for static cold storage, currently, only one type of dedicated solution is clinically used for continuous hypothermic machine perfusion: the Belzer UW Machine Preservation Solution (UW-MPS) that is used both for in-situ flushing of organs and temporary machine perfusion preservation of explanted kidneys and livers.

UW-CSS solution contains hydroxyethyl starch (HES) as a colloid. This is thought to protect against cell swelling, together with the large saccharide raffinose and anion K-lactobionate. UW-CSS also contains a phosphate buffer, adenosine to support energy metabolism, and glutathione as an antioxidant that is beneficial in protecting against oxidative damage and stabilising cell membranes (146, 148) (See Table 5-1 which illustrates the different components of each solution).

HTK does not contain a colloid but includes the impermeant saccharide mannitol as an osmotic barrier, histidine as a strong buffer and tryptophan and ketoglutarate which both help to stabilise cell membranes and can be used as substrates for anaerobic metabolism (148, 149).

IGL-1 has a similar composition to UW-CSS solution, but contains polyethylene glycol (PEG) instead of HES as a large colloid and strong anti-oxidant; it also is a high sodium and low potassium concentration solution, which is thought to reduce initial vasoconstriction (148).

UW-MPS is an isotonic solution that is slightly less viscous than UW-CSS. It contains HES since machine perfusion of an organ requires a colloid, preventing its vasculature to collapse. Its composition is similar to the static cold storage UW solution, albeit it

includes the impermeants Na-gluconate and mannitol instead of K-lactobionate and raffinose (148).

In the past decade, there has been a lot of debate about the benefit of a colloid in a hypothermic preservation solution. Inclusion of a colloid increases viscosity, and it has been argued that during initial flush-out this may have a detrimental effect on the rate at which blood is washed out and how well the solution will penetrate and preserve the parenchyma of a donor organ. In addition, there has been reported in vitro-evidence that due to HES, hyper aggregation of erythrocytes and formation of rouleaux networks in the small vessels may occur although this has not been found to be a clinical problem (88, 150).

Despite the fact that during transplant meetings many surgeons have strongly voiced that the increased viscosity of UW solution will negatively affect perfusion and preservation of organ quality, there is no evidence for this in the literature. However, as optimal preservation of donor organs is key and to find out whether this statement is based on facts or merely a general perception, we have investigated the physiological characteristics and perfusion effects during flush-out, comparing the most frequently used hypothermic preservation solutions.

	UW-CSS	HTK	IGL-1	UW-MPS
Colloids (g/L)				
HES	50	-	-	50
PEG-35	-	-	1	-
Impermeants (mM/L)				
Histidine	-	198	-	-
Lactobionate	105	-	105	-
Mannitol	-	38	-	30
Raffinose	30	-	30	-
Ribose	-	-	-	5
Dextrose	-	-	-	10
Buffers (mM/L)				
Histidine	-	198	-	-
KH_2PO_4	25	-	25	25
Electrolytes (mM/L)				
Calcium	-	0.015	0.5	0.5
Chloride	20	32	-	0.5
Magnesium	-	4	-	-
Magnesium Sulphate	5	-	5	-
Magnesium Gluconate	-	-	-	5
Potassium	120	9	25	25
Sodium	25	15	120	80
ROS Scavengers (mM/L)				
Allopurinol	1	-	1	-
Glutathione	3	-	3	3
Mannitol	-	30	-	30
Tryptophan	-	2	-	-
Additives (mM/L)				
Adenosine	5	-	5	5
Glutamic acid	-	-	-	-
Ketoglutarate	-	1	-	-
HEPES	-	-	-	10

Table 5.1 Components of preservation solutions (UW-CSS = University of Wisconsin Cold Storage Solution; HTK = Histidine-Tryptophan-Ketoglutarate solution; IGL-1= Institute of George Lopez – 1 solution; UW-MPS = University of Wisconsin Machine Preservation Solution; HES = Hydroxyethyl starch, PEG-35 = polyethylene glycol with an average molecular weight of 35kDa; ROS= Reactive Oxygen Species

5.2 Aims

To compare the perfusion characteristics of the most frequently used hypothermic preservation solutions for abdominal organs i.e. UW-CSS, HTK, IGL-1 and UW-MPS in a large animal (pigs) simulating clinical organ retrieval in a donation after circulatory death donor model.

5.3 Methods

5.3.1 Development of animal model (preliminary work)

To develop the model and ensure the methods we had planned, abattoir kidneys were used to test the use of the temperature probes, the SonoVue (sulphur hexafluoride microbubbles) and the ultrasound equipment.

A local abattoir was visited and pairs of pig kidneys retrieved by the abattoir workers were given to me. The kidneys were flushed with 0.9% normal saline (Baxter Healthcare Corporation, Illinois, USA) at the abattoir and then brought back to the laboratory in an icebox.

The temperature probes (Omega mini hypodermic probe, Omega Engineering Limited, Connecticut, USA), were checked by placing them in the kidneys, and then cold preservation solution was flushed through the kidney (after leaving the kidneys out of ice for 10 minutes). The temperature fell, but the probes kept falling out, so it was decided to suture the probes in place using 5-0 prolene. When the probes were sutured at the point of entry into the kidney and where the wire connected to the probe, they were the most secure.

The ZONARE ultrasound machine, ZSI 40 (ZONARE Medical Systems Incorporated, California, USA), was used to check for flows through the kidney. When the flushing

solution was running, flows were seen on the ultrasound, and then the fluid was clamped- the flows stopped. Different probes, modes and gains were checked, and the views were best when the L10-S probe was used in the Thyroid mode. The gain was set to 75Hz.

I explored the appropriate volume of SonoVue (Bracco, Milan, Italy) to be added to the preservation solutions. When the solution was made up, the syringe had 4.8ml of active SonoVue solution. When all 4.8ml was injected into a one-litre bag of preservation solution, and then the solution flushed into the kidney, the bubbles appeared straight away, and were very bright. The dose was halved, but this was still too bright too quickly. A quarter of the dose was injected into the litre of solution, and this meant that the Ultrasounds (USS) images initially showed no bubbles, but then the bubbles became visible becoming brighter over a short period, and then staying present. The dose was halved again, and with this smaller dose, it was difficult to see any bubbles in the USS images.

1.2ml was injected into a two-litre bag of preservation fluid (HTK), and it was difficult to see any bubbles on the images. The dose was doubled and the images were similar to those seen with 1.2ml in the litre bag (as expected).

Therefore, for the experiments it was decided that 1.2ml of SonoVue would be used for all solutions that used 1L bags, and 2.4ml for HTK that comes in 2L bags.

Three practice runs of the experiments were done. Initially it was planned that there would be 30 minutes of warm ischaemia (WI), however, 30 minutes did not give enough time to prepare and set up the experiment. The time taken to perform the laparotomy and sternotomy, ligate the vena cava, dissect the kidneys and liver, place the sutures in

the liver and kidney and to take the baseline blood and biopsy samples was 45 minutes, and this was reduced to 40 minutes after three practice runs.

During these practise runs it was also found that having a temperature probe in each lobe of the pig liver (four lobes), and in the upper and lower pole of the left and right kidney was not feasible as there were too many wires, and at the end of the procedure most of the probes were not in the organs anymore, but were found free in the abdominal cavity. Therefore, it was decided to put the probes in two of the liver lobes, and in the upper and lower pole of the left kidney. In order to take the blood and biopsy samples the kidney had to be manipulated, and as this might affect the temperature readings, these samples and biopsies were taken from the right kidney.

5.3.2 Optimised model

Female pigs (approx. 70kg) were used in a DCD retrieval model. After a schedule-I termination of the pig (overdose of barbiturate anaesthetic), a laparotomy and sternotomy were performed. The vena cava was ligated in the chest, and the liver and kidneys were dissected free to allow sutured placement of temperature probes (two probes in the left kidney, two probes in the liver (most lateral left and right lobes)). The vena cava was then ligated distally, and the aorta was dissected with ties placed around it. The distal tie was ligated to occlude the iliac arteries, and then the aorta was cannulated with a 12F aortic catheter (Medtronic, single stage venous cannula) to allow heparinised cold flush-out and perfusion to start when needed.

After 30 minutes of warm ischaemic time, liver and kidney biopsies were obtained and placed on dry ice, in formalin and glutaraldehyde. Blood from the vena cava was taken

and the viscosity measured. Blood was also collected in standard and EDTA tubes for later analysis.

Baseline (30sec) USS of liver and left kidney were recorded.

After 40 minutes of warm ischaemia, temperature recording and cold flush were commenced using the respective solution. Flush-out volumes were used according to the recommendations of the manufacturers (5 x 1L bags of UW-CSS, IGL-1 and UW-MPS, resp.; 5 x 2L bags of HTK), with 5000IU heparin in each litre. The 3rd and 5th bag contained SonoVue for contrast-enhanced ultrasound (1.2ml for 1L bags and 2.4ml to the 2L bags (HTK)).

After completion of the first either 1L or 2L bag during flush-out, effluent samples of blood mixed with preservation solution were collected from hepatic veins and right renal vein to measure viscosity and stored in standard and EDTA tubes for later analysis. After opening the third 1L or 2L bag during flush-out, an USS was performed of the liver, storing the images. After the third 1L or 2L bag, liver and kidney effluent was collected from hepatic veins and right renal vein to measure viscosity and stored in standard and EDTA tubes for analysis. Biopsies of the liver and kidney were obtained. Each biopsy was cut in three pieces, with one part placed in formalin, one in dry ice and one in glutaraldehyde. After opening the fifth 1L or 2L bag during flush-out, an USS was performed of the liver, storing the images. After the fifth 1L or 2L bag, liver and kidney effluent was collected from hepatic veins and right renal vein to first measure viscosity and then to be stored in standard and EDTA tubes for analysis. Biopsies of the liver and kidney were obtained. Each biopsy was cut in three pieces, with one part placed in formalin, one in dry ice and one in glutaraldehyde.

Following completion of flush-out and perfusion with 5L (UW-CSS, IGL-1, UW-MPS) or 10L (HTK) following the manufacturers' guidance the liver and kidneys were removed and photographed for later assessment.

5.3.3 Power Calculation

Meads' resource equation was used to calculate the number of animals in each experimental group.

E=T-N

E= Error (should be between 10-20)

T= Total number of Animals

N= Number of treatments

Equation 5.1 Meads' resource equation

This resulted in four to six animals per group.

This was a comparative study therefore it was decided to initially start with four animals per group to avoid the unnecessary use of animals. After initial analysis, however, there was considerable variation between pigs within the groups, and so it was decided to increase the number of animals in the two most important groups (UW-CSS and HTK being the most commonly used, as well as being the most, and least viscous solutions).

5.3.4 Temperature

The temperature was continuously measured and recorded during the perfusion procedure using Omega temperature probes that were connected to a thermocouple data logger and logger recorder (Omega TC-08 8 channel Thermocouple).

5.3.5 Viscosity

The kinematic viscosity was measured using U-tube, or glass capillary, viscometers (psl rheotek) (Figure 5.1). Initially blood and later effluent from the organs was drawn up into the upper bulb of the viscometer by suction, and then allowed to flow down through the capillary into the lower bulb. Two marks (one above and one below the upper bulb) indicate a known volume. The time taken for the fluid to pass between these two marks was measured and multiplied by the specific constant for the viscometer to calculate the kinematic viscosity.

$$\mathbf{v = C.t}$$

v= viscosity (mm^2/s)

C= Constant of the viscometer

t= time in seconds

Equation 5.2 Calculation of Viscosity



Figure 5.1 Capillary U Tube Viscometer

5.3.6 Perfusion

The quality of cortical perfusion in the liver and kidney was measured using contrast-enhanced ultrasonography (CEUS) and the computer data analysis software MATLAB.

USS was used to take video images of the organ during the time when the bag of preservation solution with SonoVue was infused, as SonoVue will appear on the USS images as microbubbles. The USS images were then examined to define an area of cortex remaining fairly consistent (with as little movement artefact as possible) throughout the recordings. The images were then exported to MATLAB to calculate in a 1cm square area the change in brightness over the time of the recordings compared to a baseline of zero (before the SonoVue reaches the organ). The mean increase in brightness was recorded. This process was repeated three times for each organ in each experiment to ensure robust data. The results were then compared between groups.

5.3.7 Histology

The biopsies that were obtained from the liver and kidney were placed in formalin were processed and embedded. The biopsy had its water content removed by using increasing concentrations of alcohol. The final dehydration stages used Histo-clear (a xylene substitute), so that wax could infiltrate the specimen. This was done using an automated machine overnight. Table 5.2 shows the solutions used during processing.

The biopsies were then placed in a metal specimen embedding containing to which molten wax was added. The container was then cooled to solidify the wax in a block that then had sections cut from it for use for histology and immunohistochemistry.

The biopsy sections for histology were stained with haematoxylin and eosin and reviewed by a blinded pathologist.

The slides were initially reviewed to decipher the changes that were present and the components of the score were then defined. All slides were scored in one session to minimise differences in interpretation with a blinded histopathologist.

Each kidney biopsy was scored on four morphological parameters; vacuolation, tubular integrity, oedema, and red cell presence, using a semi quantitative scale (0, normal, 1 mild, 2 moderate and 3 severe). Table 5.3a shows the components of the kidney scoring system used.

Each liver biopsy was scored on four morphological parameters as well; erythrocyte washout, dilatation of sinusoids, dilation of capillaries in septa or periductular tissue and formation of vacuoles in hepatic cytoplasm using a semi quantitative scale. Table 5.3b shows the components of the liver scoring system used. The biopsies were going to be scored for oedema, however the biopsies showed no, or very little oedema, and so was decided to be irrelevant.

The scores for the biopsies were compared across the groups.

Position	Solution	Time in Bath (mins)
Position 1:	Ethanol 70%	10
Position 2:	Ethanol 70%	10
Position 3:	Ethanol 80%	10
Position 4:	Ethanol 90%	10
Position 5:	Ethanol 100%	10
Position 6:	Ethanol 100%	10
Position 7:	Histo-clear (neat)	15
Position 8:	Histo-clear (neat)	15
Position 9:	Histo-clear (neat)	15
Position 10:	Histo-clear (neat)	15
Position 11:	Molten wax	30
Position 12:	Molten wax	30

Table 5.2 Solutions for processing formalin biopsies for histology

Component	Score
Washout	3= congestion of erythrocytes
	2= moderate amount of erythrocytes
	1= occasional erythrocytes
	0= No erythrocytes
Vacuoles	3= extensive vacuoles
	2= clearly visible vacuoles
	1= occasional vacuoles
	0= No vacuoles
Sinusoids	3= severely dilated
	2= moderately dilated
	1= slightly dilated
	0= normal calibre
Capillaries	3= obviously dilated
	2= moderately dilated
	1= slightly dilated
	0= inconspicuous

Table 5.3a Histology scoring system used to score liver biopsies

Component	Score
Washout	3= congestion of erythrocytes
	2= moderate amount of erythrocytes
	1= occasional erythrocytes
	0= No erythrocytes in PTC or glomerulus
Vacuoles	3= extensive vacuoles
	2= clearly visible vacuoles
	1= occasional vacuoles
	0= No vacuoles
Integrity of tubules	3= Lost architecture
	2= irregular architecture
	1= irregular cell membrane
	0= intact tubules
Oedema	3= Severe oedema
	2= moderate oedema
	1= mild oedema
	0= No oedema

Table 5.3b Histology scoring system used to score kidney biopsies

5.3.8 Electron Microscopy

Kidney segments were prepared for transmission electron microscopy (TEM). One-micrometre sections were stained with Azure A and examined by light microscopy to identify areas of interest. Thin sections (80 nm) of suitable areas were cut and stained with uranyl acetate and lead citrate prior to examination under electron microscopy (Jeol 1200EX, Japan). The morphology of the glomerulus, tubules and the ultra-structures were assessed qualitatively.

5.3.9 Markers of Injury

The perfusate samples were analysed for aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) To look for evidence of cell injury. AST and LDH are enzymes found in the cytoplasm of cells that are released into the serum at times of cellular injury making them common markers of cell damage.

The biopsy samples were analysed for markers of oxidative stress (4-Hydroxynonenal (4-HNE) and Heat Shock Protein 70 (HSP-70)), and apoptosis (Cytochrome C and Caspase 9). 4-HNE is released during lipid peroxidation, which is increased during times of oxidative stress. Increased concentrations of 4-HNE trigger the induction of caspase enzymes and the release of cytochrome C from the mitochondria and this leads to cell death by apoptosis and necrosis. HSP-70 helps to protect the cells from thermal or oxidative stress by preventing partially denatured proteins from aggregating and helping them re-fold to their normal structure. HSP-70 also directly inhibits apoptosis by inhibiting pro-Caspase 9. HSP-70 is released during injury, and then starts to initiate repair. Caspase 9 is an enzyme critical to the apoptosis pathway. Pro-caspase 9 is cleaved into the active caspase 9 when cytochrome C is released from the mitochondria

during oxidative stress. Caspase 9 goes onto cleave caspase 3, 6 and 7 initiating the caspase cascade, which eventually ends with apoptosis.

5.3.9.1 AST and LDH in perfusate

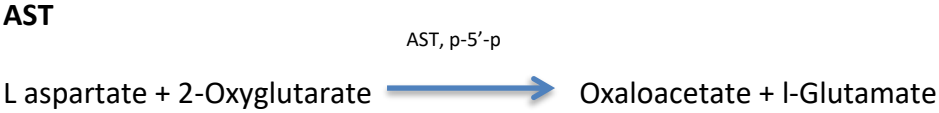
Serum-separating tubes (SST) were used to collect blood during the procedure. These were left for at least 30minutes before being centrifuged. Aliquots of the serum were taken off and stored on dry ice before being transferred to an -80°C freezer.

For AST and LDH measurements, an aliquot was removed from the freezer and defrosted. Both assays were run using the ARCHTITECT *c* Systems™ (Abbot Diagnostics, Illinois, USA).

Both assays measure the absorbance of light by NADH at 340nm, although the AST assay measures the rate of decrease in absorbance (as NADH is oxidised to NAD^+) and the LDH assay measures the rate of increase in absorbance (as NAD^+ is reduced to NADH).

The assays are based on reactions catalysed by the AST or LDH in the sample:

AST





LDH



5.3.9.2 Western Blotting

Sample Preparation

Frozen biopsy samples were kept on dry ice, and then placed into beads beater tubes and kept on ice. 300µL of ice-cold RIPA buffer (150mM NaCl, Triton-X 100, 0.5% sodium deoxycholate, 1% SDS, 50mM Tris, pH 8.0) was added to each tube.

The tubes were placed in the beads beater for 1 min and then centrifuged for 1 minute. This was repeated for three cycles, and then the samples were left on ice for an hour.

Aliquots of the liquid from on top of the beads were placed into pre-labelled tubes.

Quantification of Protein by BCA Assay

The Bicinchoninic Acid (BCA) assay exploits the reduction of Cu^{2+} to Cu^{+} by proteins in an alkaline solution (the biuret reaction). 2 molecules of BCA chelate 1 Cu+ ion, giving a purple complex. This water-soluble complex exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations (151).

Protein concentrations are determined when compared to a standard curve of known protein concentrations made up with bovine serum albumin (BSA). 25µL of the standards and samples were loaded into wells on the well plate. 200µL of assay reagent buffer was added to each well, and the plate was incubated at 37°C for 30 minutes. The plate was cooled to room temperature and then placed in the plate reader (BMG Fluostar Omega). Absorbance at 562nm was measured and corrected for a blank well measurement.

Samples were then diluted to the same concentration of 1.8µg/µL using RIPA buffer.

SDS-PAGE

15µL of sample was mixed with 15µL of Laemmli buffer (0.125M Tris-HCl (pH 6.8), 4% SDS, 20% Glycerol, 0.04% Bromophenol blue and 10% 2-mercaptoethanol), boiled at 95°C and then placed on ice.

The SDS-PAGE gel (Criterion XT Bis-Tris 4-12% gradient gel) was placed into the criterion cell tank and submerged in running buffer (MOPS, Tris Base, SDS, EDTA Free Acid)

10µL of molecular weight marker was loaded into the first well, followed by 10µL of control sample in the second well (normal rabbit IgG). 10µL of each sample was then loaded into the remaining wells of the gel. Electrophoresis was carried out at 200V for 60 minutes at room temperature.

Immunoblotting and incubation with primary antibody

Criterion XT gels were carefully removed from the cell and placed on top of a piece of pre-cut polyvinyldifluoride (PVDF) membrane which had been pre-soaked in methanol. Air bubbles were rolled out, and the gel and membrane were sandwiched between two pieces of filter paper and two sponges that had been soaked in blotting buffer (250mM Tris base (pH 8.3), 1.92M Glycine, 20% methanol in dH_2O). The sandwich was placed in the blotting apparatus with the membrane facing the anode and electro-blotting was carried out at 60V for two hours at 4°C.

The membrane was separated from the gel, and washed in PBS-T twice for five minutes each. The membrane was then placed in 5% milk in PBS-T for 60 minutes at 4°C to block any non-specific binding sites. After washing four times over 30 minutes in PBS-T the membranes were incubated with primary antibody overnight at 4°C with gentle agitation.

Primary Antibodies:

Rabbit polyclonal anti cytochrome-C	*1:1000 in PBS-T + 5% BSA*
Rabbit polyclonal anti Caspase-9	*1:500 in PBS-T + 5% BSA*
Rabbit polyclonal anti 4-Hydroxynonenal	*1:3000 in PBS-T + 5% BSA*
Mouse polyclonal anti HSP-72	*1:1000 in PBS-T + 5% BSA*

Incubation with secondary antibody and detection

The membrane was washed in PBS-T three times for five minutes each. The membrane was then incubated with polyclonal secondary antibody conjugated to horse-radish peroxidase (HRP) for one hour at room temperature with gentle agitation.

Secondary Antibodies:

Dako goat polyclonal anti rabbit HRP	*1:2000 in PBS-T + 5% BSA*
Dako goat polyclonal anti mouse HRP	*1:1000 in PBS-T + 5% BSA*

The membrane was then washed extensively in PBS-T six times over five-ten minutes. The protein band of interest was visualised by chemiluminescence (ECL chemiluminescence Detection System, Thermo Scientific). Equal volumes of stable peroxide solution and luminol/enhancer solution were mixed and the membranes incubated in this for three minutes. This was then drained off and the membrane placed in a clear sheet protector and exposed to the imaging system (Chimidoc with Imagelab software).

Strip and reprobe for loading control (beta-actin)

The membrane was washed in stripping buffer (SDS (pH 2.2), Glycine, Tween 20) twice for 10 minutes. It was then washed twice in PBS and then twice in PBS-T.

The membrane was then blocked in 5% BSA for one hour at 4°C. The membrane was then washed again in PBS-T before being incubated with anti beta-actin antibody (1:500 in PBS-T + 5% BSA) overnight at 4°C under gentle agitation.

The membrane was washed again three times in PBS-T and then was incubated with secondary antibody (Dako goat polyclonal anti-mouse HRP 1:1000 in PBS-T + 5% BSA) for one hour at room temperature.

After being extensively washed with PBS-T over 10 minutes, the ECL reagents were added and the membrane was developed as before.

Protein band intensities were quantified using ImageJ.

5.3.9.3 Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed paraffin embedded tissue.

Deparaffinisation

Slides were placed in a rack and washed in the following washes in Coplin jars to deparaffinise and rehydrated them

Xylene (1)	3 min
Xylene (2)	3 min
1:1 Xylene:100% Ethanol	3 min
100% Ethanol (1)	3 min
100% Ethanol (2)	3 min
95% Ethanol	3 min
70% Ethanol	3 min
50% Ethanol	3 min

The slides were then placed in running cold tap water to rinse off the ethanol.

The slides were kept in tap water until the antigen retrieval to stop them from drying out (to avoid non-specific binding and high background staining).

Permeabilisation

The tissue on the slides was covered with 0.2% Tween 20 (in TBS) for 10 minutes (200µL) to permeabilise them.

Antigen Retrieval- Enzymatic

The tissue on the slides was covered with 0.1% pronase solution (100µL) for 10 minutes at 35°C in a humidified container. The slides were then removed and transferred to a rack in a container of tap water. The sides were rinsed with tap water for three minutes.

Antigen retrieval- Heat induced

Antigen retrieval buffer (Sodium Citrate buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0)) was added to the pressure cooker. The pressure cooker was then placed on the hot plate with the lid resting on top. Once the buffer was boiling the slides (in the racks) were transferred from the tap water into the pressure cooker (inside a vessel that held up to 500mL). The lid was secured and once the pressure cooker reached full pressure the slides were left in for three minutes and then the cooker was taken off the heat and placed in an empty sink. The pressure release valve on the cooker was activated and cold water was run over the cooker. Once the cooker was de-pressurised the lid was opened and cold water was run into the cooker for 10 minutes.

Immunostaining

The slides were washed in TBS plus 0.025% Triton X-100 with gentle agitation for five minutes twice. A protein block (from mouse and rabbit specific HRP/DAB) was applied to the tissue on the slides and incubated at room temperature for 30 minutes (to block for non-specific background staining). The slides were then washed in buffer. The primary antibody was then applied to the tissue on the slides, and incubated at 4°C overnight.

Primary Antibodies

Anti-Von Willebrand Factor Antibody *1:1000 in TBS + 1% BSA*

The slides were then rinsed in TBS-0.025% Triton with gentle agitation for five minutes four times. Biotinylated goat anti-polyvalent solution was then applied to the tissue on the sides and incubated at room temperature for ten minutes.

The slides were then washed in buffer. Streptavidin Peroxidase was applied to the tissue on the slides and incubated at room temperature for ten minutes. Again the slides were then washed in buffer. 30µL of DAB Chromogen was added to 1.5ml of DAB substrate and this mix was applied to the tissue and incubated for five minutes. The slides were washed in buffer, and then counterstained in hematoxylin. The slides were rinsed in tap water until the washes were clear, and then the tissue was dehydrated and remounted with DPX.

50% Ethanol	3 min
70% Ethanol	3 min
95% Ethanol	3 min
100% Ethanol	3 min
1:1 Xylene:100% Ethanol	3 min
Xylene	3 min

5.3.10 Statistical Analysis of Experiments

As the sample size was small, parametric conditions could not be assumed, and so variables were assessed for normality using assessment of histograms and statistical testing using the Shapiro-Wilk test.

Parametric variables were summarised using the mean and standard deviation, and were compared using one way ANOVA.

Non-parametric variables were summarised using the median and interquartile range, and were compared using the Kruskal-Wallis test. Values of $p<0.05$ were considered statistically significant.

All statistical analysis was performed using SPSS for Mac software (IBM SPSS Statistics Version 22, Chicago IL, USA).

5.4 Results

5.4.1 Temperature

Liver

The temperature of the liver reduced throughout the retrieval procedure for all of the preservation solutions. When measuring the temperature from the start to the end of the flush-out procedure using the bespoke recommended volumes by the companies for each solution (10L HTK, 5L UW-CSS, 5L IGL-1 and 5L UW-MPS), the temperatures reduced from 38°C to 18.34°C when HTK was used, to 20.87°C with IGL-1, to 20.05°C for UW-CSS and 20.34°C for UW-MPS (*figure 5.2*). There was no difference between the end temperatures that the liver reached for each of the solutions (p= 0.625) (ANOVA), and this was still the case with the post-hoc analysis (UW-CSS vs HTK p=0.373, UW-CSS vs IGL-1 p=0.702, UW-CSS vs UW-MPS p=0.892, HTK vs IGL-1 p=0.244, HTK vs UW-MPS p=0.353, IGL-1 vs UW-MPS p=0.821).

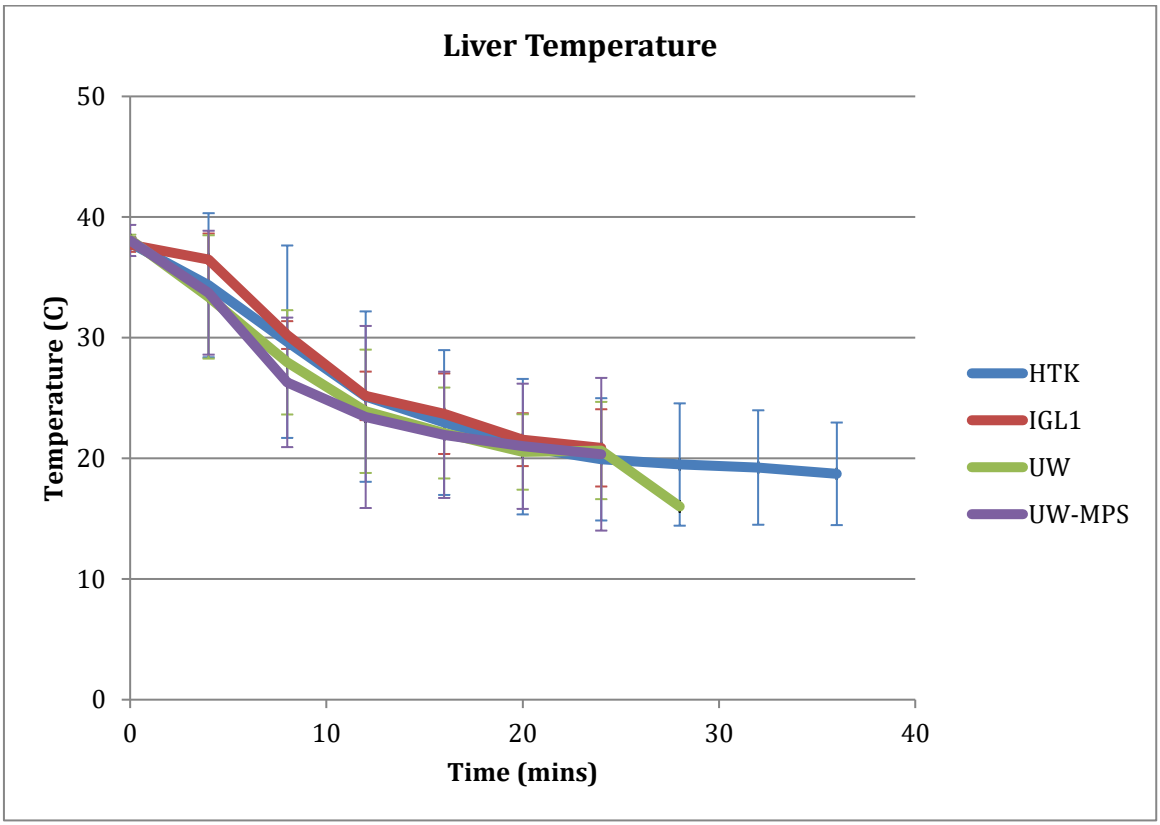


Figure 5.2 Temperature of the liver throughout the retrieval (the prescribed volume of flush by the manufacturer needed approx. 25 minutes for 5L of UW-CSS, UW-MPS and IGL-1; for HTK the required volume to obtain optimal preservation was 10L needing 38 minutes). *This graph shows that the temperature of the liver reduces throughout the retrieval procedure, and there is no significant difference in the end temperature reached when comparing the different solutions.*

Kidney

The temperature of the kidney also reduced throughout the retrieval procedure for all of the preservation solutions. The temperatures reduced from 38°C to 20.31°C when HTK was used, to 19.46°C with IGL-1, to 15.13°C for UW-CSS and 15.22°C for UW-MPS (*figure 5.3*). There was no difference between the end temperatures that the kidney finally reached when comparing all of the solutions (p= 0.095) (ANOVA), but in the post-hoc analysis, a significant difference was found when comparing UW-CSS versus HTK with UW-CSS cooling the kidney significantly more than HTK. UW-MPS also cooled the kidney better than HTK, however, this was a trend seen and this did not reach statistical significance (UW-CSS vs HTK p=0.037, UW-CSS vs IGL-1 p=0.108, UW-CSS vs UW-MPS p=0.973, HTK vs IGL-1 p=0.744, HTK vs UW-MPS p=0.063, IGL-1 vs UW-MPS p=0.147).

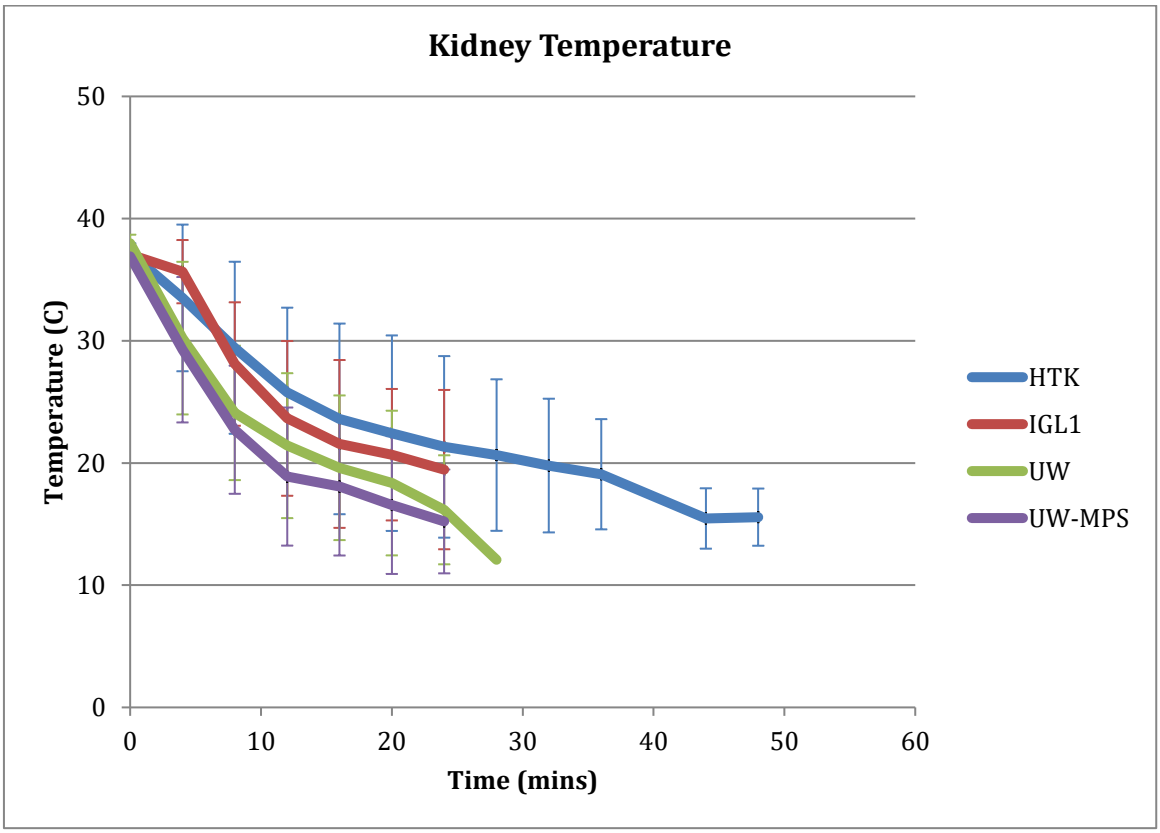


Figure 5.3 Temperature of the kidney throughout the retrieval (the prescribed volume of flush by the manufacturer needed approx. 25 minutes for 5L of UW-CSS, UW-MPS and IGL-1; for HTK the required volume to obtain optimal preservation was 10L needing 38 minutes). *This graph shows that the temperature of the kidney reduces throughout the retrieval procedure, and there is a significant difference in the end temperature reached when comparing UW-CSS with HTK (p= 0.037).*

5.4.2 Viscosity

The viscosity of each of the preservation solutions used is shown in table 5-4. The viscosity of the pig blood at the beginning of the procedure was difficult to test as it often clotted in the viscometer.

Solution	Viscosity (mm^2/sec) (sd)
Pig Blood	5.50 (±0.314)
HTK	1.12 (±0.035)
IGL-1	1.30 (±0.216)
UW-CSS	2.44 (±0.347)
UW-MPS	2.21 (±0.155)

Table 5.4 Viscosity of pig blood and preservation solutions

Liver

The viscosity of the effluent perfusate coming out of the liver reduced throughout the retrieval procedure for all of the solutions. The end viscosity reached 1.12 mm^2/s for HTK, 1.36 mm^2/s for IGL-1, 2.62 mm^2/s for UW-CSS and 2.34 mm^2/s for UW-MPS (*figure 5.4*). The end viscosity was significantly different between UW-CSS and HTK solutions (p=0.001), but there was no significant difference between the end viscosity reached when comparing UW-CSS to UW-MPS (p=1.0), UW-CSS to IGL-1 (p=0.11), HTK to UW-MPS (p=0.7) or HTK to IGL-1 (p=1.0) and UW-MPS to IGL-1 (p=0.96).

Kidney

The viscosity of the effluent perfusate coming out of the kidney also reduced throughout the retrieval procedure for all of the solutions. The end viscosity reached 1.10 mm^2/s for HTK, 1.25 mm^2/s for IGL-1, 2.26 mm^2/s for UW-CSS and 2.13 mm^2/s for UW-MPS (*figure 5.5*). Again the end viscosity was significantly different between UW-CSS and HTK solutions (p=0.001), but there was no significant difference between the

end viscosity reached when comparing UW-CSS to UW-MPS (p=1.0), UW-CSS to IGL-1 (p=0.12), HTK to UW-MPS (p=0.06) or HTK to IGL-1 (p=1.0) and UW-MPS to IGL-1 (p=0.91).

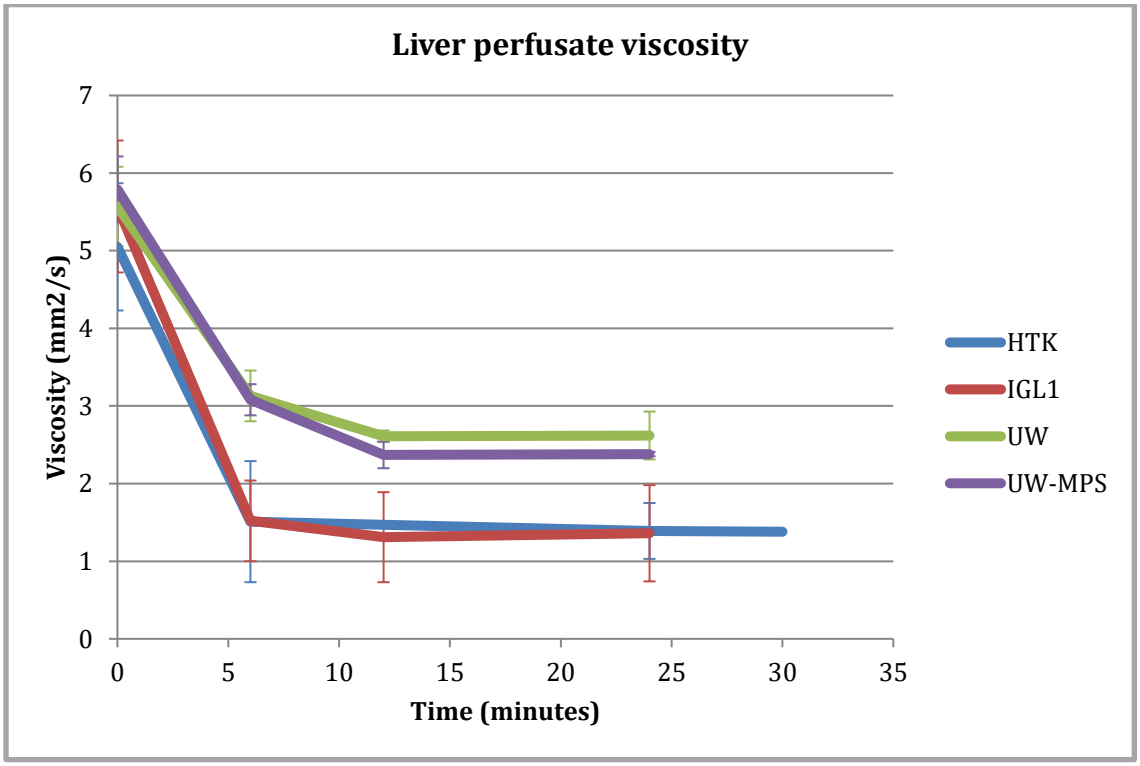


Figure 5.4 Viscosity of the effluent perfusate from the liver throughout the retrieval. *This graph shows that the viscosity of the effluent perfusate from the liver reduces throughout the procedure to a viscosity that is similar to the viscosity of the solution itself.*

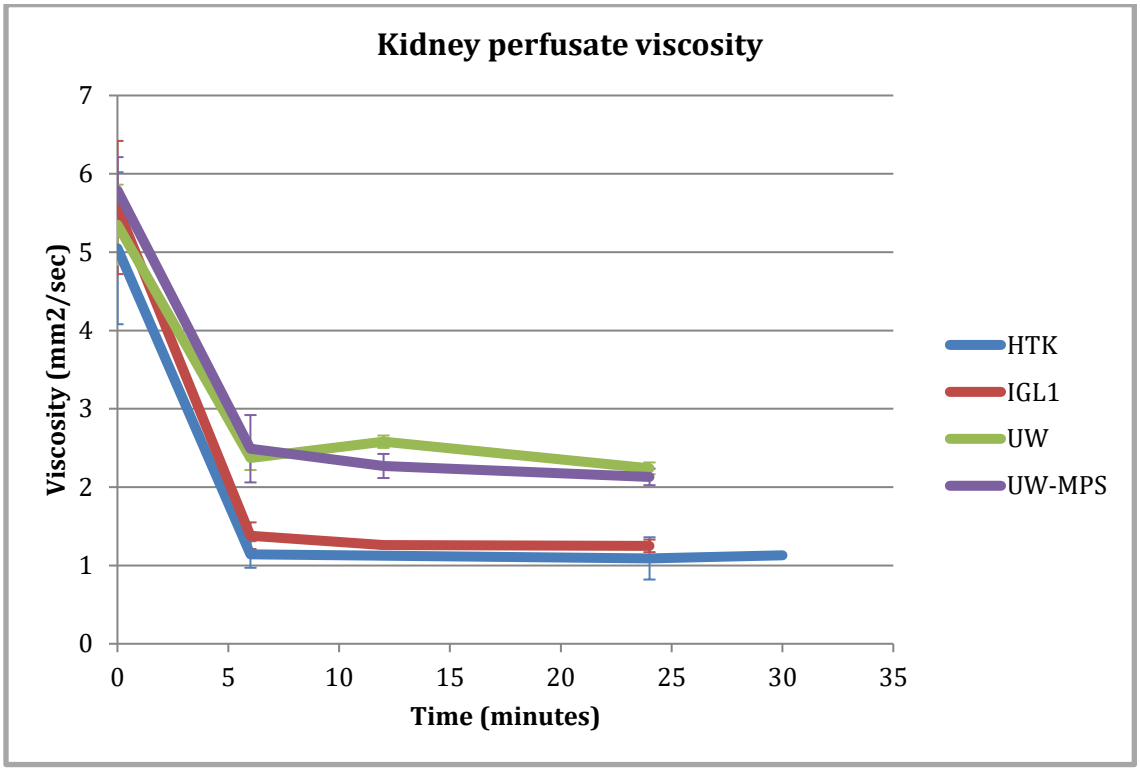


Figure 5.5 Viscosity of the effluent perfusate from the kidney throughout the retrieval. *This graph shows that the viscosity of the effluent perfusate from the kidney reduces throughout the procedure to a viscosity that is similar to the viscosity of the solution itself.*

5.4.3 Perfusion

Liver

All solutions achieved adequate perfusion of the liver, with microbubbles seen in the peripheral tissues in the microcirculation. There was no significant difference in the perfusion achieved by any of the solutions (p=0.585) (table 5-5a).

Kidney

All solutions achieved adequate cortical perfusion of the kidneys, with microbubbles seen in the microcirculation. UW-CSS and IGL-1 both achieved better cortical perfusion than HTK and UW-MPS (Table 5-5b), and this was statistically significant when comparing UW-CSS to HTK (UW-CSS vs HTK p=0.039, UW-CSS vs IGL-1 p=1.00, UW-CSS vs UW-MPS 0.266, IGL-1 vs UW-MPS p=0.330, IGL-1 vs HTK p=0.074 and HTK vs UW-MPS p=1.00).

Solution	Mean Change in Intensity Liver (SD)	P Value
UW-CSS	66.53 (45.96)	0.585
HTK	44.77 (40.08)	
IGL-1	27.18 (16.88)	
UW-MPS	26.26 (19.72)	

Table 5.5a Liver perfusion measured by the mean change in the intensity of the brightness produced by the micro bubbles. *There is no significant difference in the perfusion of the liver microcirculation by the different solutions.*

Solution	Mean Change in Intensity Kidney (SD)	P Value
UW-CSS	133.59 (70.31)	0.01
HTK	23.04 (21.00)	
IGL-1	133.30 (68.88)	
UW-MPS	30.58 (17.35)	

Table 5.5b Kidney perfusion measured by mean change in intensity in the brightness produced by the micro bubbles. *There is a significant difference in the perfusion of the kidney microcirculation when comparing UW-CSS to HTK (p=0.039).*

5.4.4 Histology

The liver and kidney biopsy scores at the start and end of the procedure were compared for all the solutions, and there was no significant difference between these scores (Liver: start p= 0.293, end p= 0.232; Kidney: start p= 0.148, end p= 0.115). The change, and the fold change in the biopsy scores were also compared for the difference solutions and again there was no significant difference in these changes of scores (Liver: change in score p= 0.360, fold change p= 0.452; Kidney: change in score p= 0.133, fold change p= 0.098).

When the separate components of the histology score were looked at, there was a significant reduction in the blood within the liver and kidney for all solutions, but no significant difference when comparing the solutions.

Figures A - H in Appendix A, show a histology slide of the liver and kidney at the start and end of the procedure for each of the preservation solutions.

Liver

The overall liver biopsy score showed a trend of reducing during the procedure for all solutions except for HTK (Figure 5.6a). The reduction in the overall score was significant for UW-CSS (p=0.04), but not for IGL-1 or UW-MPS (IGL-1 p= 0.667, UW-MPS p=0.092). The trend for increasing biopsy score when HTK was used was not a significant increase.

When the fold change was calculated (the final score compared to the start score) there was a significant change in the biopsy score for all solutions with each solution reducing the score (IGL-1 p= 0.013, UW-CSS p= 0.001 and UW-MPS p= 0.001) except for HTK when the score actually increased (the score increased in three of the experiments, and

this was due to the vacuolisation score and the sinus score increasing) ($p< 0.005$) (Figure 5.6b).

There was no significant difference in the change of biopsy score when comparing the different solutions.

When the flush out of blood in the biopsy was specifically looked at, all solutions reduced the amount of blood in the organ (figure 5.7a), and this was a significant reduction when UW-CSS and HTK were used (HTK $p= 0.001$, UW-CSS $p=0.004$, IGL-1 $p= 0.057$, UW-MPS p-0.08). When the fold change was calculated, there was a significant difference for all solutions (UW-CSS $p< 0.005$, HTK $p=0.001$, IGL-1 $p=0.036$, UW-MPS $p=0.042$) There was no significant difference when comparing the differences between the solutions $p=0.68$ (Figure 5.7b).

There was no significant difference in the change in score of sinuses, capillaries in septae and the vacuolisation throughout the procedure for any of the solutions, or when comparing the solutions.

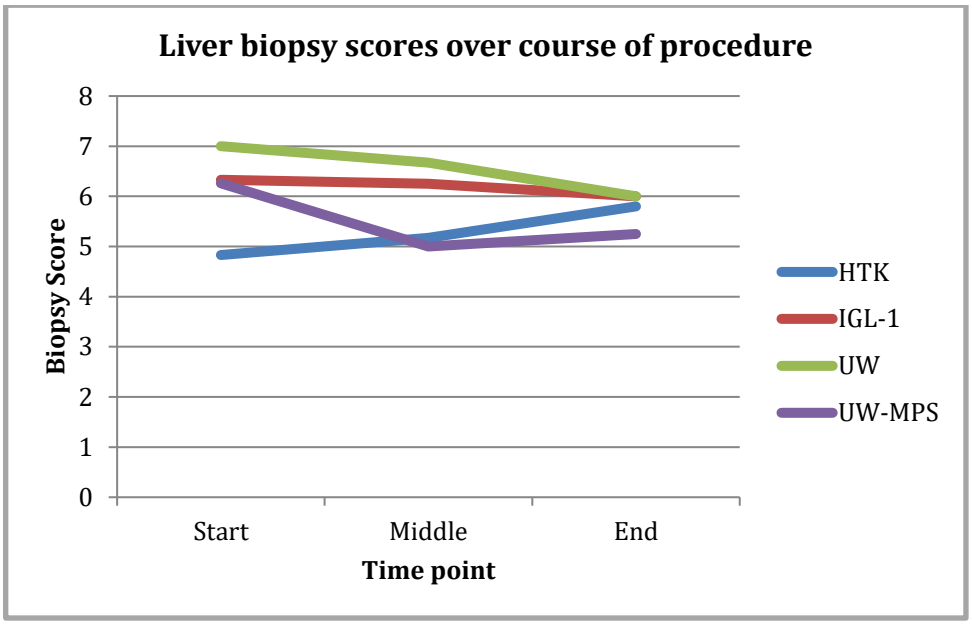


Figure 5.6a Liver biopsy score throughout retrieval procedure. *There is a trend towards an improved liver biopsy score at the end of the procedure compared to the beginning for all of the solutions except HTK where the score increased. This improvement was statistically significant only for UW-CSS (p=0.04) (HTK p=0.9, IGL-1 p= 0.667, UW-MPS p=0.092).*

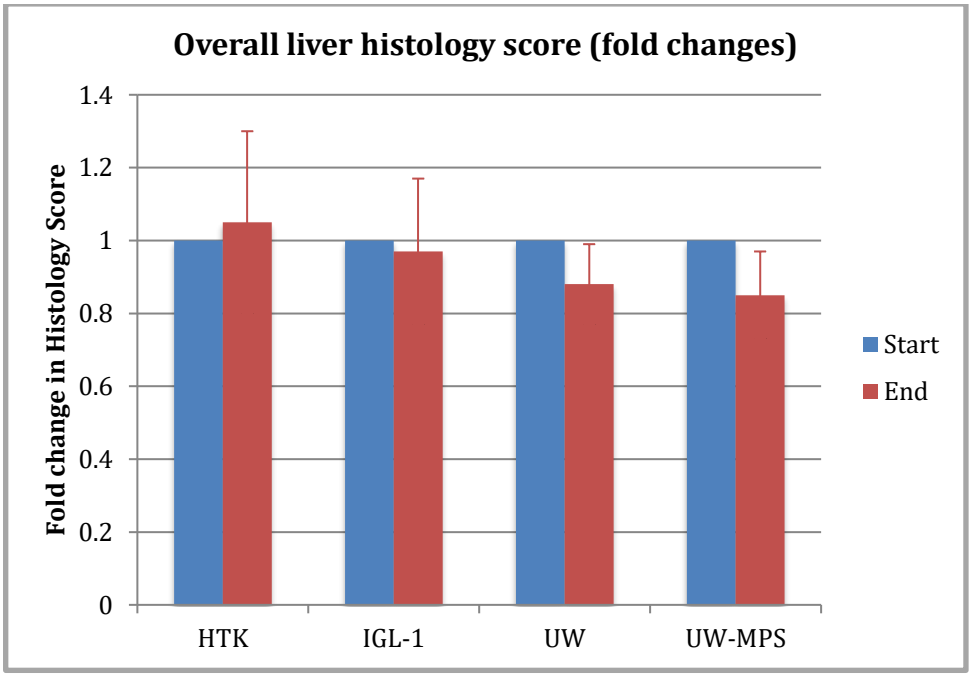


Figure 5.6b Fold change in liver biopsy score at the beginning and end of retrieval procedure. *There is an improved liver biopsy score at the end of the procedure compared to the beginning for all of the solutions except HTK where the score increased (UW-CSS p<0.005, HTK p<0.005, IGL-1 p=0.013 and UW-MPS p= 0.001).*

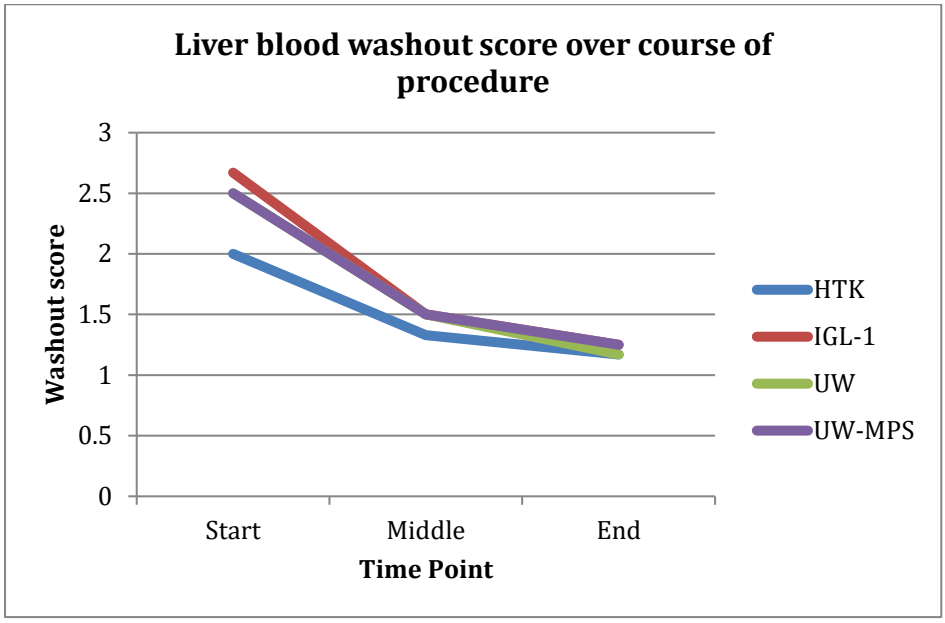


Figure 5.7a Liver blood washout score throughout retrieval procedure. *This shows that all solutions achieve a good washout of blood from the liver throughout the procedure (UW-CSS p= 0.001, HTK p=0.004, IGL-1 p=0.057, UW-MPS p=0.08).*

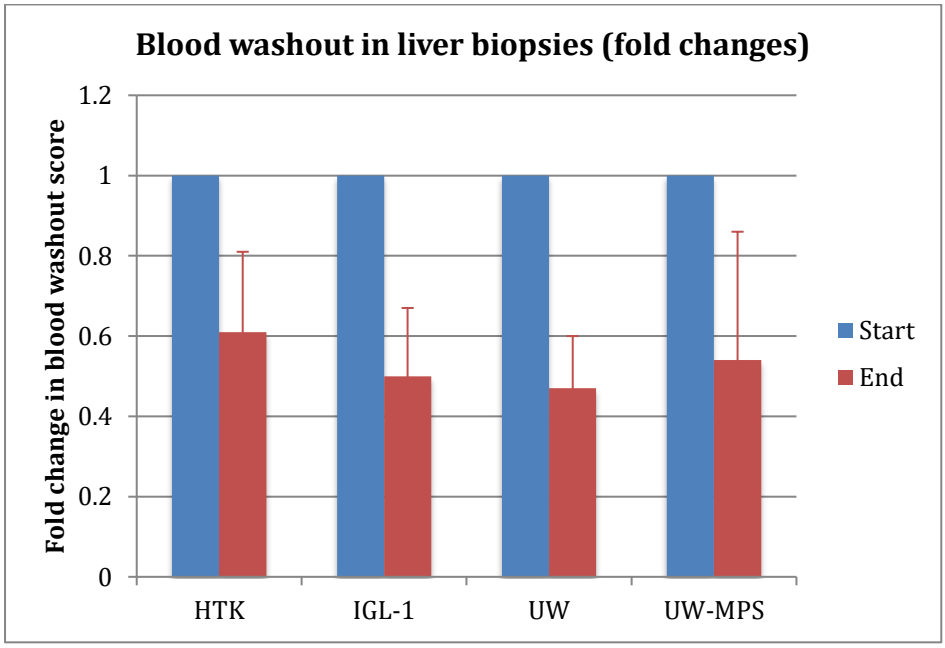


Figure 5.7b Fold change in blood washout score in liver biopsies. *This shows that all solutions achieve a good washout of blood from the liver throughout the procedure (UW-CSS p<0.005, HTK p=0.001, IGL-1 p=0.036, UW-MPS p=0.042).*

Kidney

The overall kidney biopsy score showed a trend for a decrease during the procedure for all solutions (Figure 5.8a). The reduction in the overall score was significant for UW-CSS (p=0.015), but not for HTK, IGL-1 or UW-MPS (HTK p= 0.235, IGL-1 p= 0.9, UW-MPS p=0.319).

When the fold change was calculated there was a significant change in the biopsy score for all solutions with each solution reducing the score (HTK p= 0.001, UW-CSS p= 0.002, IGL-1 p= 0.007 and UW-MPS p= 0.007) (Figure 5.8b).

When the flush out of blood in the biopsy was specifically looked at, all solutions reduced the amount of blood in the organ (figure 5.9a), and this was a significant reduction when UW-CSS, HTK and UW-MPS were used (HTK p= 0.034, UW-CSS p=0.010, UW-MPS p= 0.014), but not when IGL-1 was used (p= 0.80). When the fold change was calculated, there was a significant difference for all solutions (UW-CSS p= 0.021, HTK p=0.008, IGL-1 p=0.042 and UW-MPS p=0.011) (Figure 5.9b).

There was no significant difference in the change in score of vacuolisation, tubular integrity or oedema throughout the procedure for any of the solutions, or when comparing the solutions.

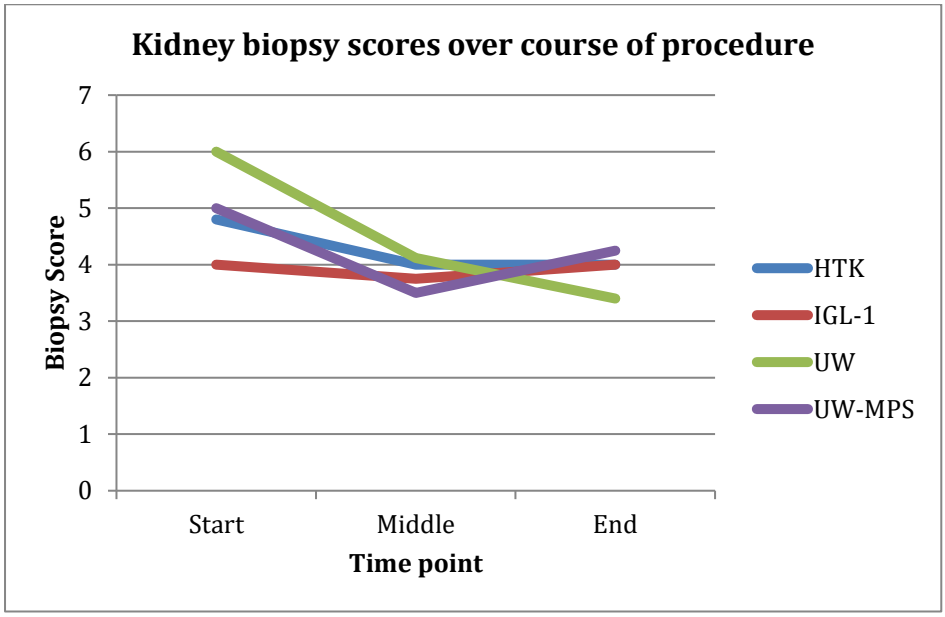


Figure 5.8a Kidney Biopsy score throughout retrieval procedure. *There is a trend towards an improved kidney biopsy score at the end of the procedure compared to the beginning for all of the solutions (UW-CSS p= 0.015, HTK p=0.236, IGL-1 p=0.9, UW-MPS p=0.319).*

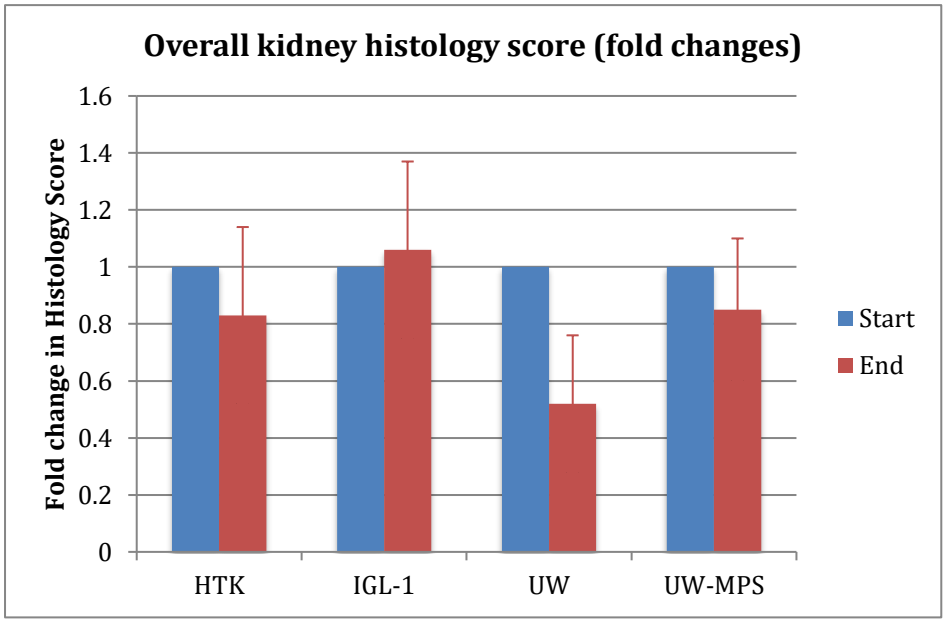


Figure 5.8b Fold change in kidney biopsy score at beginning and end of retrieval procedure. *There is an improved kidney biopsy score at the end of the procedure compared to the beginning for all of the solutions (UW-CSS p= 0.002, HTK p=0.001, IGL-1 p=0.007, UW-MPS p=0.007).*

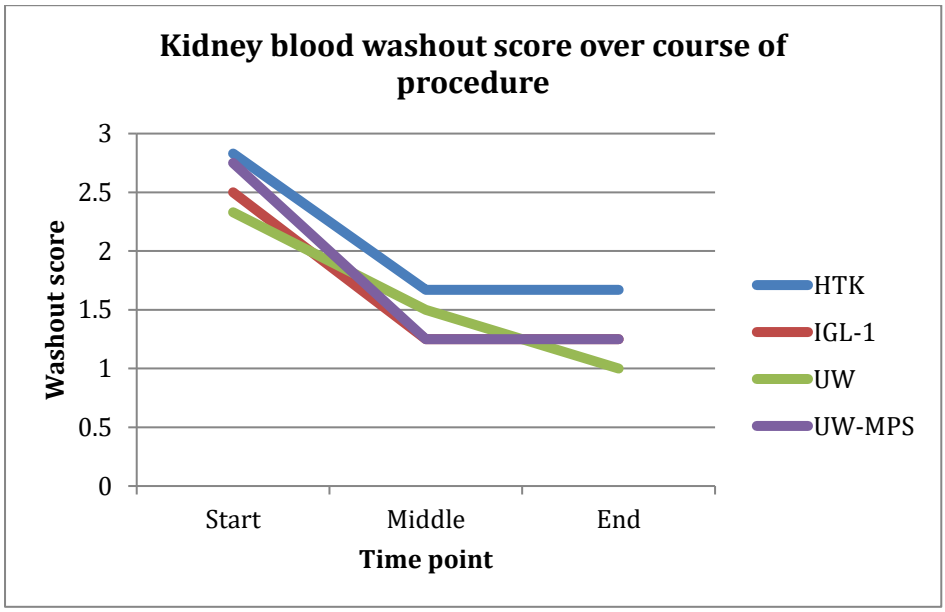


Figure 5.9a Kidney blood washout score throughout retrieval procedure. *This shows that all solutions achieve a good washout of blood from the kidney throughout the procedure (UW-CSS p= 0.010, HTK p=0.034, IGL-1 p=0.08, UW-MPS p=0.014).*

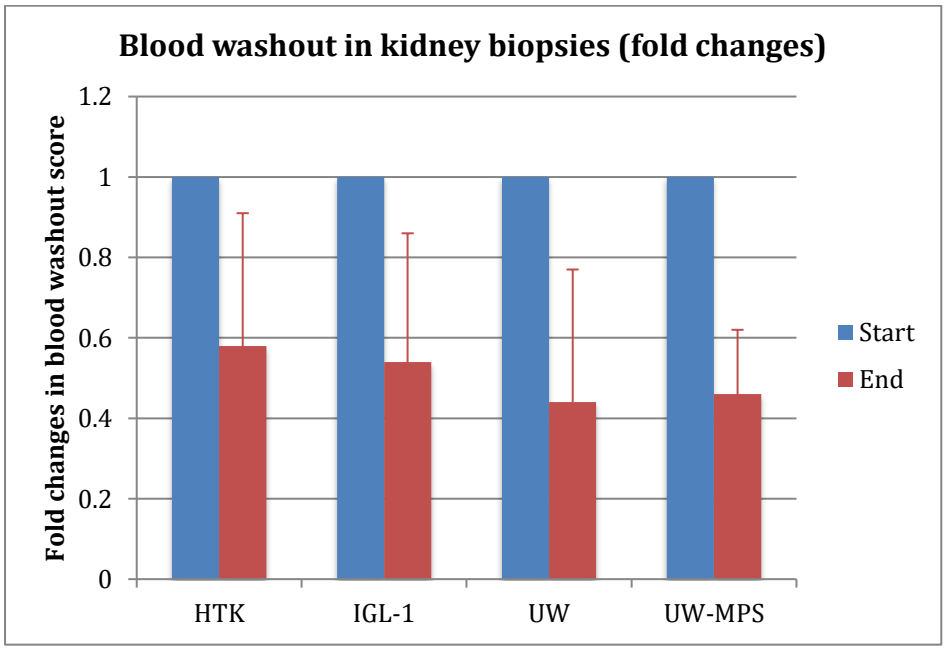


Figure 5.9b Fold change in blood washout score in kidney biopsies. *This shows that all solutions achieve a good washout of blood from the kidney throughout the procedure (UW-CSS p= 0.021, HTK p=0.008, IGL-1 p=0.042, UW-MPS p=0.011).*

5.4.5 Electron Microscopy

TEM was performed on eight renal biopsies. Two renal biopsies taken after 30 minutes of warm ischaemia (start biopsies), and six biopsies taken at the end of perfusion (three from kidneys flushed with UW-CSS and three from kidneys flushed with HTK). The start biopsies were variable in their findings. In one the structure of all components was preserved, and there were only some lipid droplet formation and inflammatory cells. In the other, there was shrinkage of the cells, and the glomerulus was unrecognisable. Post-perfusion with UW-CSS the biopsy of this kidney showed that the glomerulus had recovered with normal features and foot processes, while the proximal tubules looked intact and mitochondria were preserved. The other two biopsies from kidneys perfused with UW-CSS showed reasonable preservation of the glomerulus, although the tubules did have some shrinkage. The mitochondria structure was preserved.

Two of the biopsies from kidneys preserved with HTK had swelling of the glomerulus with loss of structure and more profound shrinkage of the cells with opening of the tubules. One of the biopsies showed that the cells were in tact with minimal shrinkage and there was preserved structure of the glomerulus.

5.4.6 Markers of Injury

5.4.6.1 AST and LDH in perfusate

Liver

The concentration of LDH measured in the effluent perfusate from the liver showed a trend of increasing concentrations for IGL-1 and stayed fairly constant or reduced slightly for UW-CSS, HTK and UW-MPS (figure 5.10a), however, this was not statistically significant (UW-CSS p=0.14, HTK p=0.97, IGL-1 p=0.41 and UW-MPS p=0.95). When the

fold change was calculated (the final concentration was compared to the initial concentration) there was a significant change in the concentration of LDH in the liver effluent when UW-CSS was used for the flush with a reduction in the concentration ($p=0.04$). There was no significant difference for the fold changes for any of the other solutions (HTK $p=0.1$, IGL-1 $p=0.14$, UW-MPS $p=0.21$). There was no significant difference when comparing the fold changes between the solutions $p=0.61$ (Figure 5.10b).

The concentration of AST in the liver effluent perfusate showed a trend of increasing over the time of the retrieval for all solutions (Figure 5.11a), but again this was not statistically significant (UW-CSS $p=0.43$, HTK $p=0.12$, IGL-1 $p=0.18$, UW-MPS $p=0.41$). When the fold change was calculated, there was no significant difference for any of the solutions (UW-CSS $p= 0.18$, HTK $p=0.09$, IGL-1 $p=0.29$, UW-MPS $p=0.25$) or when comparing the differences between the solutions $p=0.68$ (Figure 5.11b).

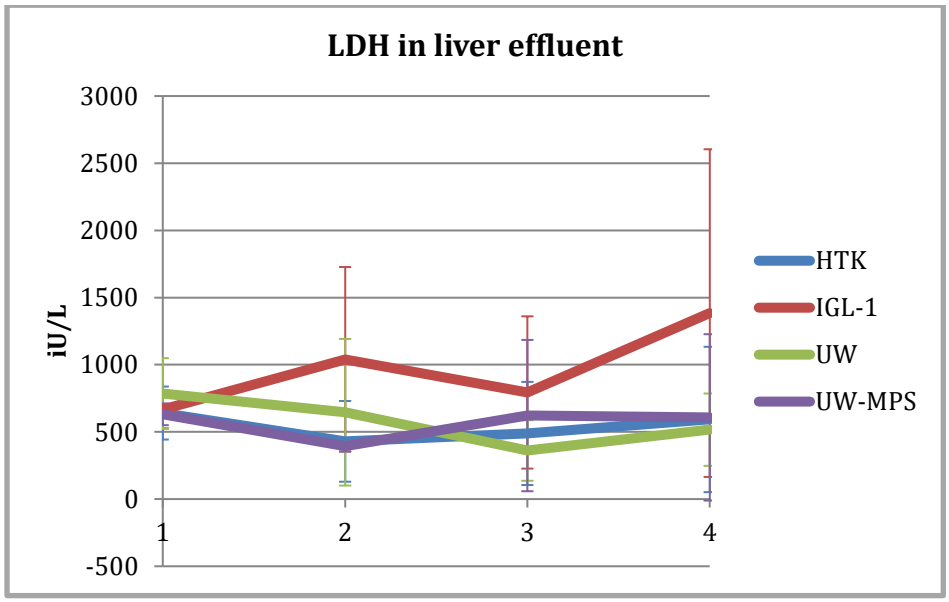


Figure 5.10a Concentration of LDH in the liver effluent throughout the retrieval procedure. *The concentration of LDH in the liver effluent showed a trend to increase throughout the procedure for IGL-1 and stayed fairly constant for the other solutions (UW-CSS p=0.14, HTK p= 0.97, IGL-1 p=0.41, UW-MPS p=0.95).*

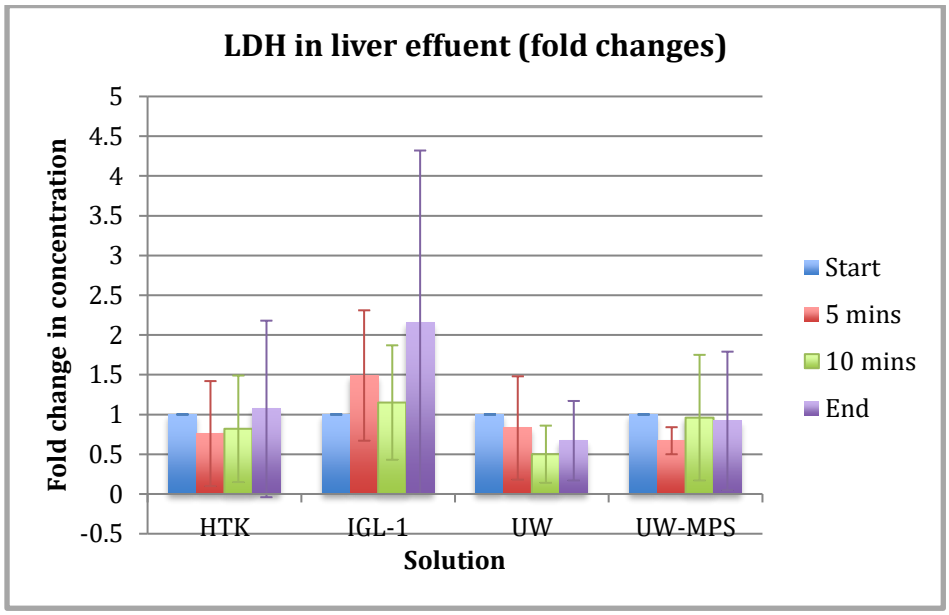


Figure 5.10b Fold change in concentration of LDH in Liver effluent throughout retrieval procedure. *This shows that the concentration of LDH in the liver effluent reduced throughout the procedure for UW-CSS (p=0.04) and stayed fairly constant for the other solutions (HTK p= 0.1, IGL-1 p=0.14, UW-MPS p=0.21) when the fold change was calculated.*

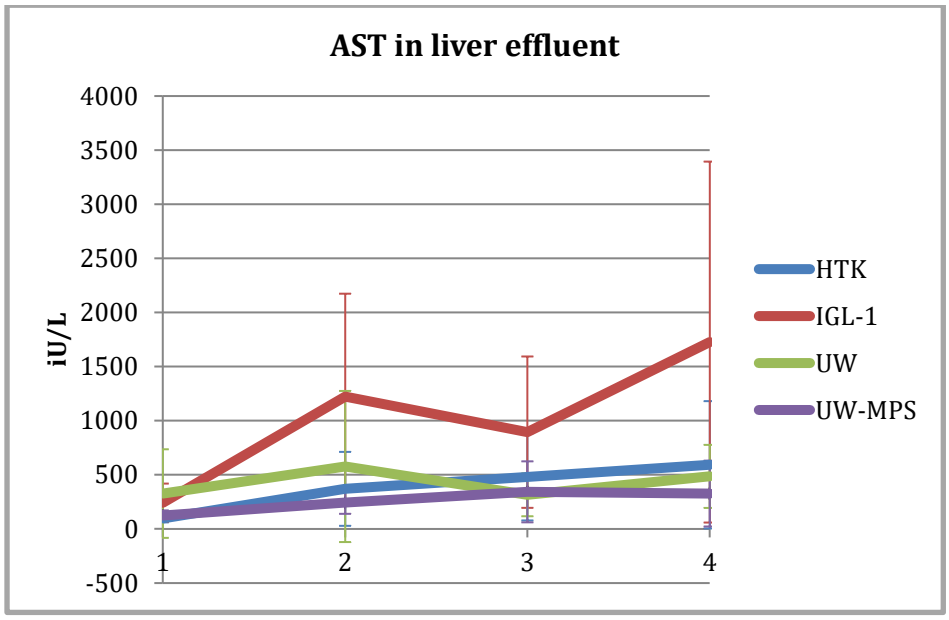


Figure 5.11a Concentration of AST in the liver effluent throughout retrieval procedure. *The concentration of AST in the liver effluent showed a trend to increase throughout the procedure for all solutions (UW-CSS p=0.43, HTK p= 0.12, IGL-1 p=0.18, UW-MPS p=0.41).*

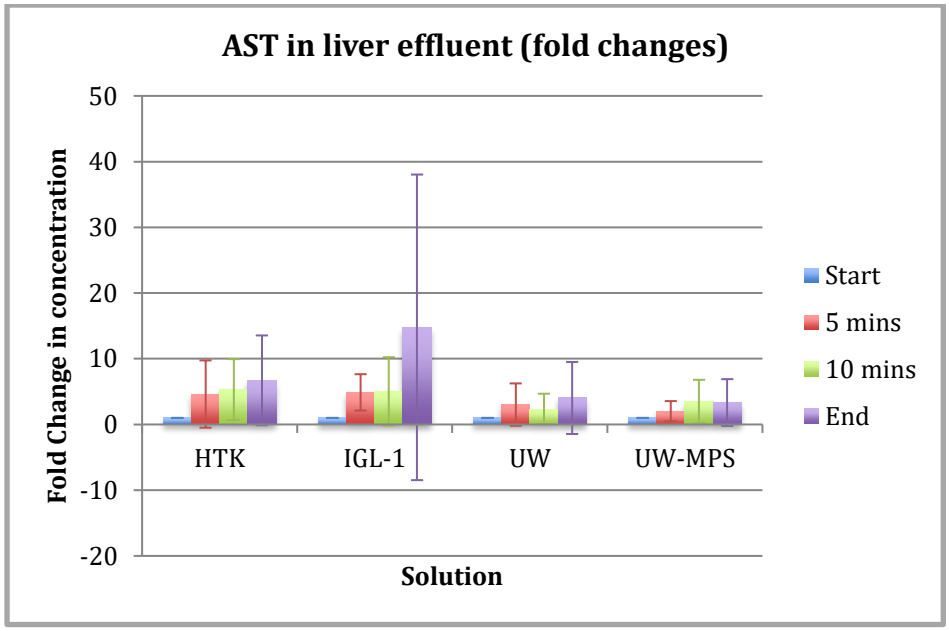


Figure 5.11b Fold change in concentration of AST in liver effluent throughout retrieval procedure. *This shows that there is no significant change in the AST concentration in the liver effluent throughout the procedure (UW-CSS p=0.18, HTK p= 0.09, IGL-1 p=0.29, UW-MPS p=0.25) when the fold change was calculated.*

Kidney

The concentration of LDH measured in the effluent perfusate from the kidney decreased throughout the course of the retrieval for all solutions (Figure 5.12a), and this reduction was significant for UW-CSS, HTK and IGL-1 (p=0.001, 0.03 and 0.005 resp). When the fold changes were compared this was also statistically significant for UW-CSS, HTK and IGL-1 but not UW-MPS (UW-CSS p=0.07, HTK p=0.02, IGL-1 p=0.03, UW-MPS p=0.09). There was no difference in the fold changes between the solutions (p=0.40) (Figure 5.12b).

The concentration of AST in the kidney effluent perfusate reduced during the course of the procedure (Figure 5.13a), and this was statistically significant for HTK and UW-MPS (p=0.007 and 0.02 resp.), but not for UW-CSS or IGL-1 (p=0.12 and 0.08 resp.) The fold change in the concentration of AST showed a trend of decreasing over the time of the retrieval, but this did not reach statistical significance (UW-CSS p=0.05, HTK p=0.05, IGL-1 p=0.07, UW-MPS p=0.92). This was not statistically different between the solutions (p=0.88) (Figure 5.13b).

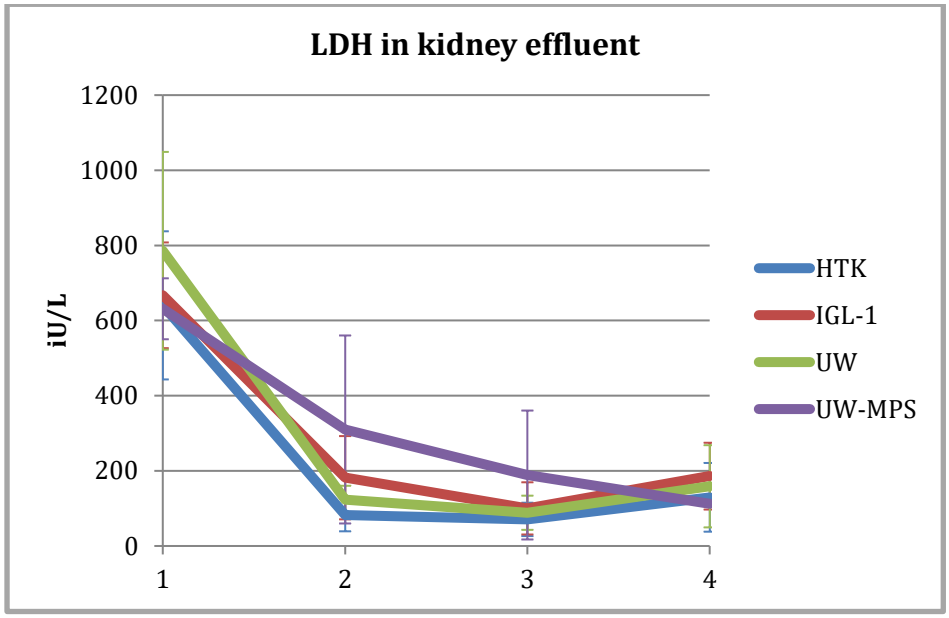


Figure 5.12a Concentration of LDH in the kidney effluent throughout retrieval procedure. *The concentration of LDH in the kidney effluent reduced throughout the procedure for all solutions (UW-CSS p=0.001, HTK p= 0.03, IGL-1 p=0.005 and UW-MPS p=0.1).*

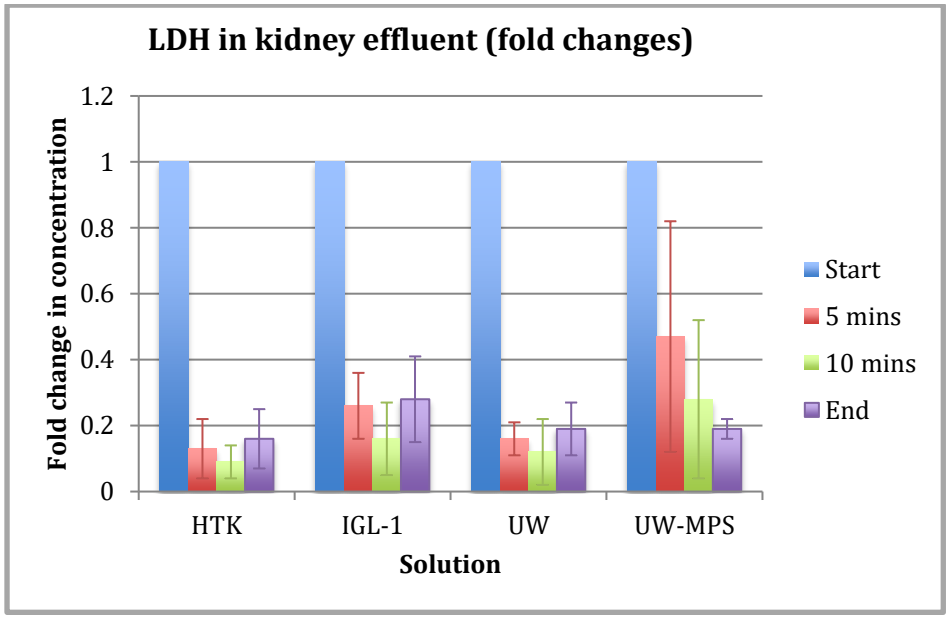


Figure 5.12b Fold change in concentration of LDH in kidney effluent throughout retrieval procedure. *The concentration of LDH in the kidney effluent reduced throughout the procedure for all solutions (UW-CSS p=0.007, HTK p= 0.02, IGL-1 p=0.03, UW-MPS p=0.09) when the fold change was calculated.*

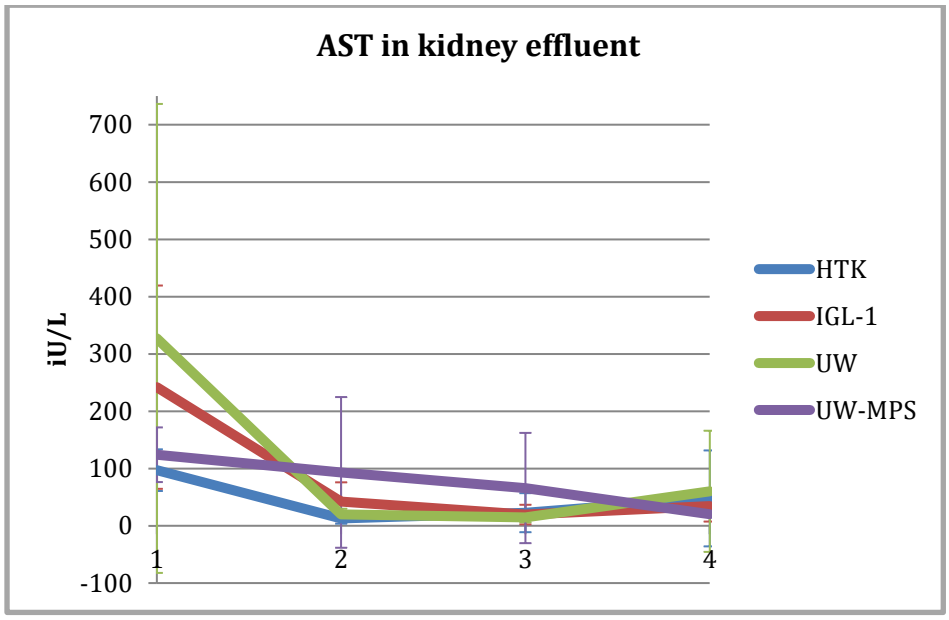


Figure 5.13a Concentration of AST in kidney effluent throughout retrieval procedure. *The concentration of AST in the kidney effluent reduced throughout the procedure for all solutions, although this did not reach significance for UW-CSS or IGL-1 (UW-CSS p=0.12, HTK p= 0.007, IGL-1 p=0.08, UW-MPS p=0.02).*

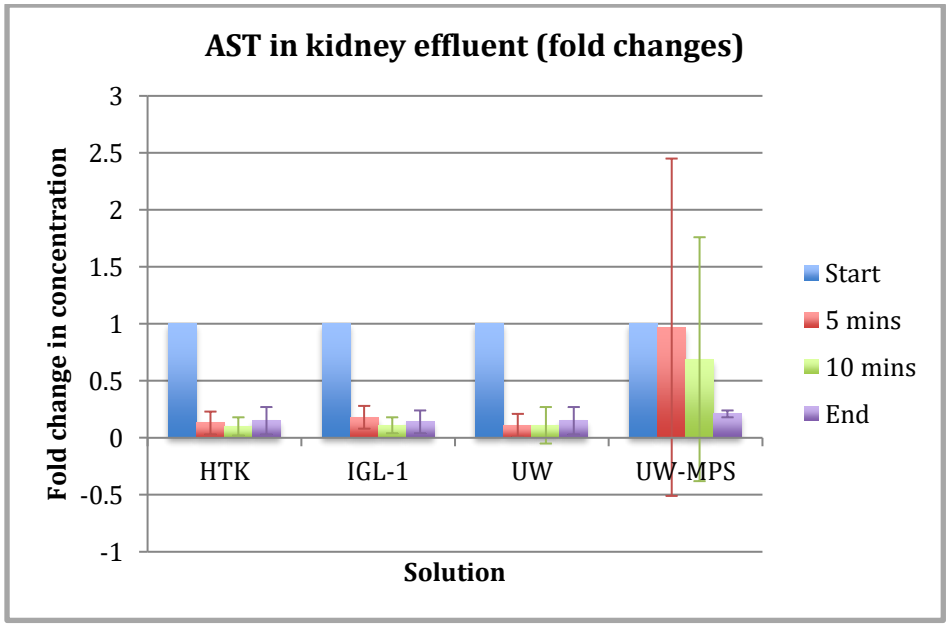


Figure 5.13b Fold change of AST concentration in kidney effluent throughout retrieval procedure. *The concentration of AST in the kidney effluent reduced throughout the procedure for all solutions although this did not reach significance (UW-CSS p=0.054, HTK p= 0.05, IGL-1 p=0.07, UW-MPS p=0.92) when the fold change was calculated.*

5.4.6.2 Western Blotting

Biopsies were collected from the liver and kidney at three time points:

i. at the beginning of the perfusion after 30 min warm ischaemia
ii. during flush out after opening the third 1L or 2L bag of solution
iii. at the end of the flush out.

Each sample was analysed for the presence of markers of oxidative stress (HSP 70, 70 kDa and 4-HNE-modified proteins, 150 kDa) and apoptosis (cleaved Caspase 9 36 kDa/pro-Caspase 9 46kDa ratio).

Following normalisation to an internal control ran in every gel, it was possible to compare the markers fold change for the four different solutions.

Liver

4-HNE, levels did not change significantly over the course of the retrieval for any of the solutions, although there was a trend for the levels to decrease in the UW-CSS and IGL-1 groups (UW-CSS p=0.065, HTK p=0.903, IGL-1 p=0.074, UW-MPS p=0.653) (Figure 5.14a). When the fold changes were analysed, there was a significant difference for the UW-CSS, HTK and IGL-1 solutions (UW-CSS p=0.009, HTK p=0.007, IGL-1 p=0.049, UW-MPS p=0.167) (Figure 5.14b).

HSP-70 levels did not change significantly over the course of the retrieval for any of the solutions, although again there was a trend towards a decrease in the IGL-1 group (UW-CSS p=0.337, HTK p=0.398, IGL-1 p=0.061, UW-MPS u=0.761) (Figure 5.15a). When the fold changes were analysed, there was a significant difference for the UW-CSS and HTK solutions (UW-CSS p=0.001, HTK p=0.002, IGL-1 p=0.071, UW-MPS p=0.142) (Figure 5.15b).

Caspase 9 levels also did not significantly reduce over the course of the retrieval for any of the solutions (UW-CSS p=0.662, HTK p=0.517, IGL-1 p=0.906, UW-MPS p=0.426) (Figure 5.16a). When the fold changes were analysed, there was a significant difference for the UW-CSS and HTK solutions (UW-CSS p=0.001, HTK p<0.001, IGL-1 p=0.152, UW-MPS p=0.183) (Figure 5.16b).

There was no difference between the levels for the different solutions (4-HNE p=0.194, HSP-70 p=0.259, Caspase 9 p=0.821).

Figures I – N in Appendix B show the western blot figures.

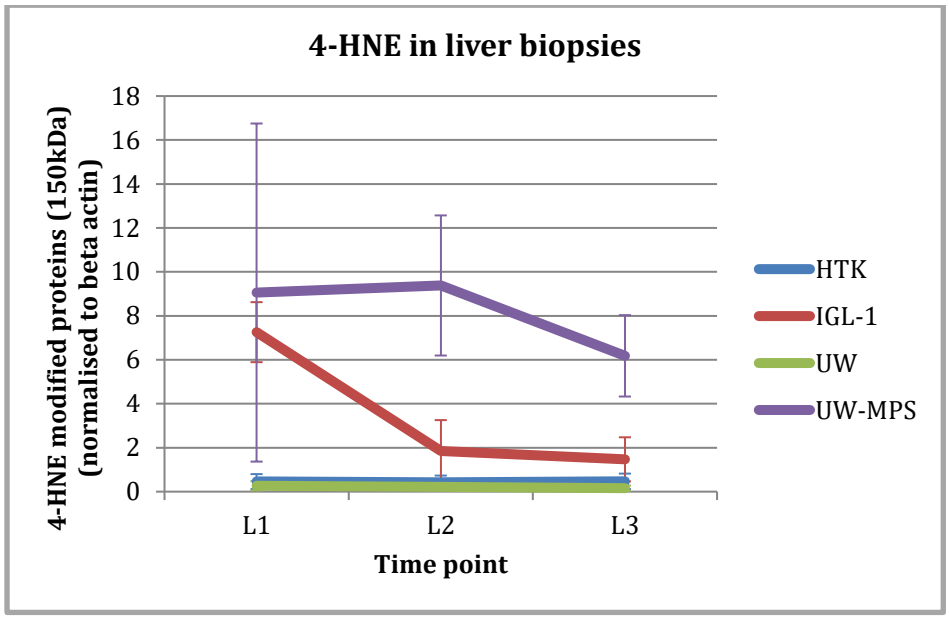


Figure 5.14a WB quantification of 4-HNE modified proteins in liver biopsies. *There was no significant change in the concentrations of 4-HNE throughout the procedure for any of the solutions (UW-CSS p= 0.065, HTK p=0.903, IGL-1 p=0.074, UW-MPS p=0.653).*

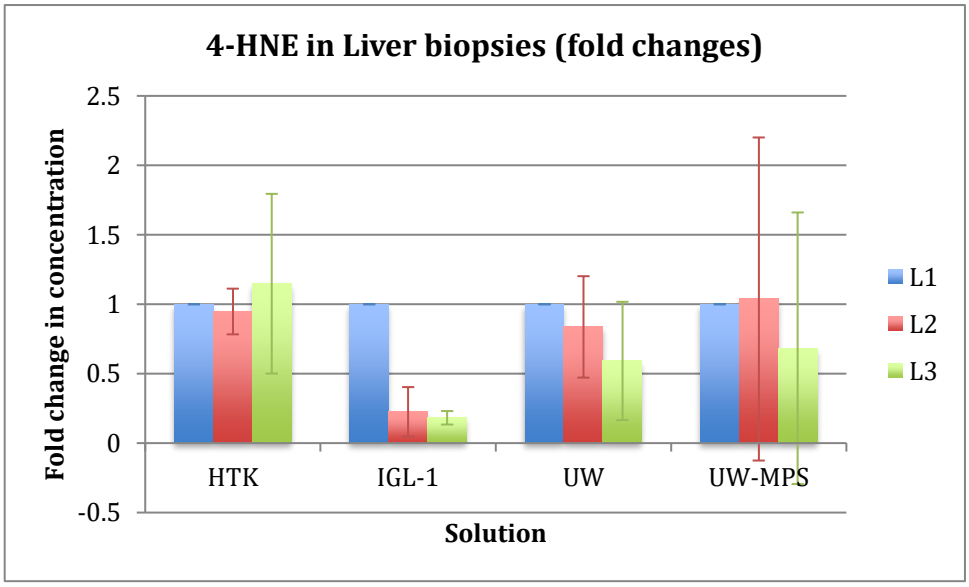


Figure 5.14b Fold change of 4-HNE modified proteins in liver biopsies. *This shows there was a reduction in the concentration of 4-HNE in the liver effluent between the start and the end of the procedure for UW-CSS, IGL-1 and UW-MPS (p=0.009, 0.049 and 0.167 resp), and there was an increase for HTK (p=0.007) when the fold change was calculated.*

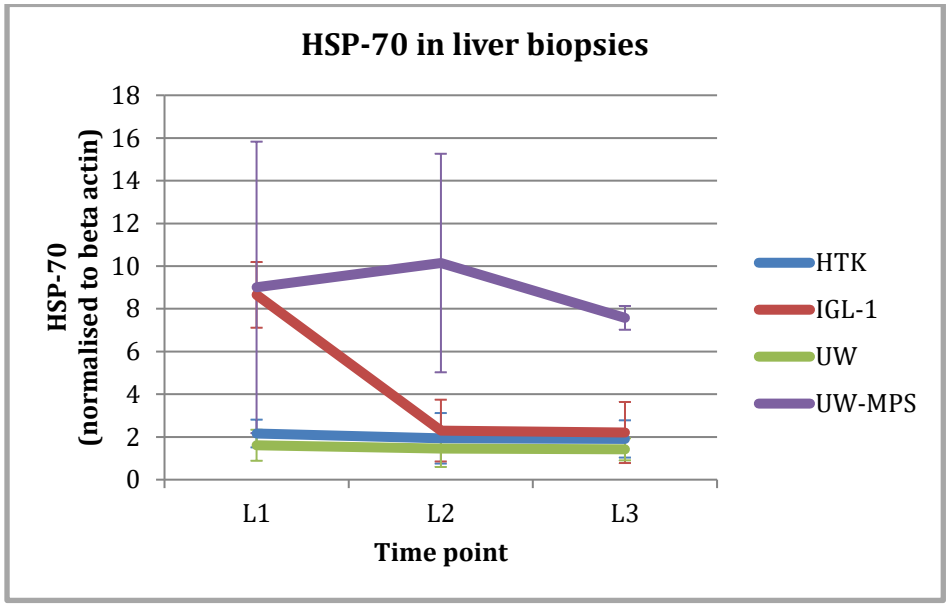


Figure 5.15a WB quantification of HSP-70 in liver biopsies. *There was no significant change in the concentrations of HSP-70 throughout the procedure for any of the solutions (UW p=0.337, HTK p=0.398, IGL-1 p=0.061, UW-MPS p=0.761).*

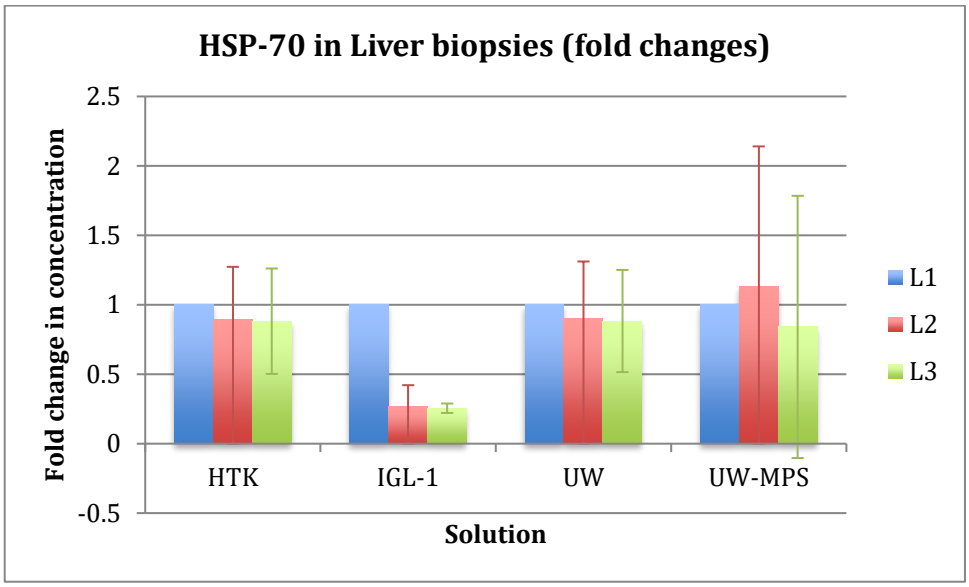


Figure 5.15b Fold changes of HSP-70 in liver biopsies. *This shows there was a statistically significant reduction in the concentration of HSP-70 in the liver effluent for UW-CSS and HTK (p=0.001 and 0.002 resp) and although there was a reduction for IGL-1 and UW-MPS, this was not statistically significant (p=0.152 and 0.183 resp) when the fold change was calculated.*

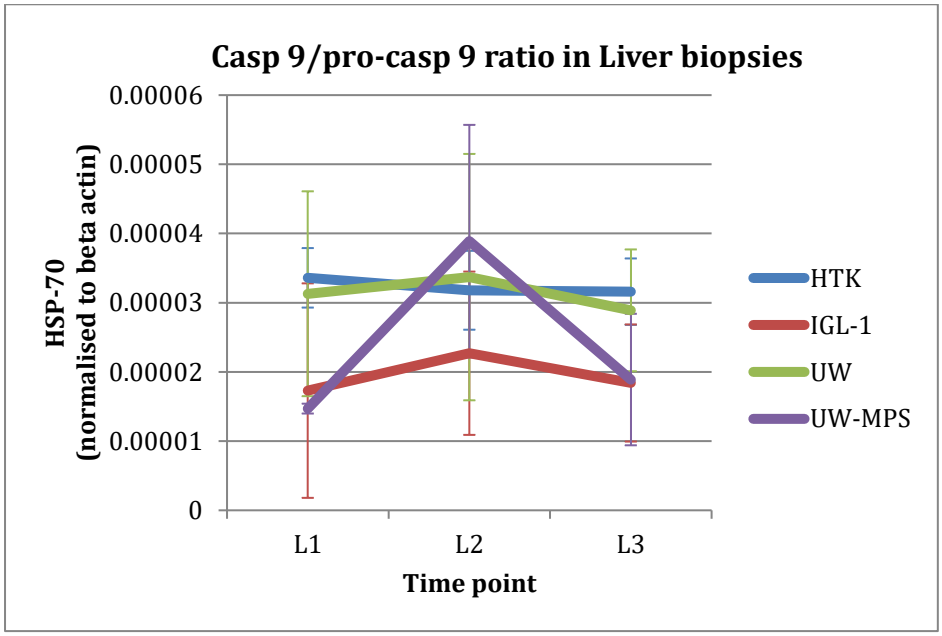


Figure 5.16a WB quantification of Caspase 9/Pro-Caspase 9 in liver biopsies. *There was no significant change in the concentrations of the Caspase9/pro-caspase 9 ratio throughout the procedure for any of the solutions (UW p=0.662, HTK p=0.517, IGL-1 p=0.906, UW-MPS p=0.426).*

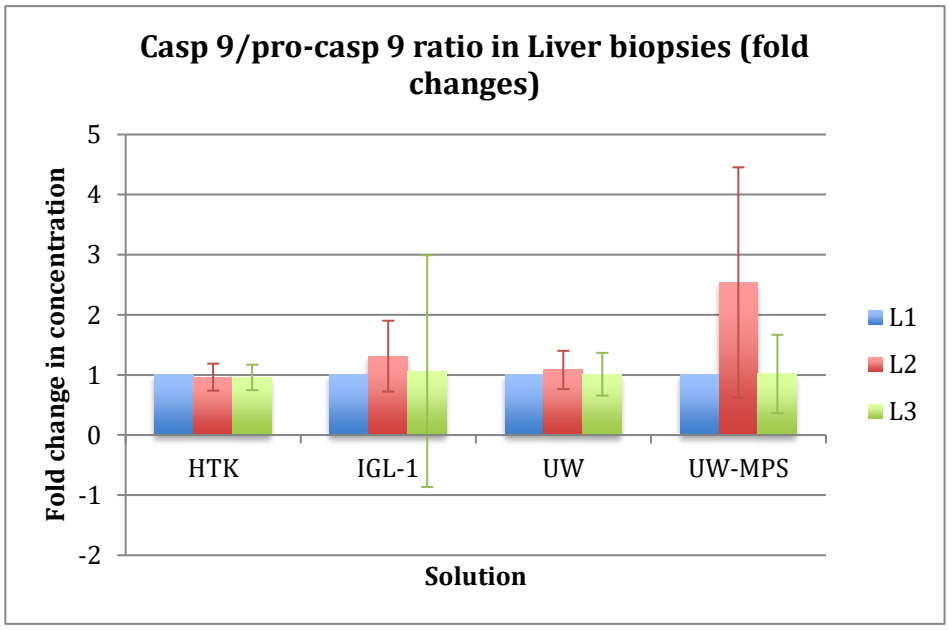


Figure 5.16b Fold changes of Caspase 9/Pro-Caspase 9 in liver biopsies. *This shows there was a statistically significant reduction in the concentration of Caspase 9/pro-caspase ratio in the liver effluent for UW-CSS and HTK (p=0.001 and <0.005 resp) and although there was a reduction for IGL-1 and UW-MPS, this was not statistically significant (p=0.152 and 0.183 resp) when the fold change was calculated.*

Kidney

4-HNE, levels did not change significantly over the course of the retrieval for any of the solutions (UW-CSS p=0.190, HTK p=0.209, IGL-1 p=0.825, UW-MPS p=0.686) (Figure 5.17a). When the fold changes were analysed, there was a significant difference for the UW-CSS, HTK and UW-MPS solutions (UW-CSS p=0.014, HTK p<0.001, IGL-1 p=0.120, UW-MPS p=0.008) (Figure 5.17b).

HSP-70 levels did not change significantly over the course of the retrieval for any of the solutions (UW-CSS p=0.248, HTK p=0.313, IGL-1 p=0.539, UW-MPS p=0.473) (Figure 5.18a). When the fold changes were analysed, there was a significant difference for all solutions (UW-CSS p=0.005, HTK p=0.001, IGL-1 p=0.034, UW-MPS p=0.003) (Figure 5.18b).

Caspase 9 levels also did not change significantly over the course of the retrieval for any of the solutions except IGL-1 when it increased (UW-CSS p=0.764, HTK p=0.899, IGL-1 p=0.021, UW-MPS p=0.379) (Figure 5.19a). When the fold changes were analysed, there was a significant difference for IGL-1 (UW-CSS p=0.187, HTK p=0.074, IGL-1 p=0.006, UW-MPS p=0.063) (Figure 5.19b).

There was no difference between the levels for the different solutions (4-HNE p=0.861, HSP-70 p=0.497, Caspase 9 p=0.437).

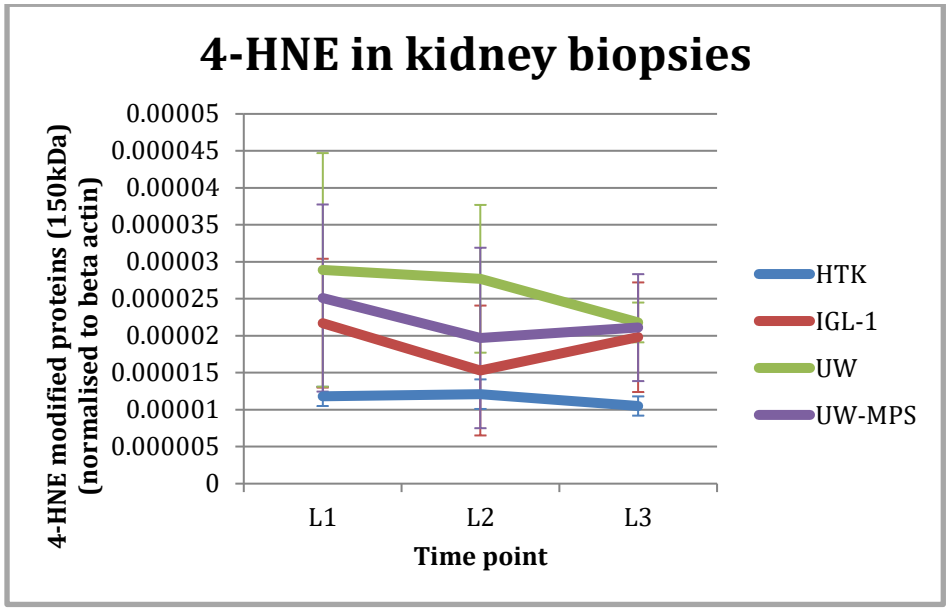


Figure 5.17a WB quantification of 4-HNE modified proteins in kidney biopsies. *There was no significant change in the concentrations of 4-HNE throughout the procedure for any of the solutions (UW-CSS p= 0.190, HTK p=0.209, IGL-1 p=0.825, UW-MPS p=0.686).*

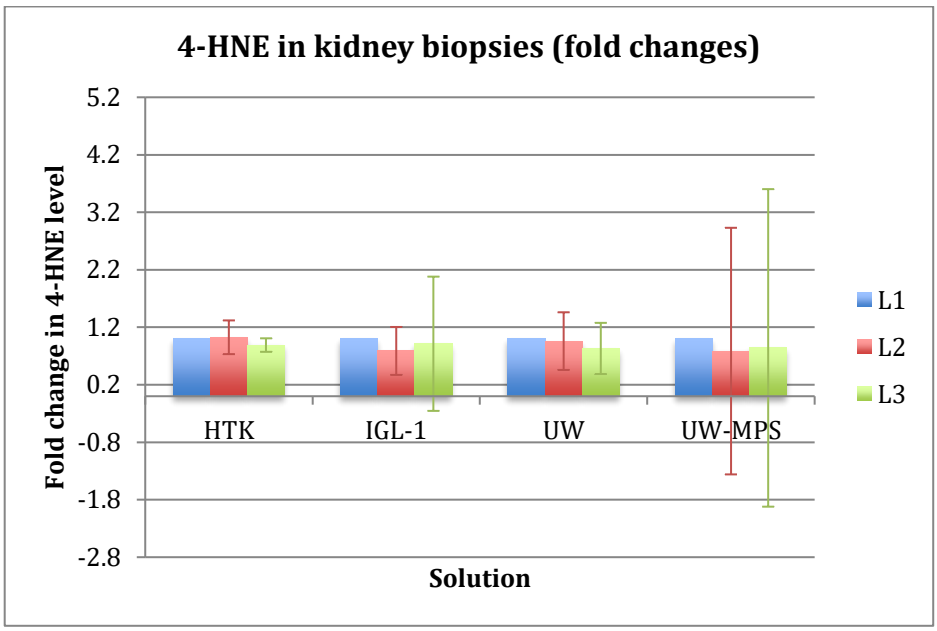


Figure 5.17b Fold change of 4-HNE modified proteins in kidney biopsies. *This shows there was a significant reduction in the concentration of 4-HNE in the kidney effluent between the start and the end of the procedure for UW-CSS, HTK and UW-MPS (p=0.014, < 0.0005 and 0.008 resp), but the reduction was not significant for IGL-1 (p=0.120) when the fold change was calculated.*

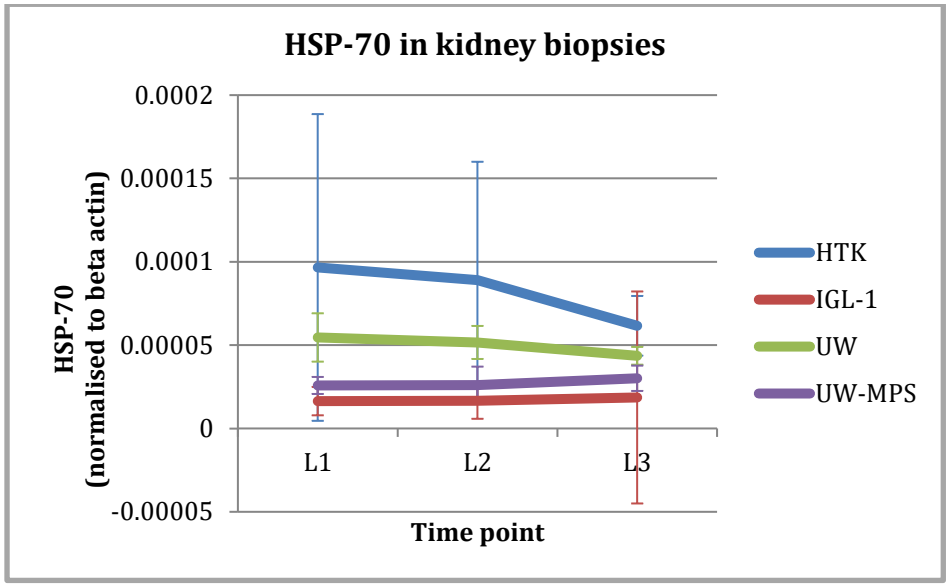


Figure 5.18a WB quantification of HSP-70 in kidney biopsies. *There was no significant change in the concentrations of HSP-70 throughout the procedure for any of the solutions (UW-CSS p= 0.248, HTK p=0.313, IGL-1 p=0.539, UW-MPS p=0.473).*

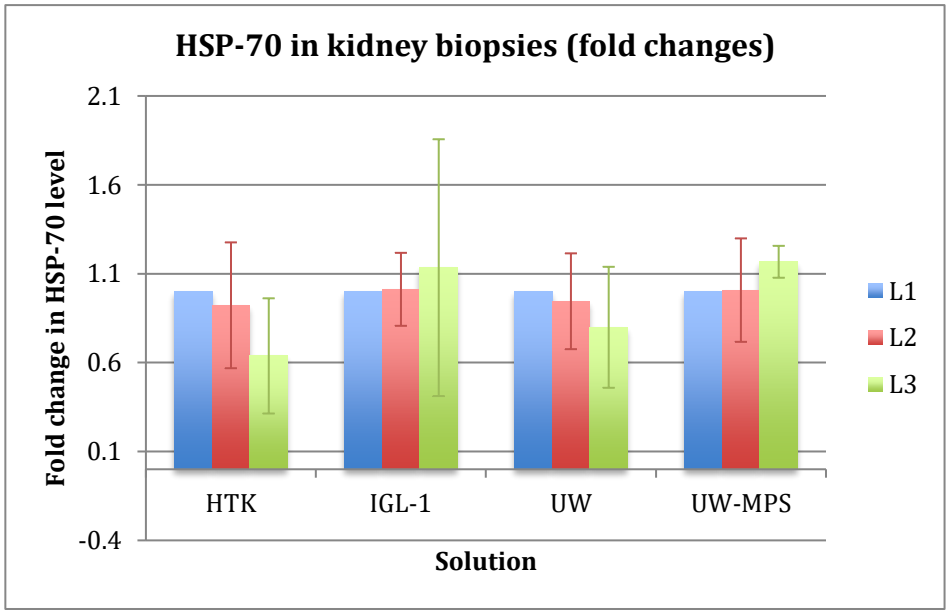


Figure 5.18b Fold changes of HSP-70 in kidney biopsies. *This shows there was a significant reduction in the concentration of HSP-70 in the kidney effluent between the start and the end of the procedure for UW-CSS and HTK (p=0.005 and 0.001 resp), but the concentration increased for IGL-1 and UW-MPS (p=0.034 and 0.003 resp) when the fold change was calculated.*

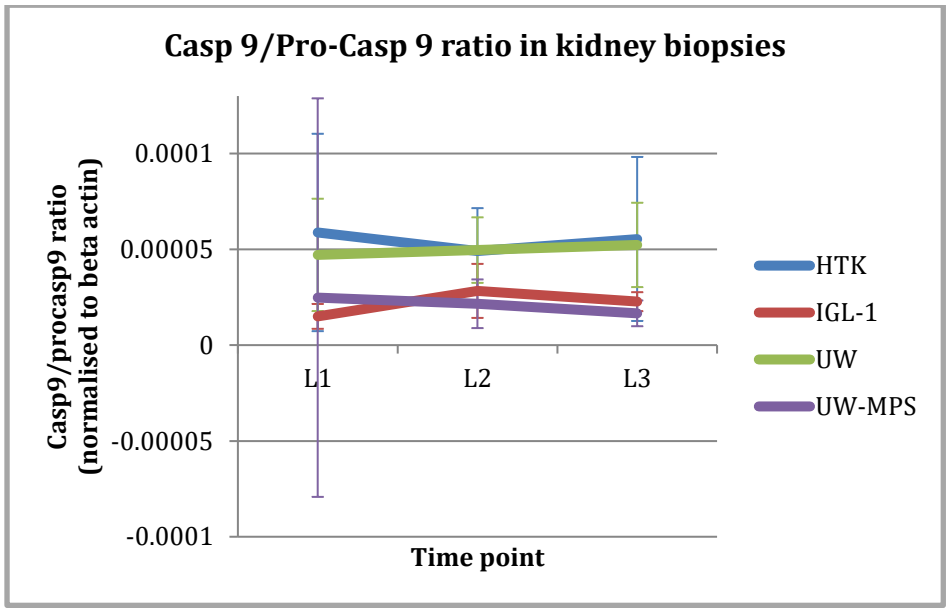


Figure 5.19a WB quantification of Caspase 9/Pro Caspase ratio in kidney biopsies. *There was no significant change in the concentrations of the caspase 9/pro-caspase9 ratio throughout the procedure for any of the solutions (UW-CSS p= 0.764, HTK p=0.899 and UW-MPS p=0.379) except IGL-1 where there was an increase (p=0.021).*

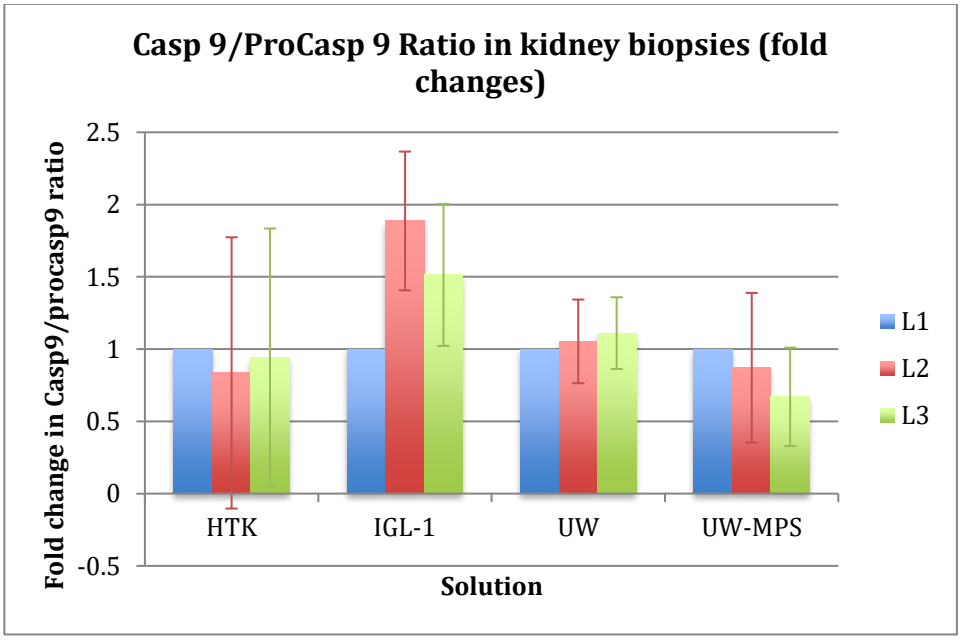


Figure 5.19b Fold change of Caspase 9/Pro-Caspase 9 ratio in kidney biopsies. *This shows there was a reduction in the concentration of HSP-70 in the kidney effluent between the start and the end of the procedure for HTK and UW-MPS (p=0.074 and p=0.063 resp) but the concentration increased for UW-CSS and IGL-1 and IGL-1 (p=0.187 and 0.006 resp) when the fold change was calculated.*

5.4.6.3 Immunohistochemistry

Unfortunately, the analysis of the IHC slides showed high background staining (when both enzymatic and heat induced antigen retrieval was used) and so no conclusive results could be drawn from these slides.

5.5 Discussion

In the past it has been suggested that hypothermic preservation solutions with a higher viscosity such as e.g. the UW solution which is currently the standard cold storage solution for many organs and used by many transplant centres have a detrimental effect on the flush-out and perfusion of donor organs rendering them prone to suboptimal preservation conditions and therefore possibly lower viability at time of transplantation. Although this appears to be merely a perception as there is a lack of evidence for this, as a result, all sort of additional precautions have been considered, e.g. the requirement to flush out using high intra-aortic pressures that can also potentially harm the endothelium under cold conditions. The aim of this study was to investigate whether there is indeed a difference in the efficacy of initial perfusion, and the achieved cooling of donor organs between the most popular preservation solutions used in clinical practice. In addition, whether there was a difference observed at the microcirculation and cellular level of the donor organ. The model used for this study simulated the clinical practice of DCD retrieval to create a more challenging condition while maintaining quality. The study question was considered important and relevant as over the past many decades' surgeons have claimed that preservation solutions with higher viscosity do not perfuse the microcirculation of organs well, and will affect viability of the graft to be (152, 153).

The findings in this study show that all the solutions, whether they are colloid containing or not, cool the liver and kidney, but none of them cool the organs to the desired temperature of below 5°C. Lower temperatures are preferable as the metabolic rate of the organ halves with every 10°C drop in temperature, protecting the cells by lowering their energy demands (as described in section 1.2.1) (84, 85). All solutions, irrespective

of their viscosity and components, achieve good perfusion of the microcirculation in both the liver and the kidney.

This model was able to demonstrate that in spite of its viscosity, UW-CSS cools the kidney to a significantly lower temperature than HTK (15.13°C vs 20.31°C), and that UW-CSS and IGL-1 achieve statistically significantly better perfusion of the kidney microcirculation than HTK and UW-MPS.

This study found no significant difference in the temperature that the liver reached when comparing all the solutions, but HTK was less efficient at cooling the kidney.

In the past, Kay et al demonstrated that UW-CSS effectively cools the kidney, but more slowly than hyperosmolar citrate (HOC) and they hypothesised that the higher viscosity of UW-CSS solution improved the heat transfer per unit volume meaning that less solution is needed (146). This may also help to explain the findings in this study for the kidney, although this was not the case in the liver. Due to its much lower viscosity HTK may flow quickly through the aorta past the renal arteries and straight to the liver where there is a large and low resistance vascular bed. Therefore, the volume of solution that reaches the liver is enough to cool the liver effectively, but consequently not enough of the solution is reaching both kidneys to also sufficiently cool them, while UW in its more viscous state will enter both renal arteries as well with sufficient solution to perfuse and cool both kidneys and liver. This may also explain why the perfusion of the kidney microvasculature was significantly less when comparing HTK versus UW-CSS.

Our study agrees with Hosgood et al that the colloid in UW-CSS leads to improved heat transfer allowing less solution to be used, as only five litres of UW-CSS through the aorta was required to reach a significantly lower temperature in the kidneys compared to ten

litres of HTK. This also is supported by the work that Bretschneider originally did when developing HTK. HTK was initially intended for cardiac surgery, but was found to be effective in both liver and kidney preservation. The main benefit of this solution is the large buffering capacity that it has (154). However, as Bretschneider argued, this means that larger quantities are required to reduce the temperature of the organs. In contrast to the concept developed and published by Bretschneider and his group, many centres now tend to use lower volumes of HTK (3-4L) (155) instead of the original 10-15L HTK used to reduce cost. This study, however, has raised concerns that even with the use of 10L HTK, the kidneys are not cooled sufficiently and therefore the use of smaller volumes may be detrimental to outcomes. In addition, it also violates the Bretschneider concept that for optimal buffering capacity a large volume of HTK is mandatory.

Most evidence is focused on the use of UW-CSS and HTK. However, our study also included IGL-1 as a more recently introduced solution that is currently used by more centres as a preservation solution. The major difference between US-CSS and IGL-1 is the use of polyethylene Glycol (PEG) instead of HES as the colloid. We found that IGL-1 cooled the kidney and liver as well as UW-CSS and achieved as effective perfusion of the microcirculation as UW-CSS. Although there was no significant difference in the temperature of the kidney when comparing IGL-1 to HTK, this may have been due to smaller numbers in the IGL-1 group. This seems to fit with the findings of a couple of recent clinical studies that have included IGL-1; Meine et al performed a prospective study in a single centre and found livers that had IGL-1 as the preservation solutions had equal PNF and graft survival when compared to HTK (156). Adam et al looked at

European Liver Transplant Registry data and found improved liver graft survival when IGL-1 and UW-CSS were used when compared to HTK (157).

As expected all solutions reduced the viscosity of the effluent perfusate from the organs to a similar viscosity of the solution by itself. This is because all the solutions flushed the majority of the blood out of the organs, so that by the end of the flush-out, it was mainly just solution that was being sampled.

In this study we found that UW-CSS and IGL-1 achieve statistically significantly better perfusion of the kidney microcirculation than HTK and UW-MPS, but all solutions do achieve adequate kidney perfusion. No differences in perfusion of the liver microcirculation were seen using different solutions. There have previously been concerns that UW-CSS, due to the increased viscosity, may not achieve adequate perfusion of the microcirculation, particularly in the liver. As the bile duct is perfused by small arteries, there are specific concerns that the flush-out of these arteries is not adequate, leading to poor perfusion and subsequently ischaemic type biliary strictures (ITBL) after transplantation (158), especially as the viscosity of UW-CSS increases at lower temperatures (152). The evidence in the literature regarding this is fairly mixed. A clinical study by Pirenne et al found that ischaemic bilary type lesions with strictures are more common in transplanted livers that were initially flushed with UW when compared to those flushed with HTK, and these strictures tend to be non-anastomotic related (152). However, other studies have found no difference in intrahepatic stricturing when comparing UW-CSS and HTK in ECD liver transplantation (155), or even increased incidence of biliary complications when using HTK in DCD liver transplantation (159).

Although we did not have a technique to specifically look at the biliary ductal perfusion, we found that all solutions achieved perfusion of the microcirculation in the liver.

Despite the significant findings in temperature and perfusion of the kidney in this study, there were no significant differences in the histological findings of the organs when comparing the solutions.

Ischaemia causes histological changes in both the liver and kidney. Cytoplasmic vacuolation is seen after mild acute and sub-acute liver injury. It is now thought that vacuolation may be transient or irreversible, and if transient it can represents an adaption of the cell that may be beneficial to the host rather than it being a degenerative change. It is possible that this vacuolation allows cells to resist further insult rather than undergoing hydropic degeneration (160, 161). In this work, it would appear that it was transient, as although there was some vacuolation seen in the first biopsy taken at the end of the period of warm ischaemia, the score had often reduced in the biopsies taken at the end of the flush out. This suggests that the initial injury is ameliorated by the preservation solutions. Ischaemia also leads to oedema in Kupffer cells and endothelial cells, as well as narrowing of the sinusoidal lumen (162). In the biopsies taken, there was no oedema seen. However, there was some narrowing of the sinusoidal lumen. This was rather variable with the scores staying the same in some, reducing in some and increasing in others.

In the kidney biopsies studied, the tubules seemed to retain their integrity and if there was disruption of the tubular basement membrane after the warm ischaemia this often improved by the end of the flush-out. Other changes seen during ischaemia are shrinkage of the glomerulus, collapsed capillaries, thickened Bowman's capsule and

wrinkled basement membranes (163). In the biopsies that underwent electron microscopy there was shrinkage of the cells and glomerulus after the warm ischaemic insult, however this recovered after the flush-out. In other biopsies there was mostly preservation of the glomeruli including the organelles. Again this suggests that the cells are injured by the ischaemic episode, but only transiently and this injury is reversed or at least halted by the flush-out using the preservation solutions.

To look for markers of injury in the effluent perfusate of the organs, I chose to look at LDH and AST concentrations. LDH is an enzyme present in the cytoplasm of all cells. During cell damage or cell death, LDH is released into serum, and therefore LDH is commonly used as a marker of cellular injury (164, 165). AST is an enzyme found in the cytosol of many cells, as well as in the mitochondria in the liver. It is released from cells into the serum when cells are injured, and therefore is commonly used as a general marker of cellular injury.

The results in this experiment show that LDH concentrations in the collected effluent perfusate tend to reduce over the course of the cold flush. This suggests that the initial ischaemic injury to the liver and kidneys injures cells causing release of LDH, but the preservation solution ameliorates this preventing on-going cellular damage and cell death. This is supported by the work done by Zager et al who found that in mice that had an AKI, plasma levels of LDH increased as the duration of ischaemia increased (165).

There was only a significant reduction in LDH concentrations for the liver when UW-CSS was the cold flush solution used, however UW-CSS, HTK and IGL-1 all significantly reduced the concentration of LDH in the kidney effluent perfusate. In the IGL-1 Liver group, there was a trend towards the LDH concentration increasing, but this was not

significant. When looking at the individual results, there was one effluent perfusate sample with a very high LDH level at the end of the procedure, and it is likely that this spurious result skewed the results for the IGL-I group.

We found that AST concentrations in the liver effluent perfusate showed a trend of increasing throughout the procedure, although this was not statistically significant. This suggests that the initial ischaemic injury causes release of the AST into the serum, but the preservation solutions ameliorate this injury meaning that there is no significant increase in the levels. It may also be that as the liver is such a large organ, the AST that is produced takes a while to wash out which is why there are still higher levels at the end of the procedure compared to the beginning, but potentially the actual release of AST has stabilised.

In the kidney perfusate effluent, the AST concentrations showed a trend of reducing throughout the procedure. Again, this suggests that the initial injury is alleviated by the preservation solutions and there is no prolonged cell damage.

For the western blot markers, we chose markers of oxidative stress and apoptosis. As expected, there was no differences in the changes of concentrations of 4-HNE, HSP-70 and Caspase-9 when comparing the solutions. There was a lot of variability in the results of the western blots and therefore it is difficult to draw definite conclusions, but it would seem that there is no significant early injury during the flush-out.

This study has its strengths but also some limitations. On the one hand, the model simulates the clinical situation, and therefore should be transferable to clinical practice. We have compared regularly used preservation solutions, as well as the newer solutions

that are starting to be used more frequently. We used contrast ultrasonography to assess the perfusion of the microcirculation with each of the solutions. Contrast enhanced ultrasonography has been used before to assess blood flow by observing the micro bubble contrast agent on ultrasound (166-169). This method has also been used to specifically look at small vessels (170), and therefore we know this is a validated method to assess microcirculation blood flows.

As it is known that the ultrasound image intensity is directly proportional to the micro bubble concentration (171), the concentration of SonoVue was kept the same in each experiment. The quantification of the intensity of the micro bubbles was measured using the purpose written Matlab program by Owen et al, which has been validated in previous studies (172, 173). However, there were limitations with this analysis. There was some movement artefact in the USS images, and this was not accounted for. Although we could assume that this movement artefact was similar for all experiments as it was the same operator using the USS each time, this has not been formally measured and therefore could affect the interpretation of the perfusion. The perfusion analysis found that HTK did not perfuse the kidneys as well as UW-CSS or iIGL-1, however, there were no significant differences between the histology scores or in the markers of injury which suggests that the perfusion of the microcirculation by all the preservation solutions studies is adequate.

Another limitation of this study that might be considered, is that it is not linked to outcomes following transplantation in the pig model. However, the main aim of this study was to assess whether the commonly used preservation solutions are equally

effective in cooling the organs and perfusing the microcirculation, and not to test the efficacy of preservation solutions on outcomes after transplantation.

Other limitations of this study include the electron microscopy and IHC. Although the electron microscopy indicated that kidneys flushed with UW-CSS managed to keep their cellular structure more intact than when using HTK, this was subjective, and some of the changes seen may have been due to sub-optimal preservation of the biopsy samples. Due to funding limitations, only a limited number of slides were reviewed, and therefore the variability between the small numbers of biopsies reviewed also makes it difficult to be certain that the differences that have been noted are true findings. To help with this, we wanted to confirm whether there was damage on a cellular level using IHC, however, we were unable to get good results with the antibodies and protocols we used, and therefore cannot draw conclusions about this.

For the IHC, we used antibodies that the manufacturers stated should work with pig tissue, however this was not guaranteed. The antibody manufacturer suggested protocols for the IHC including the enzymatic method for antigen retrieval. In preliminary cases, enzymatic antigen retrieval was tried using different lengths of time for the incubation of the tissue with the enzyme for the antigen retrieval and with different lengths of time that the primary antibody was incubated with the tissue for. As none of these protocols worked, heat induced antigen retrieval was then attempted. Again different lengths of time for the antigen retrieval and incubation of the primary antibody were tried, and there was still high background staining.

A blinded pathologist reviewed the slides, and there were occasional slides that were of good quality, however this was not consistent and it was unclear at which point along the process the problems happened.

In summary, I found that all the preservation solutions included in this study cooled the organs, but not as effectively as expected. UW-CSS achieved better cooling in the kidney. Both UW-CSS and IGL-1 accomplish better perfusion of the microcirculation in the kidney when compared to HTK, and all solutions succeed in perfusing the microcirculation of the liver.

From this study it can be concluded that the viscosity of the solution does not affect the quality of perfusion and that there are no negative effects from including a colloid in the preservation solution.

This is supported by previous clinical work that has not found inferior outcomes when using the higher viscosity UW-CSS in either liver or kidney transplantation. Stewart et al analysed UNOS data and reported an increased risk of liver and kidney graft loss when HTK was used as the preservation solution when compared to UW-CSS especially in ECD transplantation (174, 175)

Stevens et al also found that kidney graft survival and PNF rates were better when UW-CSS was used as compared to HTK (176).

This may be because despite the higher viscosity of UW-CSS, and possibly due to the properties of the other components of the solution it cools faster as well as reaching and flushing-out the microcirculation. The perception that increased viscosity is detrimental to the preservation solution has not been supported by this work, and this is not necessarily surprising as the viscosity of blood is actually greater than all the preservation solutions used, and there is never any concern about blood perfusing the microcirculation of the organs except in instances of pathology. As suggested by this study and others (146), including a colloid in the preservation solution appears to be actually beneficial, especially in DCD transplantation, as it helps to cool quicker and

potentially maintains lower temperatures for longer, achieves perfusion of the microcirculation and does not negatively affect the cellular level of the organs.

CHAPTER 6 CAN PREIMPLANTATION BIOPSIES PREDICT CLINICAL OUTCOMES FOLLOWING RENAL TRANSPLANTATION? A SYSTEMATIC REVIEW

6.1 Introduction

Despite recent increases in transplant activity, there remains a disparity between the number of patients on the kidney transplant waiting list and the number of available donor organs as discussed in Chapter 1 (figure 1.1) (12, 14). Most of the increases in transplant activity seen have arisen from unfavourable donor types including DCD and ECD donors. In the UK last year, 58% of deceased donors were over the age of 50, and over a third of deceased kidneys came from DCD (12).

DCD donor kidneys from donors under the age of 60years have been shown to have similar outcomes to kidneys from donors after brain death (DBD) (20). In contrast, whilst kidneys from ECD donors offer a survival benefit when compared to remaining on the waiting list, they demonstrate inferior outcomes to those from standard criteria donors (24).

Despite the improvements in acute rejection rates and short-term graft survival seen over recent years, improvement in longer-term graft survival has been more difficult to achieve (8, 17). Currently 5-year graft survival for kidneys from living donors is 92%, where as it is 87% for deceased donors (12). One explanation for this is that as we are using more older donors; these kidneys have a reduced nephron mass and therefore less viable nephrons, which cannot meet the metabolic demand in the recipient, or it may be that these kidneys from ECD donors with co-morbidities already have pre-existing pathological changes that persist in the recipient and are exacerbated by the warm and cold ischaemic insults (as described in section 1.1).

Currently, most transplant clinicians in the UK make the decision as to which kidneys to accept and use for transplantation based solely on clinical information about the donor, as they do not have the availability to a 24-hour histopathology service. However, some

of the existing literature suggests that donor histology may help to predict future transplant outcomes and therefore having this information may help clinicians ability to either select suitable organs, or decline organs that are less likely to survive, with resulting improvements in transplant survival and function. Kidney biopsy scores are often used to allocate kidneys to single kidney transplant (SKT) or dual kidney transplant (DKT), and many studies have shown comparative outcomes when comparing these two groups (177, 178). However, whether these kidneys that are allocated to DKT could actually successfully be used as SKT has been debated. Cambridge Transplant Centre has published outcomes from kidneys with a Remuzzi score of 4, which would have been allocated to DKT using the original protocol, used as SKT with favourable outcomes (Table 6-1) (179).

There are also reports to suggest that donor biopsy results can lead to excess discard of kidneys that would otherwise have been used (180). As described previously in section 1.4, in the US, biopsies are obtained in half of all deceased donor kidneys, and the overall discard rate is 18% (and 30% of kidneys that are biopsied are discarded). In the UK the discard rates are not as high with 10% of all kidneys being discarded in 2014, and as perhaps expected DCDs account for a higher percentage of those kidneys discarded (DCD 14.8% vs DBD 8%) (73, 98, 100, 101, 180). Therefore we need to know that pre-implantation biopsies give useful, predictive information without increasing the unnecessary discard of potentially viable kidneys.

Glomerular Sclerosis *Based on three sections (the first, middle and last sections, if available); the number of globally sclerosed glomeruli expressed as a percentage.*	
0	*None globally sclerosed*
1	*<20% global glomerulosclerosis*
2	*20 - 50% global glomerulosclerosis*
3	*>50% global glomerulosclerosis*
Tubular Atrophy	
0	*Absent*
1	*<20% of tubuli affected*
2	*20 - 50% of tubuli affected*
3	*>50% tubuli affected*
Interstitial Fibrosis	
0	*Absent*
1	*<20% of renal tissue replaced by fibrous connective tissue*
2	*20 – 50% of renal tissue replaced by fibrous connective tissue*
3	*>50% of renal tissue replaced by fibrous connective tissue*
Arterial and arteriolar narrowing *For the vascular lesions, if the changes are focal, the most severe lesion present gives the final grade.*	
0	*Absent*
1	*Increased wall thickness but to a degree that is less than the diameter of the lumen*
2	*Wall thickness is equal or slightly greater than the diameter of the lumen*
3	*Wall thickness that far exceeds the diameter of the lumen with extreme luminal narrowing or occlusion*

Table 6.1 The Remuzzi Score

6.2 Aims

In this review, we are aiming to examine the evidence for the relationship between donor biopsies and clinical outcomes. To identify the different scoring systems for assessment of donor kidney biopsies and to identify which of these have the best predictive performance for post-transplant outcomes.

6.3 Methods

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement using a pre-specified protocol (see Appendix C).

6.3.1 Search Strategy

A systematic literature search was performed using Ovid MEDLINE and EMBASE, the Cochrane Library and the Transplant Library of Randomised Controlled Trials (RCTs). Searches terms included subject headings and free text equivalents for 'kidney transplantation, biopsy, histopathology, donor, pre-transplant or pre-implantation'. Full search strategies for each database are available in the protocol (see appendix C and D). The final date for literature searches was 3rd July 2019.

6.3.2 Inclusion/exclusion criteria

Inclusion criteria specified randomised controlled trials, cohort studies, case control studies and case series of at least 20 kidney transplant recipients that related pre implantation kidney biopsies taken from any adult or paediatric deceased donor (DBD, DCD and ECD) and related these to pre-specified clinical outcomes. Pre-implantation

biopsies were defined as those taken after organ retrieval and cold storage, but prior to reperfusion in the recipient.

6.3.3 Outcomes

Pre-defined clinical endpoints included rate of graft utilisation, patient and graft survival, graft function (serum creatinine, creatinine clearance), delayed graft function (defined as need for dialysis in the first week post transplantation), primary non-function and biopsy-related complications. Studies were excluded when the biopsy was taken for diagnosis of a clinically apparent lesion or that did not report the pre-defined outcomes. Studies that included pre-procurement, post-reperfusion or protocol biopsies, live donors and animal models were also excluded.

6.3.4 Screening

A colleague and myself reviewed initial search results for duplicates. Titles and abstracts were reviewed to select studies based upon the predefined inclusion/exclusion criteria. Full texts of potentially eligible studies were reviewed, and if they were considered eligible for inclusion, data extracted. Reference lists of relevant studies were also checked for any missed studies as well as trial registries.

6.3.5 Data extraction and analysis

Demographic and outcome measures were extracted and recorded, on a pre-defined data collection worksheet, as reported in each study. Due to study heterogeneity and the small numbers of prospective comparative studies, a narrative review of the data is presented.

6.3.6 Quality Assessment

All full text studies were assessed for quality using the Newcastle-Ottawa Quality assessment scale by a colleague and myself (181). This is a semi-quantitative assessment of the risk of bias of cohort and case-control studies. It scores studies on eight items, categorised into three dimensions; selection, comparability and outcome). The total score is out of a possible nine (two points can be awarded for one item in the comparability section). Lower scores mean there is a higher risk of bias. It was decided not to score abstracts based on this assessment scale, as most abstracts did not contain all of the information required and if it was assumed these were absent, all the abstracts would therefore score poorly.

6.4 Results

The Initial literature searches generated 5,682 potentially relevant citations across all databases (Embase 4,422, Medline 1,206, Transplant Library 54). After removing duplicate references based on the title, year, author and journal 4,707 references remained. After repeating this process with author, year and journal 4,652 references remained and then finally after checking references for year and journal 4,441 references remained. The title and abstract of these references were then reviewed, and this led to excluding 4, 224 references leaving the full text of 217 papers to be assessed. 96 papers were identified to be included in the final review (49 full text articles and 47 abstracts) from 75 studies (Figure 6.1).

121 references were excluded for various reasons including: 25 included reperfusion/implantation biopsies, 7 studies included living donors and no separate analysis, 2 studies had less than 20 cases, 4 were indication biopsies post transplant, 37 references mentioned a pre-implantation biopsy, but then did not correlate them to the post-transplant outcomes. 9 studies used the biopsy score along with either clinical scores or perfusion parameters to have a combined score and the biopsy score was not individually linked to the post transplant outcomes. 11 references were review articles, 3 references used immunohistochemistry or more detailed molecular analysis of the biopsy that would not give results within 24 hours. 19 references used the biopsy score to allocate to SKT or DKT, but the outcomes were all described together. 2 references were case reports, 1 was a commentary, and 1 was an editorial comment.

All included studies were cohort studies, with no randomised controlled trials identified.

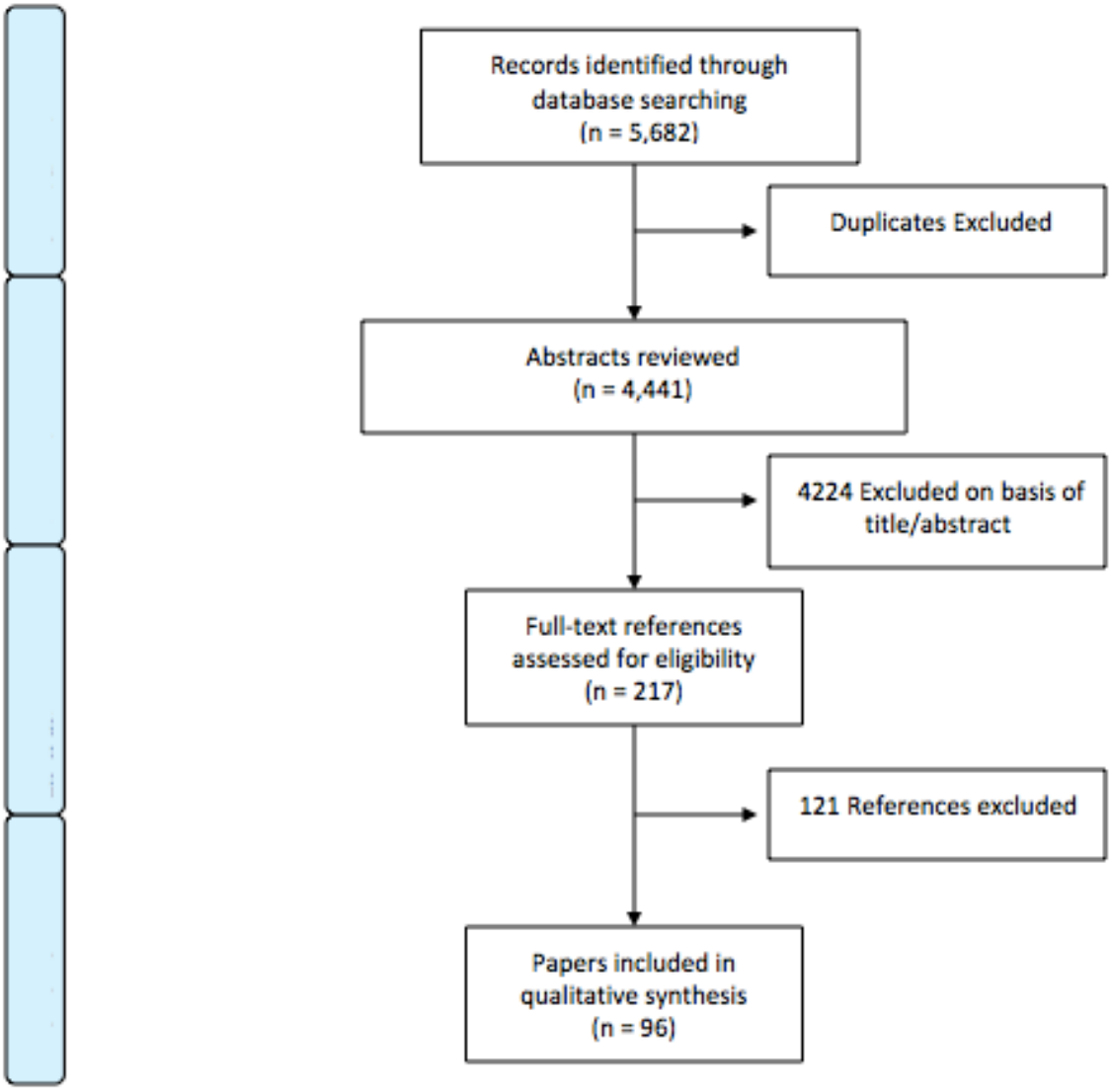


Figure 6.1 Prisma Consort Flow diagram

6.4.1 Scores identified

The main scores that were utilised were the Remuzzi Score, the Karpinski score, the Maryland Aggregate Pathology Index (MAPI) and Banff. Other papers used alternative scoring systems, including percentage of glomerulosclerosis, Acute Tubular Necrosis (ATN) and a chronic lesion or chronic damage score.

6.4.1.1 Remuzzi Score

The Remuzzi score is the sum of 4 individual components that are scored from 0-3 according to the histological findings (Table 6.1). The components include glomerular sclerosis, tubular atrophy, interstitial fibrosis and arterial and arteriolar narrowing. A score of 0 means no changes were observed, and 3 means the changes are severe. The cumulative score then gives a final grade which ranges from 1 to 12, with kidneys scoring 0-3 being classed as mild, 4-6 is moderate and 7-12 means there is severe damage (178).

This score can then be used as a decision making tool to determine use as a SKT, DKT or to discard the kidneys (in the original paper this was defined as score 0-3 SKT, 4-6 DKT and ≥7 discard). We identified 26 papers from 21 studies that used the Remuzzi score (Table 6.2) (177, 178, 182-205).

Author	Year	Type of Study	NOS				Biopsy	Nº of Pts	Type of Donor	Outcome				Bx linked to outcome
			S	C	O	T				Graft Survival	Patient Survival	Function	DGF	
Almeida	2018	Retrospective						127	'Marginal'	✗		✗		No
Brayne	2018	Retrospective						495	DD	✗		✓		GS- No <5 vs ≥5 eGFR- Yes <5 vs ≥5
Colussi	2018	Retrospective	3	0	2	**5**		132	DBD >60yrs	✗	✗	✗	✗	No
Yuan	2018	Retrospective						145	DCD	✗		✓		GS- No eGFR- Yes 0-3 vs 4-5
Yuan	2016	Retrospective					Wedge/ Needle	71	DCD	✗		✓		GS- No eGFR- Yes 0-3 vs 4-5
Assis-Borba	2014	Retrospective	3	2	1	**6**		233	ECD	✓		✗	✗	GS- Yes DGF & eGFR- No
Gandolfini	2014	Retrospective	4	2	2	**7**	Punch	690	248 SCD, 442 ECD	✓		✓		Yes
Pegas	2014	Retrospective	4	1	1	**6**	Wedge	110	47 SCD, 36 ECD	✗		✗		No
Tavares da Silva	2014	Retrospective	4	1	2	**7**	Needle	197	ECD	✗		✓		GS- No eGFR- Yes
Losappio	2014	Retrospective	4	2	1	**7**	Wedge	372	DD	✓		✓	✓	Yes
Vathsala	2014	Retrospective						61	DD			✓	✓	Yes
Garcia-Rubio	2013	Retrospective	4	0	1	**5**	Wedge	84	ECD	✗				No
Fernandez-Lorente	2012	Retrospective	2	2	2	**6**		319	ECD	✓	✓	✓		Yes
Salji	2011	Retrospective						313	DCD	✓				Yes
Re	2010	Retrospective	4	1	2	**7**	Needle	48	ECD	✗		✗	✗	No
Re	2006	Retrospective	2	0	1	**3**		95	DD	✗		✗	✗	No
Faenza	2004	Retrospective	2	1	1	**4**		153	ECD			✗	✗	No
Mallon	2015	Retrospective	2	2	3	**7**		55	DCD >70yrs	✗		✗	✗	No (Comparing SKT vs DKT)
Remuzzi	2006	Prospective	4	2	1	**7**		62	DD >60yrs	✗	✗			No (Comparing SKT vs DKT)
Rigotti	2004	Retrospective	3	2	2	**7**		84	DD >60yrs	✗	✗	✗		No (Comparing SKT vs DKT)
Remuzzi	1999	Prospective	3	2	3	**8**		72	DD >60yrs	✗	✗	✗		No (Comparing SKT vs DKT)

Table 6.2 Details of included studies that used the Remuzzi score (NOS = Newcastle Ottawa Scale. S= Selection /4, C= Comparability /2, O=Outcome /3, T= total score /9. ✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes).

6.4.1.2 Karpinski Score

The Karpinski score is similar to the Remuzzi Score, except that the vascular score is calculated by independently assessing the arteriolar narrowing and arterial sclerosis and taking the worst score (206) (Table 6.3).

We identified thirteen papers from ten studies, of which three were prospective and the other seven were retrospective (Table 6.4) (206-218).

Glomerular Sclerosis *Based on three sections (the first, middle and last sections, if available); the number of globally sclerosed glomeruli expressed as a percentage.*	
0	*None globally sclerosed*
1	*<20% global glomerulosclerosis*
2	*20 - 50% global glomerulosclerosis*
3	*>50% global glomerulosclerosis*
Tubular Atrophy	
0	*Absent*
1	*<20% of tubuli affected*
2	*20 - 50% of tubuli affected*
3	*>50% tubuli affected*
Interstitial Fibrosis	
0	*Absent*
1	*<20% of renal tissue replaced by fibrous connective tissue*
2	*20 – 50% of renal tissue replaced by fibrous connective tissue*
3	*>50% of renal tissue replaced by fibrous connective tissue*
Arterial and arteriolar narrowing *For the vascular lesions, arteriolar narrowing and arterial sclerosis are calculated and the most severe lesion present gives the final grade.*	
0	*Absent*
1	*Increased wall thickness but to a degree that is less than the diameter of the lumen*
2	*Wall thickness is equal or slightly greater than the diameter of the lumen*
3	*Wall thickness that far exceeds the diameter of the lumen with extreme luminal narrowing or occlusion*

Table 6.3 Karpinksi Score

Author	Year	Type of Study	NOS				Biopsy	Nº of Pts	Type of Donor	Outcome				Bx linked to outcome
			S	C	O	T				Graft Survival	Pt Survival	Function	DGF	
Ambrosi	2017	Retrospective						45	DD (Diabetic)	✓		✗		Yes
Carta	2013	Retrospective	3	1	1	**5**	Wedge	100	ECD	✗	✗	✗		No
Ditonno	2013	Retrospective	3	1	1	**5**		761	DD				✓	Yes
Ramalho	2013	Retrospective	4	0	1	**5**		134	DBD	✗		✓		GS-No Function- Yes
Carta	2012	Retrospective						77	ECD	✗		✗	✗	No
Karpinski	1999	Retrospective	4	0	1	**5**		34	ECD			✓	✓	Yes
Donatella	2014	Retrospective						178	ECD	✗				No (Comparing SKT vs DKT)
La Manna/ Valentini	2013	? Prospective	3	1	1	**5**		144	ECD			✗		No (Comparing SKT vs DKT)
Lavacca	2011	Retrospective						?	?	✗	✗	✗		No (Comparing SKT vs DKT)
Boggi	2005	Retrospective	2	0	1	**3**	Wedge	103	ECD	✗		✗		No (Comparing SKT vs DKT)

Table 6.4 Details of included studies that used the Karpinski score (NOS = Newcastle Ottawa Scale. S= Selection /4, C= Comparability /2, O= Outcome /3, T= Total /9. ✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes)

6.4.1.3 Maryland Aggregate Pathology Index (MAPI)

MAPI scores kidneys based on the absence or presence of specific components (Table 6.5). The scores are then added together to give a risk: low risk kidneys have a score of ≤7 and have a 5 year survival rate of 90%, intermediate risk, score 8-10 have a graft survival of 63% and high risk kidneys, score >12, have a 5 year graft survival of 53% (219).

We found seven papers from five studies that correlate the MAPI to outcomes (Table 6.6) (219-225). All studies are retrospective and come from the same group.

Arteriolar hyalinosis	
0	*Absent*
4	*Present*
Periglomerular fibrosis	
0	*Absent*
4	*Present*
Scar	
0	*Absent*
3	*Present*
Glomerulosclerosis	
0	*Absent*
2	*>15%*
Wall-to-lumen ratio of interlobular arterioles	
0	*<0.5*
2	*≥0.5*

Table 6.5 Maryland Aggregate Pathology Index (MAPI)

Author	Year	Type of Study	NOS				Nº of Pts	Type of Donor	Outcome				Bx linked to outcome
			S	C	O	T			Graft Survival	Pt Survival	Function	DGF	
Philosophe	2014	Retrospective	3	1	2	**6**	35	DD with AKI	✓				Yes
Munivenkatappa	2010	Retrospective					205	DD	✓		✓	✓	Yes
Philosophe	2010	Retrospective					140	DD	✓				Yes
Philosophe	2009	Retrospective					357	DD	✓				Yes
Munivenkatappa	2008	Retrospective	3	1	1	**5**	371	DD	✓		✓		Yes

Table 6.6 Details of included studies that used the MAPI score (NOS = Newcastle Ottawa Scale. S= Selection /4, C= Comparability /2, O= Outcome /3, T= Total /9. ✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes)

6.4.1.4 Banff

The Banff score allocates a score of 0-3 to each component of a biopsy, which are then summed up to give a final score. The components are; Glomerulosclerosis (cg), Interstitial Fibrosis (ci) and Tubular atrophy (ct), arteriosclerosis (cv) and arteriolar hyalinosis (226) (Table 6.7).

26 papers from 18 studies that used the Banff scoring were identified (Table 6.8) (227-252).

Glomerular Sclerosis	
cg0	*No Glomerulopathy – double contours in <10% of peripheral capillary loops in most severely affected glomerulus*
cg1	*Double contours affecting up to 25% of peripheral capillary loops in the most affected of non-sclerotic glomeruli*
cg2	*Double contours affecting 26 – 50% of peripheral capillary loops in the most affected of non-sclerotic glomeruli*
cg3	*Double contours affecting more than 50% of peripheral capillary loops in the most affected of non-sclerotic glomeruli*
Interstitial Fibrosis	
ci0	*Interstitial fibrosis in up to 5% of cortical area*
ci1	*Mild interstitial fibrosis in 6 – 25% of cortical area*
ci2	*Tubular atrophy involving 26 – 50% of the area of cortical tubules*
ci3	*Severe interstitial fibrosis in >50% of cortical area*
Tublular Atrophy	
ct0	*No tubular atrophy*
ct1	*Tubular atrophy in up to 25% of the area of cortical tubules*
ct2	*20 – 50% of renal tissue replaced by fibrous connective tissue*
ct3	*Tubular atrophy in >50% of the area of cortical tubules*
Arteriosclerosis	
cv0	*No chronic vascular changes*
cv1	*Vascular narrowing of up to 25% luminal area by fibrointimal thickening of arteries ± breach of internal elastic lamina or presence of foam cells or occasional mononuclear cells*
cv2	*increased severity of changes described above with 26 – 50% narrowing of vascular luminal area*
cv3	*severe vascular changes with >50% narrowing of luminal area*
Arteriolar Hyalinosis	
ah0	*No PAS-positive hyaline thickening*
ah1	*Mild-to-moderate PAS-positive hyaline thickening in at least one arteriole*
ah2	*Moderate-to-severe PAS-positive hyaline thickening in more than one arteriole*
ah3	*Severe PAS-positive hyaline thickening in many arterioles*

Table 6.7 Banff Score

Author	Year	Type of Study	NOS				Biopsy	N° of Pts	Type of Donor	Outcome				Bx linked to outcome
			S	C	O	T				Graft Survival	Pt Survival	Function	DGF	
Pegas	2018	Retrospective	2	2	1	**5**	Wedge	430	DD	✓		✓		Yes
Aubert	2017	Retrospective						882	DD	✗				No
Hyodo	2017	Retrospective						256	DD				✓	Yes
Traynor	2017	Retrospective	3	2	1	**6**	Wedge	156	DD			✓		Yes
Lee	2016	Retrospective	2	2	2	**6**	Needle	135	DD			✓	✓	
Becker	2015	Retrospective						58	DD			✓	✓	Yes
Azancot	2014	Retrospective	2	2	2	**6**	Wedge	127	ECD	✓		✓		Yes
De Vusser	2013	Prospective	3	2	3	**8**		548	DD	✓				Yes
Balaz	2013	Retrospective	2	2	1	**5**		344	ECD				✓	Yes
Malcevs	2013	Retrospective						90	DD			✓	✓	Yes
Torrealba	2011	Retrospective						283	DD			✓		Yes
Lopes	2011	Retrospective	3	0	2	**5**	Wedge	30	DD			✓		Yes
Navarro	2011	Prospective	3	2	1	**6**		136	ECD	✓				Yes
Munivenkatappa	2010	Retrospective						140	DD				✓	Yes
Gurney	2010	Retrospective						105	DD			✓	✗	eGFR- Yes DGF- No
Matignon	2008	Retrospective	2	2	2	**6**	Wedge	172	DD				✓	Yes
Arias	2007	Retrospective	2	2	2	**6**		214	DD	✓		✓		Yes
Lopes	2005	Retrospective	3	2	3	**8**	Wedge	77	DD	✓		✓	✓	Yes

Table 6.8 Details of included studies that use the Banff score (NOS= Newcastle-Ottawa Scale, S= Selection /4, C= Comparability /2, O= Outcome /3, T= Total /9. ✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes)

6.4.1.5 Glomerulosclerosis

Seven papers from six studies used the percentage of glomerulosclerosis found in the biopsy, and this was compared across groups (Table 6.9) (253-259).

6.4.1.6 Other findings/Scores

17 papers from 15 studies assessed the biopsies using scores that have not been previously described, or using a combination of components of existing scores (Table 6.10) (260-276). These included chronic damage scores, ATN, a kidney biopsy audit score and various variations using the Banff components.

Author	Year	Type of Study	NOS				Nº of Pts	Type of Donor	Outcome				Bx linked to outcome
			S	C	O	T			Graft Survival	Pt Survival	Function	DGF	
Liu	2018	Retrospective					506	DD	✗				No
Bodzin	2014	Retrospective	4	2	1	**7**	10,329	ECD	✗	✗			No
Huh	2010	Retrospective					63	DD			✓		Yes
Bajwa	2007	Retrospective	2	2	3	**7**	12,129	DD	✓		✓	✗	GS-Yes DGF- No
Edwards	2004	Retrospective	2	2	2	**6**	3,444	DD	✗				No
Randhawa	2000	Retrospective	3	2	2	**7**	78	DD			✓		Yes

Table 6.9 Details of included studies that use % glomerulosclerosis (NOS= Newcastle Ottawa Score. S= Selection /4, C= Comparability /2, O= Outcome /3, T= Total /9. ✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes)

Author	Year	Score	Type of Study	NOS				Biopsy	Nº of Pts	Type of Donor	Outcome				Bx linked to outcome
				S	C	O	T				Graft Survival	Pt Survival	Function	DGF	
Zhang	2018	ATI	Retrospective					Needle	141	DD				✓	Yes
Hall	2014	ATN	Retrospective	2	2	2	**6**	Wedge	651	DD	✗			✗	No
Hall	2013	ATN	Retrospective						635	DD				✗	No
Sulikowski	2010/ 2007	ATN	Retrospective	1	0	2	**3**		92	DBD			✓	✓	Yes
Carpenter	2018	GS, IFTA, Vascular changes	Retrospective	3	2	3	**8**		270	DD	✗				No
Rabin	2013	GS, IF, Vascular changes	Retrospective						134	ECD	✗			✗	No
Kayler	2008	GS, IF, Vascular changes	Retrospective	3	2	2	**7**	Wedge	597	DD	✓	✓			Yes
Hofer	2014	Chronic Lesion Score	Retrospective	2	2	2	**6**	Wedge	628	DD	✓		✓		Yes
Ibernon	2007	Chronic Damage Score	Retrospective	2	2	1	**5**		226	DD			✓		Yes
Anglicheau	2008	Pirani and Chronic Allograft Damage Index	Retrospective	2	2	2	**6**	Needle	122	DD >50yrs			✓		Yes
Amenabar	2016	7 histological parameters	Retrospective	2	2	2	**6**	Wedge	124	DD	✗		✓	✗	eGFR- Yes GS/DGF- No
Cahen	1995	30 histological parameters	Retrospective	3	2	1	**6**		66	DD	✗		✗		No
Jendrisak	2012	Kidney Biopsy Audit Score	Retrospective						360	DD	✓				Yes
Suhorukovas	2011	% Nephrosclerosis	Retrospective						71	DD	✓		✓	✓	Yes
Neuzillet	2004	Glomerular number and morphology	Retrospective					Wedge	110	DD			✗	✗	No

Table 6.10 Details of studies that use 'other' scores (NOS= Newcastle Ottawa Scale, S= Selection /4, C= Comparability /2, O= Outcome /3, T= Total /9. ✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes)

6.4.2 Outcomes

The studies presented their outcomes in a variety of ways.

Some used the biopsy score to allocate kidneys to either SKT or DKT and then compared the outcomes between these two groups. In other retrospective studies the biopsy scores were grouped and then the groups compared (for example outcomes of kidneys with scores of 0-3 were compared to outcomes of kidneys with scores of ≥4).

Different studies looked at different donor types, and reported different outcomes. Some reported rates of DGF, some looked at graft survival or looked at graft function at different time points, and some reported combinations of these outcomes. Because of this, it has only been possible to perform a narrative review and it has not been possible to do a meta-analysis.

No study linked biopsy findings to PNF, and only a few studies report the PNF rates and therefore PNF was not included in the outcome analysis.

6.4.2.1 Remuzzi Score

6.4.2.1.1 Quality of studies

All studies identified were observational cohort studies. Two were prospective and the remaining nineteen appeared to be retrospective, although some of the studies do not indicate whether it is prospective or retrospective and therefore they have assumed to be retrospective. The prospective studies were both by Remuzzi and had a Newcastle Ottawa score of 7 and 8, which is at the higher end of the scale. 69% of the retrospective studies (9/13) had a score of 6 or 7, with only one having a score of 3, and one of 4, and two having a score of 5. These lower scoring studies tended to lose points

because they did not describe the ascertainment of exposure; they did not adjust for confounders in the analysis or did not account for all the subjects in the follow-up.

6.4.2.1.2 Comparing outcomes between Dual and Single Kidney Transplants

The original paper that conceived the Remuzzi score was by Remuzzi et al in 1999 (178). This was a prospective, case controlled study done between November 1996 and September 1998. They used the histological score of the kidney in donors over the age of 60 years to allocate the kidney to a SKT (if the score was 0-3), a DKT (if the score was 4-6) or the kidney was discarded (score ≥7). They compared 24 DKTs with 48 SKTs and found that the DKT had comparative outcomes to SKT in regards to serum creatinine and DGF, and 6-month graft and patient survival was 100% in both groups. This study concluded that transplanting two marginal kidneys otherwise destined for discard is safe and offers improved filtration power. Following on from this, there have been three other prospective studies published that used the Remuzzi score to allocate kidneys to SKT or DKT and compare the outcomes between these groups. Remuzzi repeated their study, which included 62 deceased donors over the age of 60 in a period between 1997 and 2002, and allocated the kidneys to SKT or DKT as in the original study. Again they found no difference in graft survival between the two groups (196). Rigotti et al looked at 84 deceased donors over the age of 60 years, and used the biopsy score to allocate to SKT or DKT as per Remuzzi (197). After discard, they had 40 SKT and 21 DKT to compare outcomes, and found that there was no significant difference in 1-year graft or patient survival or in 1-year serum creatinine. The most recent prospective study was from Fernandez-Lorente et al (188). In this study the Remuzzi score of 319 ECD kidneys was used to allocate the kidney to either SKT or DKT. These biopsy samples were then blindly

re-evaluated by two renal pathology experts. Patient survival was similar in the SKT and DKT groups. Long-term graft survival (10yr) was significantly lower in the SKT group compared to the DKT (p=0.03), although this was not death censored and there were more deaths in the SKT group due to cardiac events. The eGFR at 1- and 3-years was significantly better in the DKT group than the SKT group (51 ± 16 vs 42 ± 14 ml/min (p=0.0006) and 50 ± 17 vs 43 ± 19ml/min (p=0.02) resp.), however by 5 years there was no difference in the eGFR between the two groups (44 ± 23 (DKT) vs 45 ± 18 mls/min (SKT) (p=0.93)). The histological re-evaluation identified some kidneys that were transplanted as SKT had scores of greater than 3, and therefore these were analysed as a separate group; kidneys with a score of 4 had similar outcome to kidneys that scored 3 or less, but kidneys with a score of 5 that were used as a SKT had very poor graft outcomes. The authors of this study suggest that a combination of macroscopic inspection, renal function and histological assessment provide adequate long-term results for kidneys from older donors.

Mallon et al also compared outcomes of SKT and SKT, but in DCD kidneys from donors over the age of 70years (177). They used the experience from Fernandez Lorente and allocated kidneys with a score of 4 to SKT not DKT. They found that the SKT group had more DGF than the DKT group (65% vs 25% p=0.01), but 3-year graft survival was comparable between the SKT and DKT (95% vs 100% p=0.4) as was eGFR at 1-year.

All of the studies that compared the outcomes of SKT with DKT found that there was no difference between these two groups, suggesting that DKT is safe and allows acceptable, perhaps even good, outcomes (177, 178, 196, 197).

6.4.2.1.3 Comparing outcomes of Single Kidney Transplants with different scores

Since these studies, many centres have retrospectively looked at the outcomes of their transplants and linked this to a pre-implantation biopsy score if available (the biopsy was not always used to allocate kidneys prior to transplantation).

Four studies looked at all deceased donors, ten at ECD donors and three at DCD donors. Each study reports different outcomes.

Deceased Donors

A recent abstract from a study by a group in Cambridge reports the 1-year graft survival and 1-year eGFR in 495 deceased donor SKTs (185). They compared the outcomes between SKTs using kidneys with a score of one, two, three, four or five to seven. They found no difference in the graft survival of these kidneys, but there was a difference in 1-year eGFR when comparing SKTs with a score of less than five to SKTs with a score of five to seven (1=58mls/min 2 = 45mls/min, 3=42 mls/min, 4=46mls/min and 5-7=35mls/min p=0.02). They concluded that the Remuzzi score was effective at predicting function at 1-year, and that selected kidneys with a score of ≥5 could give reasonable outcomes.

Losappio et al analysed 372 SKTs and compared outcomes between SKT using kidneys with a score of 0, 1, 2, 3, 4 and 5 (205). They found that kidneys with a score of 0 had significantly better 5- and 10-year graft survival compared to all other scores ($p<0.001$). Kidneys with a score of 1, 2, 3 and 4 had comparable graft survivals, but kidneys with a score of 5 allocated to SKT had significantly worse graft survival. DGF rates were significantly worse when the kidney had a score of 5 compared to scores of 1-4 and 0 (DGF rates 63.1%, 30.3% and 17.9% resp. ($p<0.0001$)). When looking at graft function,

SKTs using kidneys with a score of 0 had significantly better eGFR at 1- and 5- years when compared to SKTs using kidneys with score of 1-4 and 5, but there was no difference between these groups (1-4 vs 5). This study concluded that pre-transplant histological score is a helpful tool to predict long-term outcomes, and they also concluded that kidneys with a score of 4 could be allocated to SKT.

In a few studies, it was not entirely clear what the comparisons were being made between, but they do comment on whether the biopsy scores correlate with outcomes. Vathsala evaluated short-term outcomes (DGF and 1- and 3- month serum creatinine) in 61 deceased donor kidney transplants (?$SKT_{\leq 4}$ vs $SKT_{>4}$) and found that the Remuzzi score was an independent risk factor for eGFR but not DGF (201).

A group in Buenos Aries report the outcomes from 95 deceased donor kidney transplants, and then the outcomes of 48 ECD kidney transplants (191, 194, 195). In both studies they report that the Remuzzi score does not correlate with DGF, graft function (1- and 2-year creatinine in the first study, 1- and 2-year eGFR in the second study) or graft survival when comparing $SKT_{0\text{-}3}$ with $SKT_{4\text{-}6}$.

ECD Donors

Recently Colussi et al described the outcomes from their centre in a retrospective review. They introduced a protocol to allocate kidneys with a pre-implantation score of 4-6 to DKT in 2010, and looked at the outcomes of these compared to all SKT transplants they did between January 2000 and October 2017 (186). They included 102 SKTs using kidneys with a score of 1-4 and compared the outcomes with 30 SKTs using kidneys with a score of greater than 4, and compared these outcomes to 53 DKTs. They looked at DGF, graft and patient survival and eGFR at 3- and 12-months and found there was no significant difference in any of the outcomes between the groups and concluded that if

a kidney was thought to be suitable for transplantation clinically, histology was not useful.

Gandolfini looked at the outcomes of 690 kidney transplants, 248 from SCD kidneys and 442 from ECD kidneys from 2001 to 2009 (189). Pre-implantation donor biopsies were taken from the ECD kidneys. Between 2001 and 2005 kidneys that scored <4 were allocated to SKT and those that scored 4-6 were allocated to DKT. From 2005, there was a change in the allocation policy, and so kidneys that scored 4 were allocated to SKT. There were 102 DKT, 278 $SKT_{<4}$ and 62 $SKT_{=4}$. 3-year graft survival of DKTs was not significantly worse than SCD kidney transplants, but it was lower in $SKT_{<4}$ and $SKT_{=4}$. $SKT_{=4}$ also had inferior graft survival when compared to $SKT_{<4}$. 1-year eGFR was significantly worse in DKT, $SKT_{<4}$ and $SKT_{=4}$ than in SCD kidney transplants. eGFR was similar in $SKT_{<4}$ compared to $SKT_{=4}$ (43.6 vs 43.8mls/min), but significantly worse in $SKT_{<4}$ compared to DKT (43.6 vs 51.1mls/min). The authors concluded that allocating ECDs to SKT or DKT can give excellent outcomes. This agrees with the study by Fernandez-Lorente that kidneys that have a Remuzzi score of 4 can be used for SKT, although as they have inferior graft survival, caution needs to be used in recipients with longer life expectancies.

Two studies by Assis-Borba and Pegas compared the outcomes of SKTs, and grouped them as mild (score 0-3), moderate (score 4-6) and severe (score ≥7). Assis-Borba found a significant difference in 3-year graft survival between the groups, however, Pegas did not find a difference in 1-year graft survival. In the Assis-Borba study, there were 233 transplants from ECDs, however, Pegas did not separate out the 36 ECD transplant from the 47 SCD transplants, and had much smaller numbers which may account for the different findings.

Tavares da Silva compared the outcomes of 197 ECD kidneys and found the Remuzzi score correlated with eGFR at all time points (3- and 6-months and 2- and 3-years) but not with graft survival (200).

In an analysis looking at the discrepancies in biopsy scores between pairs of kidneys, biopsy findings were also correlated to outcomes (190). This study found no significant difference in graft survival between favourable (low score) and non-favourable (higher scores) kidneys (p=0.945). The scores that made up these groups were not defined.

Almeida et al reported their findings in an abstract that was presented at the European conference of Pathology (182). This study looked at 127 biopsies from marginal donors between 2009 and 2016 and found that there was no correlation between biopsy score and graft function or graft survival. In this study kidneys with a biopsy score of ≥4 were excluded, and they do not report the outcomes for the kidneys with different scores, and they don't define what a 'marginal' kidney is.

Faenza et al look at donors over the age of 60years in two time periods and found that there was no correlation between biopsy score and kidney function or DGF (187).

DCD Donors

Yuan et al reported the outcomes of 145 DCD kidneys transplanted between 2013 and 2016 (203). They compared the outcomes of SKTs using kidneys with a score of 0-3 with SKTs using kidneys with a score of 4 and 5. They found no difference in graft survival between the two groups, but they did find that the eGFR was significantly better when the score was 0-3 when compared to 4 and 5 at 12 months (76.3 ± 21.4ml/min vs 44.0 ± 10.9ml/min $p<0.001$). This study concluded that DCD kidneys with a score of 4 and 5 have a worse graft function at 1-year when used as a SKT, but have acceptable short-term survival. This study repeats what this group have reported before when they

looked at 71 DCD transplant between 2011 and 2014 (202). This study compared the same groups as the 2018 study, and the results were similar with no difference in graft survival between SKTs score 0 -3 vs 4-5, but a difference in eGFR at 1 year (76.3 ± 21.4ml/min vs 44.0 ± 10.9ml/min p<0.001). This study also concluded that DCD kidneys with a score of 4-5 used as SKT had poor function, but good short-term survival.

Salji also looked at outcome of DCD SKT_{0-3} and SKT_{4-6} and reported that graft survival was better in the SKT_{0-3} group (198, 199). They looked at kidney with a score of 4-6 in donors >55 and found that only 40% survived past 3 years, but concluded that overall SKT_{4-6} had acceptable results.

6.4.2.2 Karpinski Score

6.4.2.2.1 Quality of studies

All ten studies (from 13 papers) that utilised the Karpinski Score (K-score) were retrospective studies. Five full text studies had a Newcastle Ottawa score of 5, and one had a score of 3. Again the studies lost points for not describing the ascertainment of exposure; they did not adjust for confounders in the analysis or did not account for all the subjects in the follow-up or explain how they assessed the outcome.

6.4.2.2.2 Comparing outcomes between Dual and Single Kidney Transplants

Three studies scored the biopsies according to Karpinski and allocated the kidneys according to Remuzzi, although all three of these studies had kidneys with scores of 4 as SKT (208, 214, 215) and one also had kidneys with a score of 5 as SKT (214). All these studies were looking at ECD kidneys, and they all found that outcomes were comparable between SKT and DKT although they used kidneys with different scores as SKT. Donatella

et al looked at 10 year GS comparing DKT with $SKT_{\leq 3}$ and SKT_{4-5} and found no difference between all three groups (214). La Manna compared DKT with $SKT_{<3}$ and SKT_{3-4} and found that $SKT_{<3}$ and DKT had better 3-year eGFR compared to SKT_{3-4} ($p<0.05$) (215, 217). Boggi compared SKT_{1-4} with DKT_{5-7} and found no difference in rate of DGF, 3- and 5-year graft survival or 3- and 5-year serum creatinine (208).

Lavacca et al looked at the outcomes of their kidney transplants following on from changing their protocol to allocate kidneys with a score of 4 to SKT rather than to DKT, and they found that there was no significant difference in-patient and graft survival when comparing DKT to SKT including kidneys with a score of 4 (218). They did note a trend to worse creatinine when SKT_4, but this did not reach significance.

6.4.2.2.3 Comparing outcomes of Single Kidney Transplants with different scores

The remaining six studies compared the outcomes of SKTs using kidneys with different Karpinski scores (206, 207, 209-213, 216, 217). Two describe the outcomes in deceased donors, and four in ECD donors.

Deceased donors

Ambrosi specifically looked at the outcomes of 45 diabetic donors and used the K-score as well as histological classifications of diabetic nephropathy (micro aneurysms, hyaline droplets, hyalinosis of basement membrane of glomerulus and thickening of basement membrane of tubules) (207). They found that the K-score does correlate to graft function, but individual histological parameters do. They found that interstitial fibrosis and thickening of basement membrane of tubules are associated with graft duration ($p=0.007$ and 0.009 resp.), and vascular damage is associated with eGFR ($p=0.012$) and

serum creatinine (p=0.008) at 1-year. They suggest that diabetic donors require a more complex histological scoring system than the K-score alone to help predict outcomes. Ditonno et al found that deceased donor kidneys with a higher K-score had more DGF (213).

ECD donors

Carta et al in 2013 compared the outcomes of kidneys from donors >60 years of age that were scored as K-score of ≤3 to those that were scored 4-5 (209, 212). They found there was no significant difference between the groups in renal function or graft survival at 3-years. This confirmed the results of a previous study the group had done in 2012 in kidneys from donors >65 years of age that also found no difference in 1-year graft survival or renal function (210, 211).

Ramalho et al also found that the K-score had no effect on 1-year graft survival in both SCD and ECD kidneys, although they found that higher scores were correlated to worse renal function at 1 year (eGFR 60ml/min (K-score <2) vs 53ml/min (K-score ≥2) p=0.02) (216).

The earliest study was by Karpinski in 1999, and they looked at the outcomes of 34 donors over the age of 60years (206). 100% of kidneys with K-score ≥6 had DGF compared to 43% of kidneys with a K-score ≤5 (p=0.001), and kidneys with a K-score ≥6 had higher creatinine levels. They found that the vascular component of the score was the most significant and they suggest that if the creatinine clearance of the donor is <100mls/min, and there is more than 20% glomerulosclerosis and severed vascular disease on the biopsy then these kidneys should be considered for use as a DKT.

6.4.2.3 MAPI Score

6.4.2.3.1 Quality of studies

Of the five studies that used the MAPI score, only two were full text studies and the others were abstracts presented at various conferences. The two full text studies had Newcastle Ottawa scores of 6 and 5 (mid-range).

6.4.2.3.2 Comparing outcomes between Dual and Single Kidney Transplants

None of the studies that used the MAPI score compared outcomes between DKT and SKT.

6.4.2.3.3 Comparing outcomes of Single Kidney Transplants with different scores

All five of the studies that use the MAPI score report the outcomes in all deceased donors. The initial study in 2008 by Munivenkatappa used biopsies from 259 deceased donors and the findings were correlated to the outcomes of these kidneys to develop a pathological scoring system (219). This was validated in 112 random biopsy samples and found that graft survival was reduced if any component of the MAPI was present on the pre-transplant biopsy. They specifically looked at the 5-year graft survival and found it was 90% for low-risk kidneys (MAPI ≤7), 63 in intermediate-risk kidneys (MAPI 8-11) and only 53% in high-risk kidneys (MAPI 12-15).

A second study in 2009 confirmed these findings in 357 donors, including 47 donors >70years (225).

A study of 140 deceased donor kidney biopsies in 2010 found that MAPI had a 14% increased risk of graft loss for every point increase in score and found that a higher

MAPI is associated with lower graft survival (2.5-year graft survival 83.1% (low risk) vs 55.8% (intermediate risk) vs 50% (high risk) p=0.004) (221, 222). Another study from 2010 looked at 205 deceased donors (140 from the centre used in the other abstract, and 65 from a second centre) (223, 224). They found that 1-year graft survival was better for low MAPI kidneys than high MAPI kidneys in both centres and therefore conclude that the MAPI can be used in different institutions with different donor and recipient characteristics.

In the final study that uses the MAPI, 196 deceased donors are analysed (140 from one centre and 56 from a second centre) (220). This study looks at both 3-year graft survival and graft function, and found that the MAPI correlates to both with low MAPI having better graft survival and function at 3 years compared to kidneys with a high MAPI.

6.4.2.4 Banff Score

6.4.2.4.1 Quality of studies

All were retrospective studies, except two that stated they prospectively collected the patient and donor data (238, 251). One prospective study scored 8 on the NOS, which is at the higher end of the scale, only dropping a point for not having a control/comparative group (238). And the other scored six (251). The retrospective studies mostly scored a 5 or 6 on the NOS, with only one other study scoring 8 (see table 6.4).

6.4.2.4.2 Comparing outcomes between Dual and Single Kidney Transplants

There was one study that compared the outcomes between SKT and DKT. Traynor et al looked at the outcomes of 156 SKT and 15 DKT that had biopsies and also compared

these outcomes compared to standard kidney transplants (that had not had a biopsy) (232). This study found that there was no significant difference in the outcomes of SKTs and DKTs, and there was no significant difference in eGFR between DKTs and Standard SKTs, although there was a difference in eGFR between SKTs that were biopsied compared to SKTs from standard donors (44.4 ml/min vs 53.9ml/min). This study looked at the individual components of the Banff score and found that the glomerulosclerosis score had a significant association with 5-year eGFR, although it was not helpful when added to a predictive model using clinical information.

6.4.2.4.3 Comparing outcomes of Single Kidney Transplants with different scores

Thirteen of the studies analysed the different components that make up the Banff score separately (227, 229, 233, 235, 237-240, 243, 245-250, 252) (see Table 6.11). Five papers from four studies reported the outcomes of the Banff score as a total all components combined (230, 231, 234, 236, 251).

The thirteen studies that looked at the components separately looked at a variety of outcomes. Some looked at DGF, some graft survival and some graft function and others a combination of these outcomes. Twelve studies report the outcomes from all deceased donors, and one from ECD donors.

Deceased donors

There were six studies that looked at DGF (229, 237, 246-248, 250, 252). Of these studies one found that none of the components were linked to DGF (247, 248), and all other studies found that at least one component was associated with DGF, although none of them found that all the components were. Three studies found that two

components were associated with DGF (although different components in each study) (237, 250, 252), one study found that three components were associated with DGF (interstitial fibrosis, tubular atrophy and arteriolar hyalinosis) (229), and one study found one component was associated with DGF (246). The interstitial fibrosis and arteriosclerosis score were most commonly associated with DGF. Four studies associated arteriosclerosis with DGF and three studies associated interstitial fibrosis. The other components (glomerulosclerosis, tubular atrophy and arteriolar hyalinosis) were associated with DGF by two studies each.

Three studies investigated whether the Banff score was associated with early graft function (serum creatinine at discharge or, SCr or eGFR at 3- or 6- months) (237, 245, 250). One study associated one component (interstitial fibrosis) (245), one study associated four components (glomerulosclerosis, interstitial fibrosis, tubular atrophy and arteriosclerosis) (250), and one study found two components were associated with short-term function (glomerulosclerosis and interstitial fibrosis) (237). All three studies found that interstitial fibrosis was associated with short-term function. None of these studies found that arteriolar hyalinosis was linked to short-term function.

Three studies report the association between Banff components and graft survival (227, 238, 249). Two of these studies found that four components affect the graft survival (glomerulosclerosis, interstitial fibrosis, tubular atrophy and a vascular component) (227, 249), and one found that three components (glomerulosclerosis, interstitial fibrosis and tubular atrophy) do (238). All three studies found that glomerulosclerosis, interstitial fibrosis and tubular atrophy are associated with graft survival, two studies found there was a vascular component also associated with graft survival, but the two

studies differed in which one (one study found arteriosclerosis and one found arteriolar hyalinosis).

Five studies looked at the effect of the Banff components on longer-term graft function (227, 233, 243, 244, 247-249). One of these studies report 1-year eGFR as well as 1-year creatinine and found different components were associated with these outcomes and therefore will be included as two separate studies (247, 248). Two studies found that four components were associated with eGFR (227, 233). They both found glomerulosclerosis, interstitial fibrosis and tubular atrophy were, but differed as t the vascular component that was linked to this outcome, with Pegas reporting arteriosclerosis and Lee reporting arteriolar hyalinosis. Gurney found interstitial fibrosis, arteriosclerosis and arteriolar hyalinosis were associated with eGFR (247, 248). Gurney also found that glomerulosclerosis, interstitial fibrosis, tubular atrophy and arteriolar hyalinosis were associated with serum creatinine. Two studies found that interstitial fibrosis and arteriolar hyalinosis were associated with longer-term graft function. All six studies found that interstitial fibrosis was associated with function, and five studies found arteriolar hyalinosis was. Tubular atrophy and arteriosclerosis were found to be associated with graft function by three studies and glomerulosclerosis by two.

ECD Donors

There was one study that looked at the separate Banff components in ECD donors. Balaz found that interstitial fibrosis, tubular atrophy and arteriosclerosis correlate with DGF, whereas glomerulosclerosis and arteriolar hyalinosis do not (239, 240).

Study	cg	ci	ct	cv	ah	Outcome
Early Outcomes- All donors						
Hyodo 2017	✗	✓	✓	✗	✓	DGF
Malcevs 2013	✓	✓	✗	✗	✗	DGF
Munivenkatappa 2011	✗	✗	✗	✓	✗	DGF
Gurney 2010	✗	✗	✗	✗	✗	DGF
Matignon 2008	✗	✗	✗	✓	✓	DGF
Lopes 2005	✓	✗	✗	✓	✗	DGF
Malcevs 2013	✓	✓	✗	✗	✗	SCr (at D/C)
Lopes 2011	✗	✓	✗	✗	✗	SCr @ 3 & 6/12
Lopes 2005	✓	✓	✓	✓	✗	eGFR @ 3/12
Early Outcomes- ECD donors						
Balaz 2013	✗	✓	✓	✓	✗	DGF
Later Outcomes						
Pegas 2018	✓	✓	✓	✓	✗	GS
De Vusser 2013	✓	✓	✓	✗	✗	GS
Arias 2007	✓	✓	✓	✗	✓	GS
Pegas 2018	✓	✓	✓	✓	✗	eGFR
Lee 2016	✓	✓	✓	✗	✓	eGFR
Torrealba	✗	✓	✗	✗	✓	eGFR and SCr
Gurney 2010	✗	✓	✗	✓	✓	eGFR
Gurney 2010	✗	✓	✓	✓	✓	SCr
Arias 2007	✗	✓	✗	✗	✓	CrCl

Table 6.11 Studies that looked at the components of the Banff score separately and linked them to outcomes (✓= biopsy findings correlated to outcomes, ✗= biopsy findings did not correlate with outcomes)

Of the four studies that reported the outcomes of the Banff score as a total and not as the individual components, one found the Banff score did correlate with 1- and 5-year graft survival (251), although another found it did not (230, 231), and two found that the Banff score correlated with 1-year SCr and 1-year eGFR (234, 236).

6.4.2.5 % Glomerulosclerosis

6.4.2.5.1 Quality of studies

Four of the studies were full text articles and so were assessed for quality using the NOS. Three studies scored 7 and one scored 6.

6.4.2.5.2 Comparing outcomes between Dual and Single Kidney Transplants

None of the studies compared DKT with SKT outcomes.

6.4.2.5.3 Comparing outcomes of Single Kidney Transplants with different scores

All of the studies examined the percentage of glomerulosclerosis present in pre-implantation biopsies and linked this to outcomes in SKT. Only one study looked at this in ECD donors.

Deceased donors

Two studies used UNOS/OPTN/SRTR data on biopsies over different time periods and investigated whether the percentage of glomerulosclerosis affected the outcomes. Edwards et al looked at over 3,000 deceased donor transplants done between 1999 and 2001 (257). They divided the biopsy findings into ≤20% glomerulosclerosis and >20% glomerulosclerosis. They further stratified the donors according to their creatinine

clearance (≤80ml/min vs >80ml/min) and donor age (≤50 years vs > 50 years). They report that if the amount of glomerulosclerosis in the biopsy is ≤20% there is no significant influence on graft survival whatever the creatinine clearance is. However the graft survival is better if the donor is ≤50 years. If the percentage of glomerulosclerosis in the biopsy is >20%, there is worse graft survival when the creatinine clearance is >80ml/min. Bajwa also looked at OPTN/UNOS data but between 2000 and 2005 and reported outcomes comparing groups with 0-5%, 6-10%, 11-15%, 16-20% or >20% glomerulosclerosis (256). This study found that if the glomerulosclerosis was <5% graft survival was significantly better at 1-, 3- and 5-years compared to >5% glomerulosclerosis. There was no difference in graft survival rates in the groups where the amount of glomerulosclerosis is >5%. There was no difference in DGF rates when comparing <5% vs ≥5%, although serum creatinine at 1-year was better in the <5% group compared to the other groups ($p<0.001$).

The other three studies were single centre studies. Liu looked at 500 deceased donor transplants between 2009 and 2014 and found that there was no association between glomerulosclerosis and 1-, 2- and 3-year graft survival (253), whereas Randhawa found that increased percentage of glomerulosclerosis led to reduced graft function at 1-year (258) and Huh et al found the same in the short-term (>10% glomerulosclerosis led to reduced eGFR at 6 months) (255).

ECD Donors

In the most recent study using OPTN/UNOS data, Bodzin analysed outcomes of ECD transplants between 1994 and 2010 comparing the percentage of glomerulosclerosis grouped as in Bajwa's study (254, 259). They found that the graft survival of kidneys with <5% glomerulosclerosis was better when compared to the 6-10% group only, and if

the glomerulosclerosis is >10% there was no difference in graft survival between the different groups (11-15%, 16-20% >20%).

6.4.2.6 Other Scores

6.4.2.6.1 Quality of studies

Most of the studies scored six on the Newcastle Ottawa Scale, with one study scoring only a three and one scoring five. Two studies scored better than six, with one scoring seven and one scoring eight. Most studies lost points for selection; for not having a control group and for not explaining how they collected the data. They also lost points for not explaining how the outcomes were assessed.

6.4.2.6.2 Comparing outcomes between Dual and Single Kidney Transplants

There were no studies that compared the outcomes of single kidney transplants with dual kidney transplants.

6.4.2.6.3 Comparing outcomes of Single Kidney Transplants with different scores

All studies except two looked at outcomes in all deceased donors. One study looked at biopsy scores and outcomes in ECD donors and one in DBD donors.

Deceased donors

Three studies looked at whether Acute Tubular Necrosis/Acute Tubular Injury (ATN/ATI) had an affect on outcomes following kidney transplantation (260, 264, 267). Zhang defined severe ATI as the presence of flattened tubular epithelial cells or exposure of the basement membrane, and Hall defined ATN as the presence of any tubular injury.

They all looked at the association between ATN/ATI and DGF, and Hall (2014) also looked at the effect on graft survival. Zhang, and the first study by Hall (2013) found that if there was ATN/ATI on the biopsy there was a significant increase in the incidence on DGF, although Hall found that there was no association between ATN and graft survival. Three studies report the association of glomerular sclerosis, interstitial fibrosis and vascular changes (261, 265, 271). Two of these were in all deceased donors and one in ECD donors (results explained below). The study by Carpenter also included tubular atrophy and found that none of these components correlated with graft survival (261). Kayler however found that glomerular sclerosis and vascular changes were associated with inferior graft survival (271).

Three studies looked at a chronic damage or chronic lesion score (266, 272, 274), although the definitions for these were different in each study. Hofer defines a chronic lesion score as a sum of scores given to whether there are globally sclerotic glomeruli, arterial/arteriolar narrowing, tubular atrophy and interstitial fibrosis present. Each of these components (which also make up the Remuzzi score) is scored 1-3 based on whether there is none, mild, moderate or severe changes and these scores added together to give a score out of twelve. This large study of 628 deceased donors found that chronic damage on the biopsy correlates to graft survival (266). Anglicheau used a chronic allograft damage index (CADI), which was based on the individual component scores for interstitial inflammation, interstitial fibrosis, glomerular sclerosis, glomerular mesangial matrix increase, vascular intimal proliferation and tubular atrophy, with each component being scored from 0 to 3 (272). They report that the CADI is associated with a lower eGFR at 1-year in deceased donor transplants from donors older than 50-years. They also use the Remuzzi score and Banff components and found that the Remuzzi

score and the Banff components glomerulosclerosis and arteriolar hyalinosis correlate to eGFR at 1-year. They use these components with clinical parameters to form a composite scoring system that they find is the best predictive tool for outcomes. Ibernon uses a chronic damage score (CDS), which is calculated by the percentage of glomerulosclerosis combined with the interstitial fibrosis index and fibrous intimal thickening. In their study of 226 deceased donor transplants the CDS correlates with 3-month eGFR.

Two studies used a combination of some or all of the components of Banff with the addition of additional components (262, 276). Amenabar scored kidneys based on glomerulosclerosis, glomerular capsular fibrosis, cystic glomeruli, arteriolar hyalinosis, myointimal elastosis, tubular atrophy and interstitial fibrosis. The scores were grouped into 0-3, 4-5 and 6-8 and the outcomes for the groups were compared. There was no difference in DGF or 3-year graft survival rates between the groups, but there was a difference in 1-year eGFR (0-3 49.6 ± 12.5ml/min, 4-5 46.3 ± 15.7ml/min, 6-8 38.5 ± 14.1ml/min p=0.01). They also looked at the individual components and found ≥20% glomerulosclerosis, vascular and tubulointerstitial lesions were associated with poor clinical outcomes (262). Cahen looked at biopsies from 65 deceased donors and scored the biopsy on 30 different histological parameters (10 glomerular, 8 tubular, 4 interstitial and 8 vascular). Each component was scored 0-2 (0= none, 1= mild, 2=marked). They report that 6-, 12- and 18-month creatinine and graft survival was comparable between the normal and abnormal groups (these groups are not defined) (276).

A study by Jendrisak analysed the kidney biopsy audit score (KBAS) in 360 deceased donor biopsies, and found that if the KBAS is >40, this correlated to poorer graft

survival. The KBAS is a summary of the biopsy findings representing the aggregate severity of injury found within each of the four renal components with higher scores corresponding to more severe injury (268). Suhorukovs scored biopsies from 71 deceased donors according to the percentage of nephrosclerosis and found that higher percentages led to increased rates of DGF, poorer function and reduced graft survival (269).

Neuzillet looked at the number and morphology of the glomeruli in a biopsy, as well as the morphology of the interstitial space and vessels (275). The biopsies, from non-borderline donors, were classed as normal or abnormal and they report that the rates of DGF, and transplant function (measured by creatinine and creatinine clearance) were similar between the groups. They suggest that biopsies from non-borderline donors are not useful on their own to predict outcomes.

DBD Donors

Sulikowski looked at the intensity of ATN and correlated it to serum creatinine at 6-months and 1-year, and then in a later paper serum creatinine at 3-years (270, 273). They found that the presence of ATN correlated with a worse 6-month serum creatinine, but did not correlate with 1- or 3-year creatinine. They also looked at other histological parameters (tubulitis, inflammatory infiltration, glomerulonephritis, arterial hyalinization, arteritis, fibrosis, tubular atrophy, fibrosis of arterial intima, growth of mesangial matrix and percentage of glomerulosclerosis). They found that fibrosis of the arterial intima, interstitial inflammation and interstitial fibrosis were associated with higher serum creatinine concentrations at 12-months, and tubular atrophy had a borderline association with serum creatinine at 1 and 3 months.

ECD Donors

Rabin was the final study that reports the effect of glomerular sclerosis, interstitial fibrosis and vascular changes on outcomes, and found that these components have no association with graft survival or DGF in ECD kidney transplants (265).

6.5 Discussion

This systematic review has identified multiple studies that have used biopsies of donor kidneys prior to transplantation to guide kidney allocation protocols to SKT or DKT, or have investigated whether the biopsy findings can predict outcomes. The review has found that there is no consistency between biopsy findings and outcomes with a large variability between studies. Different studies have looked at different donor types (deceased donors, DCDs, ECDs), used different histological scores and investigated different outcomes. Therefore, it has not been possible to perform a meta-analysis of the included studies.

Looking at the results of each scoring system to try and see whether one scoring system has better correlation between biopsy findings and outcomes than others, it would seem as though the Banff scoring system correlated with outcomes more often than not in deceased donors, although there were not many studies that looked at ECD donors specifically. The MAPI score was consistent in predicting outcomes in all of the studies that used it, but these studies all came from the same group, so it has not been validated by an independent centre.

Whether biopsies are predictive to guide the use of kidneys as either SKT or DKT is also difficult to conclude as in the studies that compare DKT with SKT there is no control group, for the DKT kidneys, of an SKT. So it is possible (although perhaps unlikely) that higher-scoring single kidneys would have done just as well as the lower-scoring single kidneys if directly compared, and DKT is actually unnecessary.

When looking at whether the biopsy findings correlate with outcomes in specific donor types, using the most used scores; Remuzzi, Karpinski, MAPI, Banff and

glomerulosclerosis there were 32 studies that looked at the outcomes in all deceased donors. Eleven found the biopsy findings correlated to graft survival (192, 207, 219-225, 227, 238, 249, 250, 256); with six finding there was no correlation (185, 194, 216, 230, 231, 253, 257). Eighteen studies found biopsy findings correlated to graft function (185, 192, 201, 216, 223, 224, 227, 228, 233, 234, 237, 243-245, 247-250, 252, 255, 256), and three found they did not (194, 207, 258). Eleven studies (192, 201, 213, 223, 224, 229, 233, 234, 237, 246, 250, 252) found DGF was related to biopsy findings whereas three studies found there was no relationship (194, 247, 248, 256).

There were 17 studies that looked at ECD donors. Five found that the biopsy findings correlated with graft survival (184, 188, 189, 236, 251) and nine found they did not (182, 186, 190, 193, 200, 209-212, 254). Five found the biopsy score was related to graft function (188, 189, 200, 206, 236) whereas eight found there was no relationship (182, 184, 186, 187, 193, 195, 209-212). And two found the biopsy score correlated with DGF (206, 239, 240) and five found it did not (184, 186, 187, 195, 210, 211).

There were only two studies that looked at biopsy findings and outcomes in DCD donors. One study found there was a correlation with graft survival (198, 199), whereas the other study found there was not (202-204). The one study looked at graft function, found there was a correlation between biopsy findings and function (202-204).

Therefore, it seems that although the use of biopsies has been advocated in older more marginal donors (ECDs and DCDs), the biopsy findings do not seem to correlate as consistently with outcomes in this group.

Most of the scores used are based on similar renal components identified on biopsy; glomerulosclerosis, tubular atrophy, interstitial fibrosis and a vascular component

(arterial or arteriolar). The scores do not give more weight to any individual component, but many studies, particularly the ones that use the Banff score, report how each component relates to outcomes. However, even the studies that do this do not have consistent findings, with many studies suggesting that a different component is the most important, or different combinations of components on multivariate analysis.

It has been suggested that the different components of the biopsy score are important for different reasons:

Glomerulosclerosis refers to scarring of the glomeruli, and is a marker of irreversible nephron damage. Kaplan et al found that the incidence of glomerulosclerosis increased with age in normal adults (277). Hypertension can also contribute to the degree of glomerulosclerosis and this is why it has been suggested that it is an important to report. However, different studies report different thresholds of glomerulosclerosis to be significant from 5% up to 20%, and there are more studies reporting that it does not correlate to outcomes.

Interstitial fibrosis and tubular atrophy are markers of progression towards kidney disease and are associated with vascular insults including ischaemia and hypertension (278). Several studies felt that these components were the most important predictors of post transplant function as they are often later markers of kidney damage seen in older donors, or donors with a history of hypertension (238, 240, 243-245). This is consistent with a recent analysis of living donors from the Mayo clinic (279). This large study of 2,673 living donors found that microstructural findings on the biopsies predicted changes in kidney function early after donation. Specifically they found that low nephron number, larger nephron size and age specific nephrosclerosis all contributed to poorer post donation measured GFR.

The process of donation and transplantation has an effect on the renal vasculature, and therefore pre-existing vascular injury may be a useful marker of post transplantation outcomes (280). This has been confirmed in a few studies that report the vascular component being predictive of post transplant outcomes (233, 234, 271).

As it is not clear which component of the biopsy score is the most important, the added concern that the scores are not reproducible make it difficult to use the biopsy to predict outcomes. The reports may be different depending on the experience of the pathologist, and also the speciality (281). It has been reported that there is a discordance in the biopsy score for all components of the score (glomerulosclerosis, interstitial fibrosis, tubular atrophy and vascular component) in both wedge and needle biopsies (282). Azancot addresses this in their study, and found that the on call pathologist over-estimated the amount of chronic change on the biopsies, when compared to renal pathologists (236). They report that the scores assigned for global glomerulosclerosis correlated well, but there was only fair correlation for arterial intimal fibrosis and interstitial fibrosis, and poor agreement for arteriolar hyalinosis and tubular atrophy. This was significant as the donor damage score determined by the renal pathologist was independently associated with 1-year eGFR, but this was not the case when the on-call pathologist determined the chronic damage score. Fernandez-Lorente also found that when biopsy samples were blindly re-evaluated by renal pathology experts the scores changed. In their study this meant that kidneys that had been allocated to SKT had worse scores than originally stated, and the outcomes were inferior (188).

Another inconsistency in the studies identified is the biopsy technique that was used. Some centres used a wedge biopsy and others a core needle biopsy, and many of the studies did not describe what biopsy technique was used. Of the studies that reported what biopsy technique was applied, more report using a wedge biopsy rather than a needle biopsy. There are advantages and disadvantages to each of these techniques (wedge or core needle).

Wedge biopsies are less likely to damage larger blood vessels, but they overestimate the glomerulosclerosis and underestimate the severity of vascular changes (283).

Core needle biopsies have increased sensitivity in estimating cortical lesions, but increase the risk of large vessel damage (281, 282, 284).

The correlation between the biopsy findings and outcomes for both wedge and needle biopsy were very variable in this review.

None of the studies report complications from the biopsies when either wedge or core needle biopsy was done.

There is a concern that the use of biopsies may increase the discard rate of potentially transplantable kidneys. As already mentioned biopsies are more frequently used in the US and they do have a higher discard rate of kidneys that we do here in the UK without having better outcomes (14, 180). In the UK, some centres are increasingly using biopsies, especially in an attempt to increase the use of older DCD donor kidneys that would otherwise be discarded. The group in Cambridge have successfully used biopsies to increase their deceased donor program, especially their DCD program with good outcomes (285).

In this review the rate of discard was reported by some studies and it ranged from 9% to 32% of biopsied kidneys being discarded (177, 188-190, 195, 200, 215, 264, 268).

Obviously there is no way of knowing how discarded kidneys may have functioned once transplanted, and this is a limitation of all of the studies. Some of the studies only had the biopsy reports available after transplantation, and in these studies, kidneys that would potentially have been discarded due to poor scores were used. One study within this group that specifically report the outcomes of kidneys that had particularly bad scores was from Assis-Borba. They found a significant difference in eGFR in kidneys with a Remuzzi score of >7 (184). Another way to try and look at what the outcomes of kidneys that could potentially be discarded is to look at studies that use re-perfusion biopsies. As these obviously would not be available before transplantation, there is a chance that kidneys with unfavourable scores would have been transplanted. This is the case in a recent study by Phillips who found that the Karpinski score had no significant effect on graft survival (including kidneys with a score of 8), although kidneys with a score of 6-8 had worse function (eGFR) at 1- and 3-years (286). This review does not include studies that used re-perfusion biopsies. The reason for this is that there is a question as to the concordance of pre-implantation and reperfusion biopsies. In the study by Carpenter, they report that the overall histological classification was the same in 64% of cases. Kidneys that were classed as sub-optimal on pre-implantation biopsies were deemed as optimal in 13% of reperfusion biopsies. And kidneys that were deemed optimal on the pre-implantation biopsy were reported as sub-optimal on the reperfusion biopsy in 23% of cases (261).

A limitation of this study is the quality of all the studies that were identified. Most were retrospective observational studies and there were no randomised controlled trials. The Newcastle Ottawa scale was used to assess the risk of bias of the studies. The lower the

score the greater the risk of bias in the study. This is a widely used tool recommended by the Cochrane collaboration, however there is no specific cut-off value for what score is low or high risk for bias (287). The average NOS for all the studies included in this review was 5.9 (Remuzzi 6.1, Karpinski 4.7, MAPI 5.5, Banff 6.1, Glomerulosclerosis 6.8 and other 5.9) which would suggest that there is a moderate risk of bias in all the studies included. Most of these studies do not have a comparative group, which is reasonable given that they are not comparing the outcomes, however this means that they score less on the NOS.

As previously mentioned, another limitation is that a meta-analysis could not be done, as the studies were so heterogeneous in their donor types, scores and outcomes.

Overall this review can conclude that centres use biopsies for varying donors, and that the different biopsy scores used have mixed results as to whether the scores relate to post-transplant outcomes. Many groups suggest that the biopsy information should be used in conjunction with clinical details to make the decision about whether to use the kidney or not. Anglicheau has suggested a composite scoring system, consisting of donor creatinine, donor hypertension and glomerulosclerosis, which strongly predicts graft function and survival (272). De Vusser derived the Leuven Donor Risk Score, which was a combination of donor age along with the interstitial fibrosis, tubular atrophy and glomerulosclerosis scores and they found that this was highly predictive for 5-year graft survival (238).

The lack of consistency between biopsy scores and outcomes, along with the discrepancy of biopsy reports suggests that the use of kidney biopsies to decide whether to accept or decline a kidney is currently not justified. This is in addition to the added

cost that processing and reading a biopsy prior to transplantation adds, as well as the logistical challenges that taking a biopsy poses, including who is going to read the biopsy (as most UK units do not have a 24-hour histology service). There is also the risk that waiting for a biopsy result to decide whether to accept or decline a kidney will prolong the cold ischaemic time, and this may have more effect on the outcome than the biopsy result. This is particularly important in more high-risk organs when the cold ischaemic time should be kept as short as possible. In the UK there is currently a randomised controlled trial running which is looking at a 24-hour renal pathology service to report biopsies (PITHIA), and hopefully this will give a greater understanding on how to interpret pre-implantation biopsies in respect to their predictive value of post-transplantation outcomes as well as answering some of these logistical issues (288).

CHAPTER 7 SUMMARY

7.1 Summary of Thesis Findings

It should have become clear from this thesis that the current era of transplantation, despite all its achievements, is presenting clinicians with many challenges. Donors are becoming older with more co-morbidities, but yet the number of potential transplant recipients on the transplant waiting list is still increasing (14). Potential donor organs are subjected to injury at all stages of the deceased donation process. This concept was explored in the introductory chapter where I discussed many of the issues facing kidney transplantation today. Currently, there are more patients on the waiting list for a kidney transplant than kidney transplants performed per year. In order to bridge this gap clinicians are accepting organs from more marginal, higher-risk donors. Although these offer a survival benefit for individual patients versus remaining on dialysis, they may not always result in the same good outcomes as kidneys from the so-called standard criteria donor. However, as these higher-risk kidney offers are becoming more the norm, it is important to know that using them for transplantation is not exposing patients to unnecessary or additional risk, and that outcomes are not compromised. Having as much information as possible about outcomes from these types of donors will help transplant clinicians to make informed decisions about whether to accept or decline the donor kidney.

As I found in **chapter 3**, the donation process nowadays is taking longer, and therefore the duration of brain death is significantly extended. I have shown that this prolonged period of brain death does not negatively affect graft survival following kidney, liver and pancreas transplantation, and actually appears to be beneficial for kidney graft survival (if the ensuing CIT is longer than 12hours). This could be particularly useful in more

marginal donors when this time period can be utilised for observation, investigation, or to deliver targeted therapeutic interventions in an attempt to improve organ conditions. The duration of brain death is longer, in part due to the allocation and logistic process becoming more complex. As already mentioned, donors are more likely to be older with co-morbidities themselves, and some recipients are also becoming more complex, therefore ensuring that the organs are offered to appropriate patients will take more time.

As established in **chapter 4**, the use of donor kidneys with acute kidney injury (AKI) can give good outcomes following transplantation, which is in contrast to the perception of many clinicians who tend to decline these organs. In fact, the time during donor management can be used to observe whether kidney injury is worsening or actually improving. Although this analysis has shown that kidneys with an AKIN stage 1 or 2 AKI can be used with outcomes (graft survival and function) equalling those of kidneys with no AKI, some clinicians remain hesitant about using these kidneys. Creating time to observe and assess AKI may give clinicians more reassurance about transplanting the organs. In the analysis, 30% of kidneys that were declined were kidneys with AKI; if not unnecessarily declined and discarded these donor kidneys could help to expand the donor pool and be a valuable resource of additional organs.

If we accept higher risk organs such as ECDs, kidneys with AKI and DCDs we need to be sure that the injury they are subjected to during the actual process of the retrieval does not compromise the viability of the organs, and this was explored in **Chapter 5**. This study utilised an animal model to simulate the DCD retrieval using commonly used preservation solutions. Many authors have stated that including a colloid in a cold storage preservation solution such as hydroxy-ethyl starch increases viscosity and will

have a negative impact on the quality of flush-out at time of retrieval, preventing good preservation of, especially, the cortical areas of the parenchyma of donor organs. In my study I found that all preservation solutions currently used to cool the kidney flush well and provide adequate preservation, although UW-CSS cools the kidney more efficiently. The study found that all solutions reach the microcirculation of the organs to ensure that the blood is washed out and prevent micro thrombi formation. It also showed that there were no differences in the cellular markers of injury found in kidneys when different preservation solutions were used reassuring us that the preservation solution used currently in practice (UW-CSS in the UK) is not inferior to other available solutions. Once the kidney has been retrieved and is placed on the back-table, the final decision as to whether to use or decline the kidney is often made after inspecting the organ. It has been advocated to take a biopsy of the kidney as a standard procedure to see whether there are histological changes in the donor kidney that might help making the right decision. In **Chapter 6,** I performed a systematic review of studies that have investigated whether the biopsy findings do actually correlate to outcomes. This review found that the available evidence is of a fairly poor quality, and does not support the conclusion as to whether the histological changes found in a kidney will affect the way it may function post transplantation. I found that there are multiple different methods to score the biopsy findings, and that there is no consensus as to which scoring system or which renal component best predicts outcomes.

Therefore, and in order to make the decision as to whether to accept or decline an organ, it is clear that multiple factors need to be taken into account. This includes donor but also recipient factors. This thesis provides information about donors and the donation process that can be used by clinicians together with other evidence on

outcomes on how best to determine the risk benefit of using specific organs for specific recipients.

7.2 Future Work

Although this thesis has provided evidence that may help clinicians to decide as to whether to use or decline a kidney, it has also has given the foundations of further work that could be done to enhance the information to make these decisions easier still.

Quality in Organ Donation (QUOD) sample analysis

The QUOD national biobank is a bioresource that integrates biological samples with clinical data from deceased donors and outcomes after transplantation. This biobank was established by the Oxford group in close collaboration with other academic centres and within the strategic research framework of NHSBT for improving donor organ quality, increasing donor organ utilisation and obtaining better transplantation outcomes for organ recipients (101, 289). Blood, urine and tissue biopsy samples from kidney, liver and spleen, are collected. All samples are transferred to the QUOD laboratory facility at the University of Oxford for long-term storage. These samples are accessible for researchers once their study application has been approved by a steering committee. This resource is one that I hope to be able to use for several future projects.

In **chapter 3** I found that the duration of brain death does not lead to detrimental outcomes for kidneys, livers and pancreas after transplantation. However, it would be useful to know if there are specific markers of injury or repair in donor blood or biopsy samples at different time points. In previous work done using the QUOD samples it has been shown that pro-fibrotic, apoptotic and catabolic pathways are enriched in DBD

kidneys that have poorer outcomes, and cytoprotective pathways are activated in DBD kidneys with good allograft function (290). I would like to stratify biopsy samples according to brain death duration and look for markers of these pathways and see whether there is a minimum duration before the pathways of injury start, or after which repair pathways are initiated. Blood samples could also be analysed to see if they show any markers of these pathways.

Following on from the registry analysis in **chapter 4** that found kidneys with AKI can have good outcomes following transplantation, I would like to further investigate whether kidneys with AKI that develop poor outcomes have specific histological findings, or molecular markers of injury that differ to AKI kidneys with good outcomes, or standard donors without AKI. Specifically, I want to address whether kidneys with AKI have different molecular markers of injury to kidneys with no AKI, have different mitochondrial bioenergetics to kidneys with no AKI and whether kidneys with AKI have specific histological findings that relate to outcomes. By stratifying the samples according to good and poor outcomes in the recipient it may be possible to identify molecular profiles or pathways in donors with AKI that could predict or correlate to the outcome post transplantation.

As seen by the systematic review on donor biopsies and whether they relate to outcomes, it is clear there is no consensus view. Therefore, I would like to use the QUOD biobank to try and investigate this further. Discussion with renal histo-pathologists has led to the decision that alongside the usual components that are scored on a biopsy, other components may also need to be scored. These scores will then be related to the clinical outcomes using NHSBT registry data on UK transplants. The other components assessed can also be related to outcomes, and then validated in a further set of biopsies

to come up with a more reliable score that does relate to outcomes.

Retrieval studies

Following on from the DCD retrieval model work, it would be useful to extend the experiments and use a normothermic perfusion model to simulate transplantation in order to assess organ quality. This might enable us to know whether the difference in the cooling actually makes a difference to outcomes.

As the animal retrieval model showed that the temperatures reached during the retrieval are not as low as it was thought, it would also be beneficial to focus studies on improving retrieval technique. This would be particularly useful in donors after circulatory death, concentrating on keeping temperatures down and extraction times short. This will determine whether organs are cooled to a lower temperature in clinical retrievals, and whether the temperature reached does affect outcomes.

Summarising, the assessment of donor organ quality and better insight in mechanisms of injury and repair possibly leading to targeted therapeutic interventions have become key in organ transplantation. Novel technologies aim to identify important molecular pathways and clinically relevant biomarkers to enhance clinical decision making at the time of offering. The detection of promising signals of repair and stimulation of regeneration in damaged donor organs may allow us to reduce discard and increase organ utilisation whilst avoiding a negative impact on post transplant function as well as graft and patient survival.

7.3 What this Thesis means to me

Spending three years doing this work has taught me so much about the different formats research can take. I have learnt more about the laboratory based techniques-

particularly Western blot analysis and immunohistochemistry. It has shown me how sensitive these methods are to small changes and how the results need to be interpreted in combination with other findings. I definitely learnt to use patience and perseverance when working in the lab!

Doing the registry analyses taught me not to try to predict the outcomes. I learnt a lot about statistical analysis from the team at NHSBT and this has helped me understand and critically appraise new and existing studies/literature, which in turn will help me alter my practice in a more meaningful way. The registry analysis of donors with AKI has given me reassurance to use kidneys with an AKI, however the colleagues I work with are more conservative and I am working to educate them with the outcomes of my work, and the experience of others. Colleagues in other units have told me that they have used this work to increase their use of kidneys with an AKI, which is great to hear.

Doing the systematic review helped me consolidate my knowledge on pre-implantation biopsies. When I started this, I thought the results would be conflicting, and this is what was found. In terms of how this affects my practice; it has given me confidence to accept kidneys based on the clinical information available, but shows that having the baseline biopsy findings are useful to compare future biopsies to and also to guide certain aspects of a patients management as well as expectations of outcomes.

Overall all the work I have done for this thesis means that I can confidently form my own opinions on how I want to deliver my practice, and am able to give my patients the information they require to make decisions along with my guidance. It adds to the current literature available to help all transplant clinicians make as informed a decision as possible about the quality and potential outcomes of a kidney offered for transplant.

CHAPTER 8 APPENDICIES

Appendix A- Liver and Kidney biopsy histology slides

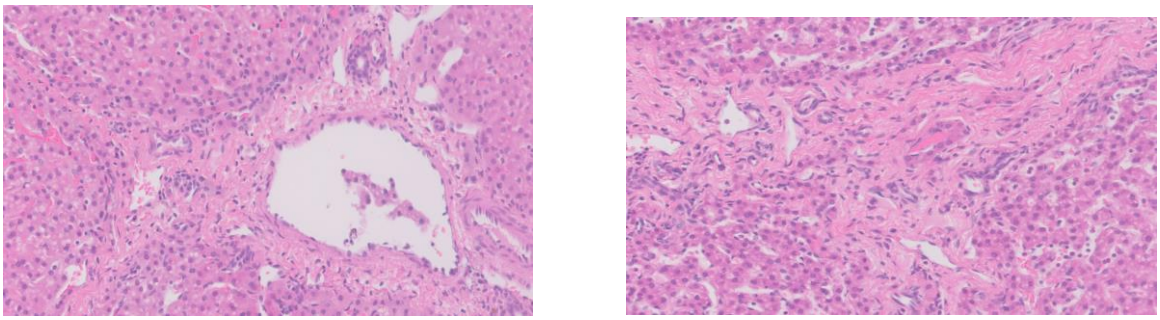


Figure A Liver biopsy at start and end of procedure when using UW-CSS

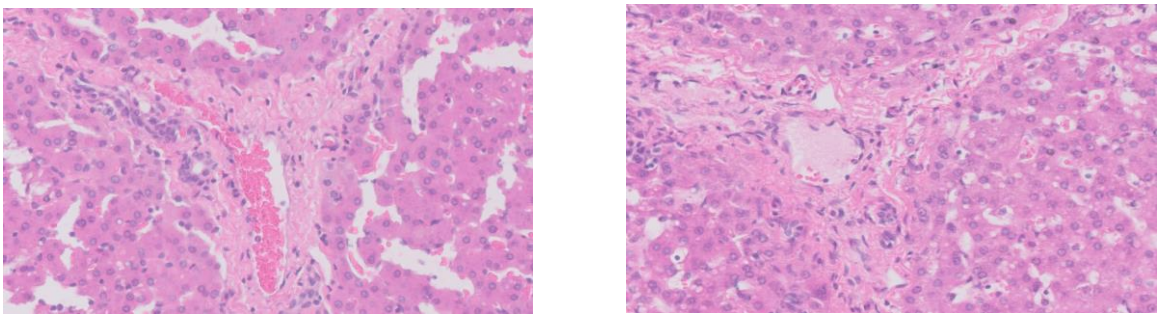


Figure B Liver biopsy at start and end of procedure when using UW-MPS

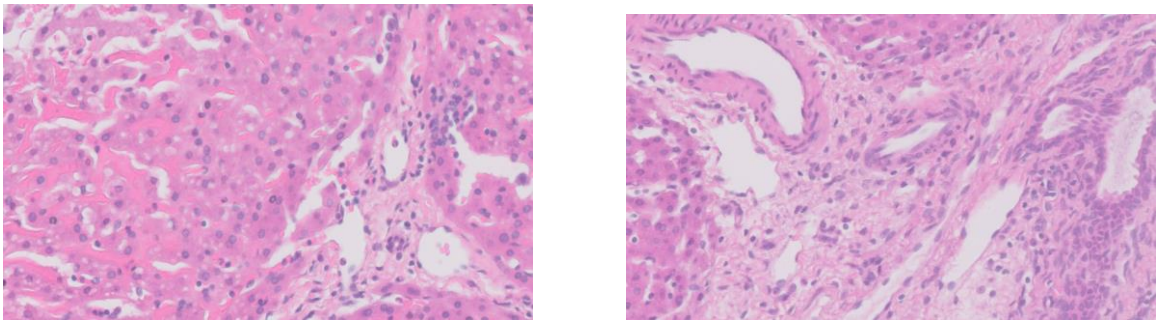


Figure C Liver biopsy at start and end of procedure when using IGL-1

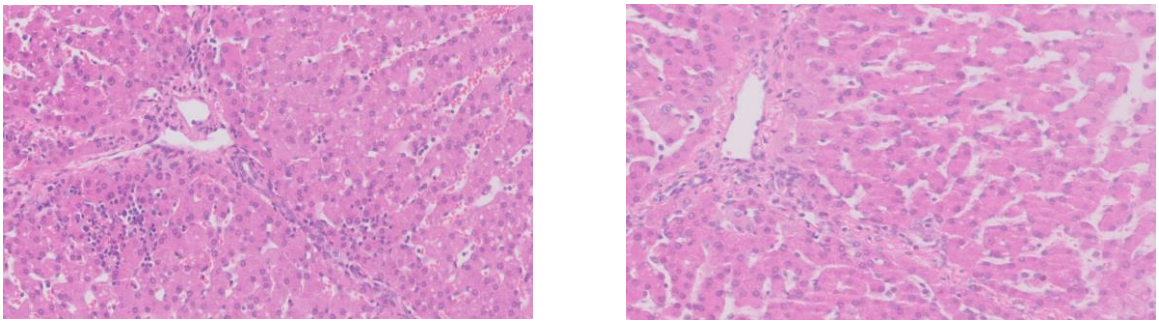


Figure D Liver biopsy at start and end of procedure when using HTK

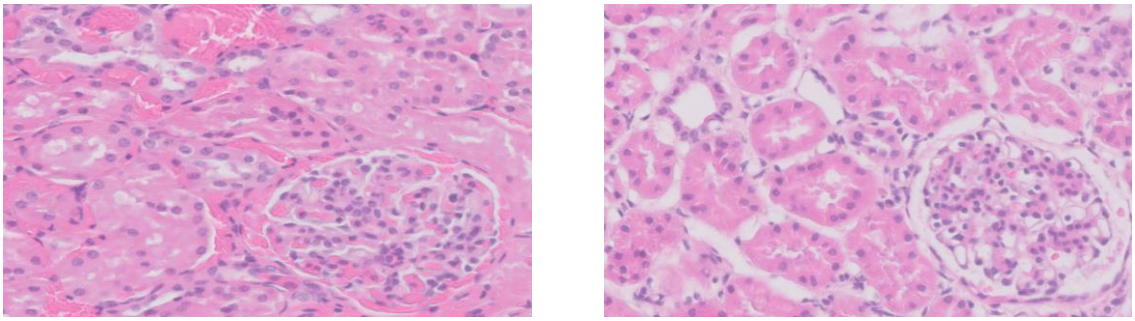


Figure E Kidney biopsy at start and end of procedure when using UW-CSS

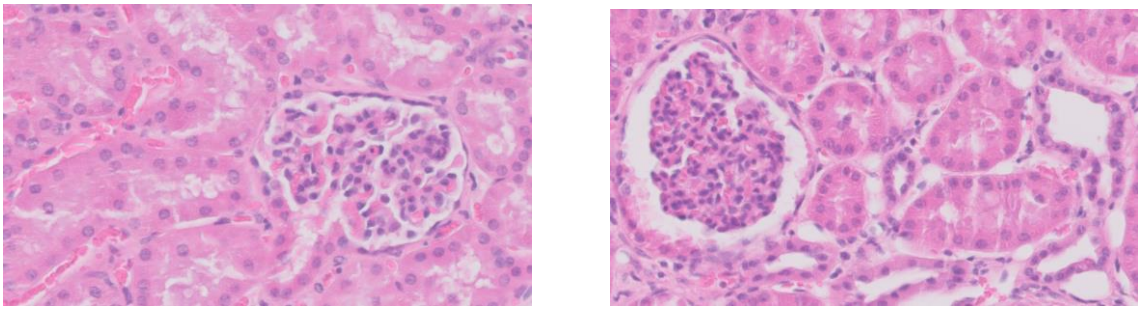


Figure F Kidney biopsy at start and end of procedure when using UW-MPS

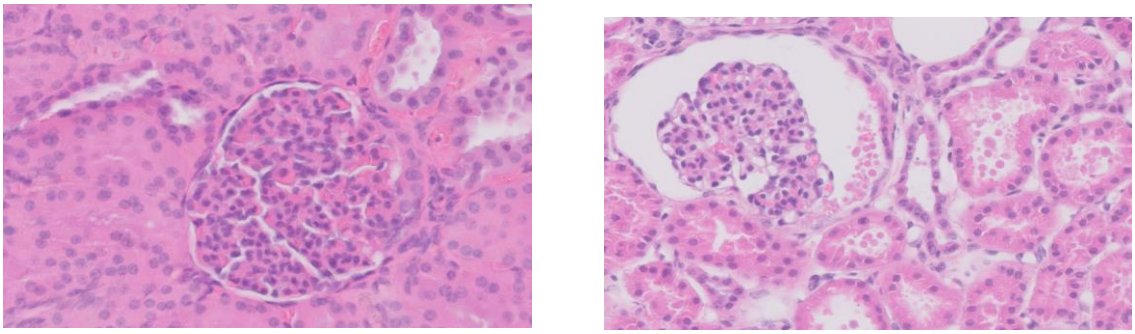


Figure G Kidney biopsy at start and end of procedure when using IGL-1

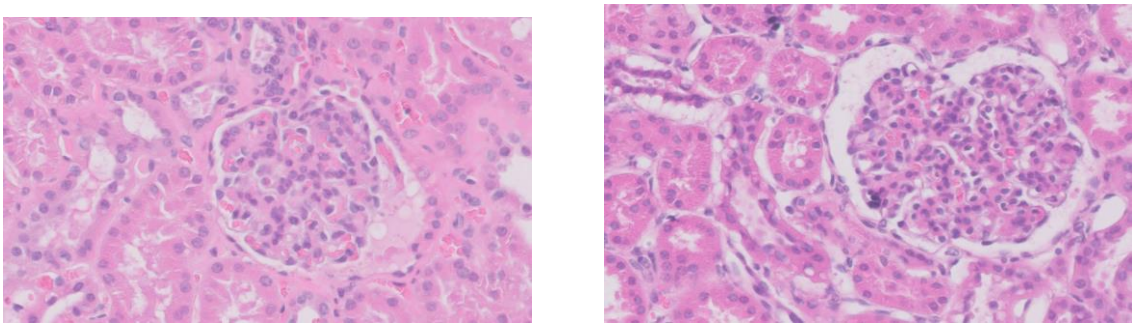


Figure H Kidney biopsy at start and end of procedure when using HTK

Appendix B- Western Blot Figures

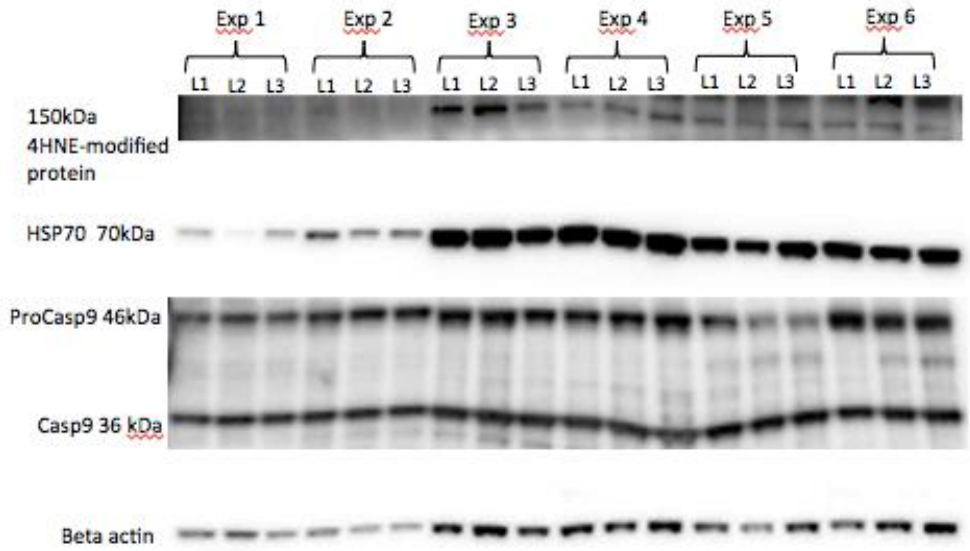


Figure I Western Blot analysis for 4-HNE, HSP70, Pro Caspase 9 and caspase 9 with the control Beta Actin for Liver biopsies from the UW perfusion experiments

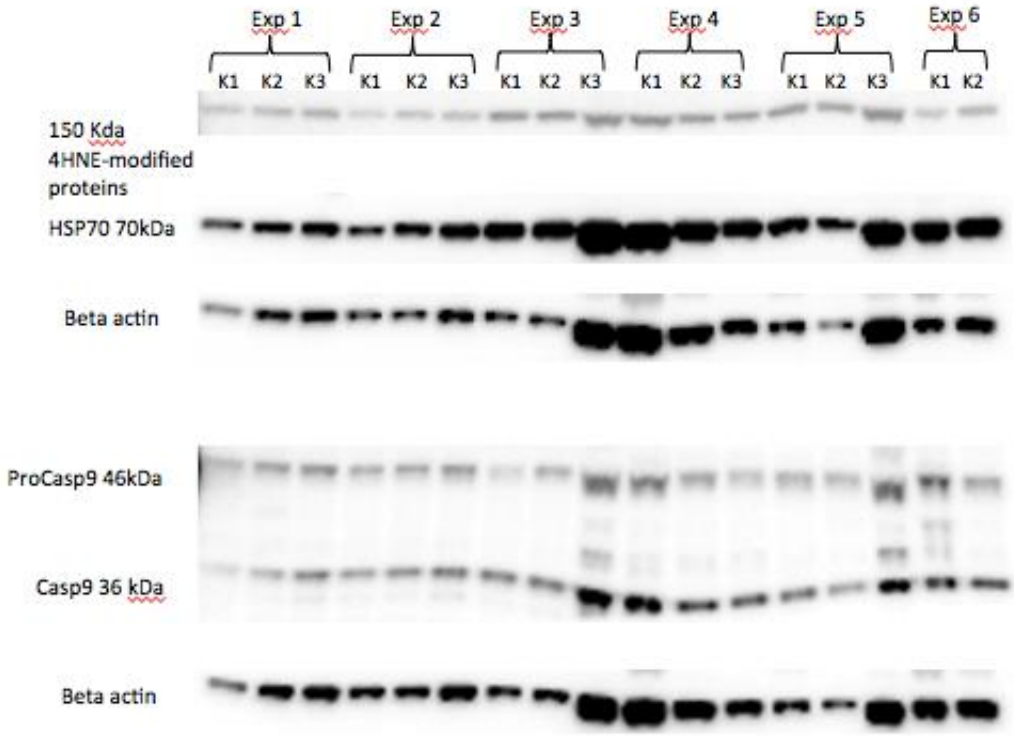


Figure J Western Blot analysis for 4-HNE, HSP70, Pro Caspase 9 and caspase 9 with the control Beta Actin for Kidney biopsies from the UW perfusion experiments

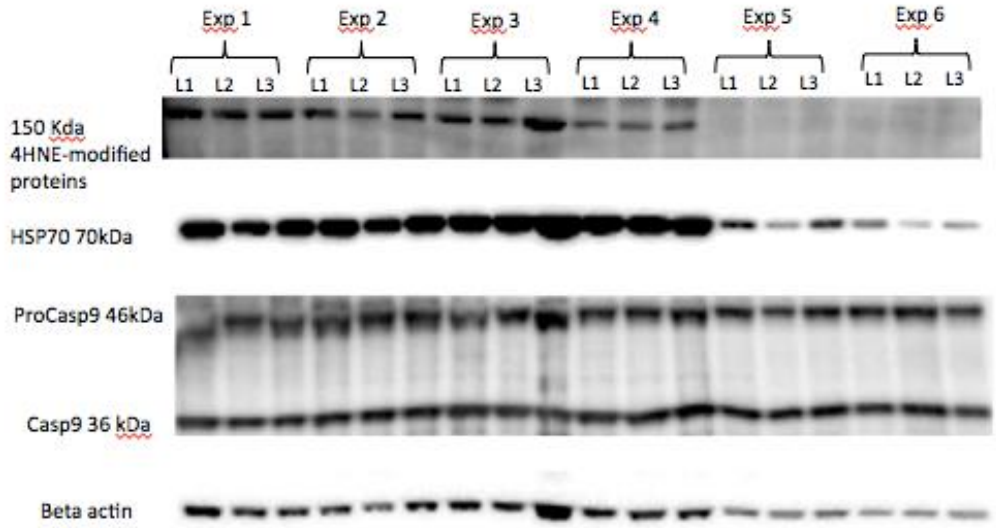


Figure K Western Blot analysis for 4-HNE, HSP70, Pro Caspase 9 and caspase 9 with the control Beta Actin for Liver biopsies from the HTK perfusion experiments

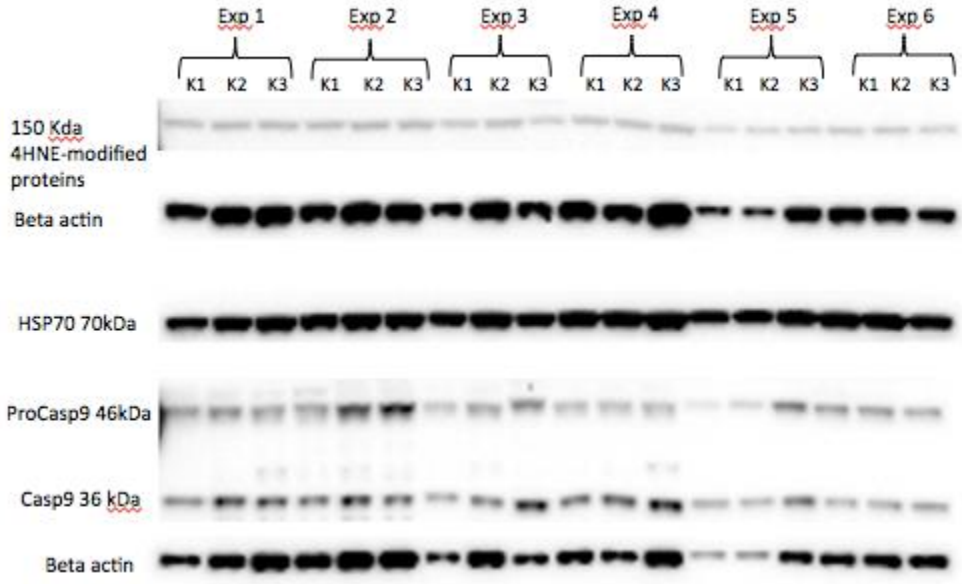


Figure L Western Blot analysis for 4-HNE, HSP70, Pro Caspase 9 and caspase 9 with the control Beta Actin for Kidney biopsies from the HTK perfusion experiments

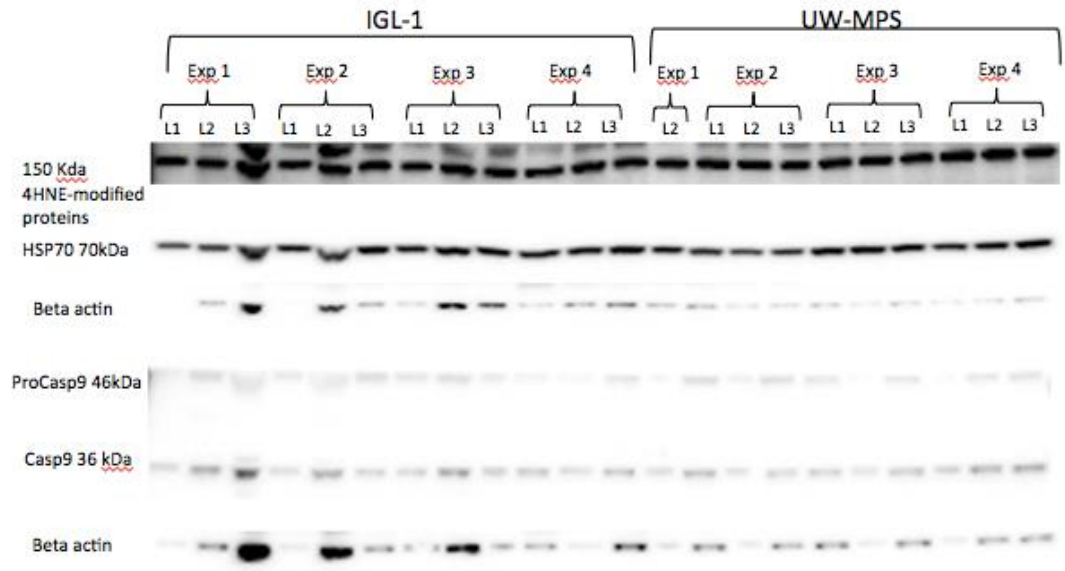


Figure M Western Blot analysis for 4-HNE, HSP70, Pro Caspase 9 and caspase 9 with the control Beta Actin for Liver biopsies from the IGL-1 and UW-MPS perfusion experiments.

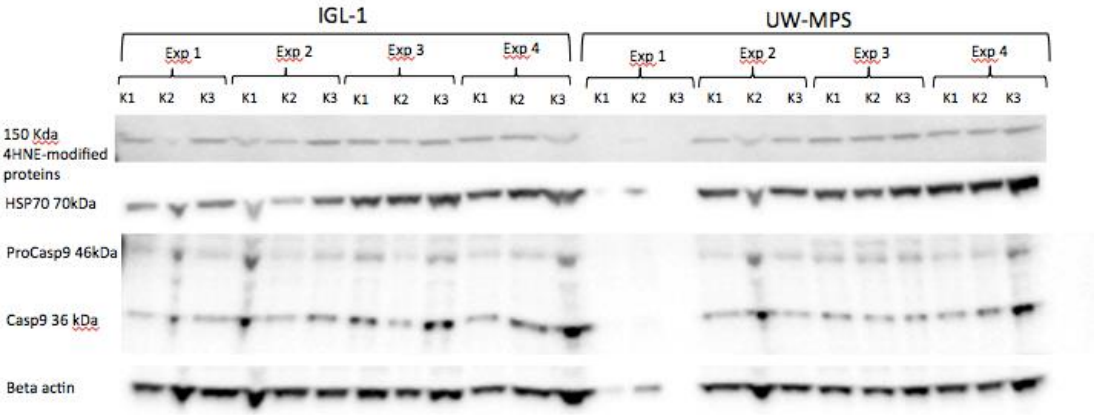


Figure N Western Blot analysis for 4-HNE, HSP70, Pro Caspase 9 and caspase 9 with the control Beta Actin for Kidney biopsies from the IGL-1 and UW-MPS perfusion experiments.

Appendix C- Systematic Review Protocol

"Do pre-implantation biopsies affect patient management or outcomes following renal transplantation?"

Patient/problem:	Deceased donor transplant grafts, adult or paediatric, DCD, DBD
Intervention:	Pre-implantation: Biopsy taken post retrieval (after cold perfusion) and before reperfusion (before removal of arterial clamps).
Comparison:	No biopsy taken[1]
Outcome:	Rate of graft utilisation Transplant outcomes (graft/patient survival, graft function (serum creatinine, creatinine clearance, delayed graft function, primary non-function) Post transplant surgical complications (bleeding, AV malformations, haematuria, transfusion, lymphocele, need for intervention (interventional radiology/operative), graft loss) Immunosuppression used

Type of Studies:
Cohort Studies, Case control studies, RCTs
Case series of at least 20 patients

Alternate terms:
Pre-implantation, preimpantation, retrieval, pre-retrieval, preretrieval, implantation
Biopsy, trucut, needle biopsy, core biopsy, histopathology, cytopathology
Renal, graft, allograft

Inclusion Criteria:
All deceased donor renal grafts for transplantation- DBD, DCD, ECD
Adult and paediatric donors
Adult and paediatric recipients
Publications in any language

Exclusion Criteria:
Biopsies taken for diagnosis of clinically apparent lesion within kidney
Living Donors
Studies not reporting any of the pre-defined outcomes
Pre-procurement biopsies (prior to cold perfusion)
Reperfusion Biopsies (taken after arterial and venous clamps removed)
Protocol biopsies (after skin closure)
Animal model studies

Searches:
Medline
Embase
Transplant Library

Cochrane Library
Trial registries
Reference lists

Appendix D- Search Strategies used for systematic review

Embase Search Strategy

1	ORGAN TRANSPLANTATION/	35129
2	TRANSPLANTATION/	148120
3	exp KIDNEY TRANSPLANTATION/	145432
4	((kidney$ or renal$) adj5 transplant$).tw.	160536
5	((kidney$ or renal$) adj5 graft$).tw.	64567
6	((kidney$ or renal$) adj5 allograft$).tw.	27029
7	or/1-6	316736
8	((histolog$ or biops$ or histopath$ or lesion$ or patholog$) adj5 scor$).tw.	44258
9	((histolog$ or biops$ or histopath$ or lesion$ or patholog$) adj5 (donor or pretransplant$ or pre-transplant$ or preimplant$ or pre-implant$ or baseline)).tw.	15238
10	(KDPI or "kidney donor profile index" or "remuzzi score$" or "donor chronic damage score$" or "Leuven donor risk score$").tw.	627
11	or/8-10	59012
12	**7 and 11**	**4422**

MEDLINE Search Strategy

1	ORGAN TRANSPLANTATION/	12237
2	TRANSPLANTATION/	8865
3	exp KIDNEY TRANSPLANTATION/	92146
4	((kidney$ or renal$) adj5 transplant$).tw.	84596
5	((kidney$ or renal$) adj5 graft$).tw.	13661
6	((kidney$ or renal$) adj5 allograft$).tw.	16739
7	or/1-6	134233
8	((histolog$ or biops$ or histopath$ or lesion$ or patholog$) adj5 scor$).tw.	24201
9	((histolog$ or biops$ or histopath$ or lesion$ or patholog$) adj5 (donor or pretransplant$ or pre-transplant$ or preimplant$ or pre-implant$ or baseline)).tw.	6849
10	(KDPI or "kidney donor profile index" or "remuzzi score$" or "donor chronic damage score$" or "Leuven donor risk score$").tw.	140
11	or/8-10	30773
12	**7 and 11**	**1206**

Transplant Library Search Strategy

1	ORGAN TRANSPLANTATION/	196
2	TRANSPLANTATION/	42
3	exp KIDNEY TRANSPLANTATION/	8036
4	((kidney$ or renal$) adj5 transplant$).mp.	8416
5	((kidney$ or renal$) adj5 graft$).mp.	2378
6	((kidney$ or renal$) adj5 allograft$).mp.	1117
7	or/1-6	8595
8	((histolog$ or biops$ or histopath$ or lesion$ or patholog$) adj5 scor$).mp.	39
9	((histolog$ or biops$ or histopath$ or lesion$ or patholog$) adj5 (donor or pretransplant$ or pre-transplant$ or preimplant$ or pre-implant$ or baseline)).mp.	46
10	(KDPI or "kidney donor profile index" or "remuzzi score$" or "donor chronic damage score$" or "Leuven donor risk score$").mp.	2
11	or/8-10	83
12	7 and 11	54

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