Evidence Based Hypothermic Preservation of the Kidney and Liver for Transplantation

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Abstract

Improving the techniques used for the preservation of livers and kidneys for transplantation has the potential to enhance the outcomes of transplant procedures. With an ever increasing demand for suitable organs, we now turn to the use of organs that may once have been considered to have lower success rates. The importance of preventing further damage to these organs during preservation is even greater than for standard organs. National and international guidelines for the use of such techniques in health service provision should be based upon the best level of evidence available; this is a particular challenge in a specialty with relatively few numbers of procedures each year.

This thesis describes a cohesive group of complementary studies which analyse the comparative effectiveness of preservation fluids for the cold storage of kidney and liver allografts from deceased donors. It also describes the comparison of hypothermic machine perfusion with static cold storage of kidneys. The systematic reviews carried out have a wider scope than any previously conducted. A novel approach was also taken towards the analysis of survival outcomes after transplantation as described in multiple studies. This work includes an analysis of national registry data that is the only large-scale comparison between Marshall's Solution and University of Wisconsin Solution for kidney preservation.

There is limited trial evidence that suggests similarity between the effectiveness of current preservation fluids for kidney preservation, and good evidence that Celsior and University of Wisconsin Solutions are equivalent for liver preservation. Good randomised trial evidence suggests that hypothermic machine perfusion reduces delayed graft function of kidneys. Hypothermic machine perfusion may also result in improved graft survival, but robust evidence is not available for this. The continued use of Marshall's Solution for kidney preservation in the UK results in equivalent adjusted outcomes compared to University of Wisconsin Solution.

Role of the candidate

I developed and designed the research studies here included and the thesis outline together with my supervisors. The contents of this thesis have been written by me. For the systematic reviews I developed search strategies and conducted all database searches myself. I extracted data and did the quality assessments for each study; this was done in duplicate with Robert Morgan and/or Simon Knight to improve accuracy. I wrote the analytical syntax required for meta-analysis, except for the graphical functions written by Simon Knight to enhance the forest plots. I performed other associated statistical analyses myself. For the registry analysis I was provided with a raw data file by NHS Blood & Transplant (Kidney Advisory Group). I cleaned the file, prepared it for analyses and wrote all the analytical syntax required. I conducted all the data analyses.

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Firstly I must thank my supervisor Professor Sir Peter Morris who has been as helpful, reliable and supportive as a student could hope for. Our regular discussions, meetings and musings drove the work forward. Without his enthusiasm and dedication much of this work would not have been possible. I am grateful to the input and supervision from Professor Peter Friend. I am thankful to him, along with Professor Rutger Ploeg, for the many discussions we have had about organ preservation. Their vast experience in this field has been vital in maturing my understanding of the many interacting factors. We wrote two book chapters together on the theme of organ preservation, and this experience was invaluable in setting up the introduction for this thesis.

My collaborators for the three systematic reviews, Simon Knight and Robert Morgan, worked tirelessly and without complaint to assist me. To Robert I am grateful for assisting with the data extraction and assessing the quality of studies. Simon taught me how to use "R" and his previous work in systematic reviews of transplantation was vital. Liset Pengel has provided advice on quality assessment of trials and the conduct of systematic reviews.

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Abbreviations

95%CI- 95% Confidence Interval
AC- Allocation Concealment
ALT- Alanine Aminotransferase
AST- Aspartate Aminotransferase
ATP- Adenosine Triphosphate
Aza- Azathioprine
BPAR- Biopsy Proven Acute Rejection
Chi²- Chi² Test for heterogeneity
CIT- Cold Ischaemic Time
CsA- Ciclosporin
CPP- Cryoprecipitated Plasma
CRF- Calculated Reaction Frequency
CTS- Collaborative Transplant Study
DBD- Donation after Brain-Death
DCD- Donation after Cardiac/Circulatory Death
DGF- Delayed Graft Function
EC- Eurocollins Solution
ECD- Expanded Criteria Donor
ECMO- Extra-Corporeal Membrane Oxygenation
ED- Early Dysfunction
FEM- Fixed Effect Meta-Analysis
eGFR- Estimated Glomerular Filtration Rate
HES- Hydroxyethylene Starch
HLA- Human Leukocyte Antigen
HMP- Hypothermic Machine Perfusion
HOC- Hyper-osmolar Citrate, Marshall's Solution
HR- Hazard Ratio
HTK- Histidine-Tryptophan- Ketoglutarate, Bretschneider Solution
I^2- I^2 Test for heterogeneity
ICTRP- International Clinical Trials Registry Platform
IGL-1- Institut Georges Lopez-1 Solution
IRI- Ischaemia Reperfusion Injury
ITT- Intention-to-treat
MeSH- Medical Subject Heading
MMF- Mycophenolate Mofetil
MPS- Belzer Machine Perfusion Solution
NA- Not Applicable
NHSBT- NHS Blood & Transplant
NIHR- National Institute for Health Research
NMP- Normothermic Machine Preservation
NORS- National Organ Retrieval Service
NR- Not Reported
NRP- Normothermic Regional Perfusion
OKT3- Orthoclone, Muromonab-CD3
OR- Odds Ratio
ORS- Organ Recovery Systems
OPTN- Organ Procurement and Transplantation Network
PBS- Phosphate Buffered Sucrose
PEG- Polyethylene Glycol
PNF- Primary Non-Function
PPF- Plasma Protein Fraction
RCT- Randomised Controlled Trial
REM- Random Effects Meta-Analysis
RL- Ringer's Lactate
ROS- Reactive Oxygen Species
RR- Relative Risk
RTA- Road Traffic Accident
SCD- Standard Criteria Donor
SCS- Static Cold Storage
SSC- Sample Size Calculation
Tac- Tacrolimus
TPII- Hyperosmolar Colloid Solution
UK- United Kingdom
UNOS- United Network for Organ Sharing
USA- United States of America
UW- University of Wisconsin Solution, Belzer Solution
WIT- Warm Ischaemic Time

1. Introduction

The transplantation of solid organs form one person to another is a life-changing, if not life-saving, procedure for the recipient. The outcomes of organ transplantation have continued to improve, and the demand for suitable organs has consequently increased, Table 1.1 (1). There has been an increase in the use of organs from both living and deceased donors, however the waiting list for organs remains a considerable challenge; there are currently over 7,600 people waiting for an organ transplant in the UK (2). This shortfall of suitable organs is mirrored in many other developed nations. The contrast is markedly shown in the USA, where over 54,000 patients are still active on the kidney waiting list despite almost 18,000 kidney transplants per year (3).

Deceased and living human donors currently provide the only source of viable solid organs for transplantation. Transplantation programs are therefore dependent upon the transfer of a fragile and complex set of interacting tissues (the organ) from one person to another. It is hoped that transplanted tissues function as near to normal as possible after transplantation, and to be life-sustaining in order to justify the risks of surgery and the lifetime of immune suppressing drugs.

The cells that comprise human tissues are sensitive to even the slightest changes in their environmental conditions. Any degradation that occurs at a cellular level can have dramatic effects on the function of the organ as a whole, and hence the success of the transplant. If the full potential of a transplant is to be achieved, then cellular changes during the preservation period must be reduced, or completely prevented. Patient and graft survival has improved since the first transplants were undertaken, making organ transplantation a safe and effective treatment for end-stage organ failure. There is however room for

improvement; the current five year graft survival rate for kidneys transplanted from deceased donors is 86%, and five year patient survival following liver transplantation is 78% (1).

By improving preservation methods, the function and life-span of transplanted organs can be increased. We may also expand the potential donor pool, by increasing the use of organs from donor types that are more sensitive to preservation damage.

Table 1.1. Transplant activity in the UK by organ and financial year. Wait list= patients on waiting list as of 31st March 2013. NA= Not Applicable. Data available from NHS Blood and Transplant (1). In the UK last year, 4,210 organ transplants were carried out, 3,113 of these organs were from deceased donors.

Transplants	2008-09	2009-10	2010-11	2011-12	2012-13	Wait List
Deceased donor organs transplanted						
Kidney	1403	1482	1502	1599	1750	6111
Liver	651	666	668	726	775	462
Pancreas	54	40	41	37	38	86
Kidney & Pancreas	152	160	156	173	166	210
Heart	129	121	131	141	142	196
Lung	143	145	169	175	188	227
Heart & Lung	3	5	3	5	3	16
Live donor organs transplanted						
Kidney	927	1038	1021	1009	1066	NA
Liver	27	20	21	38	31	NA

1.1 Delays to the transplantation of an available organ

The preservation of deceased-donor organs, sometimes for several hours after retrieval from the donor, is important for two main reasons: firstly there are preparatory processes that the recipient must go through before being ready for surgery; secondly, the donor and suitable recipient are not always in the same hospital, city or even country.

Patients on transplant waiting lists are selected for transplantation using complex algorithms that take into account variables such as age, HLA match with the donor and waiting time (1, 4-6). These algorithms differ by country and organ, but all require that patients on the list wait for the all-important telephone call, sometimes for years. The recipient's journey from home to hospital is the first delay.

Once admitted to hospital the recipient undergoes a review by a doctor to check that they are currently fit to undergo major surgery. They will have routine blood tests done and also have blood units matched by the hospital blood bank. This clinical review and processing is the potential second delay.

For most organs, HLA-matching is conducted to determine the mismatch grade between the donor and recipient as there are implications for long-term graft survival and acute rejection episodes (7). It is also necessary to complete an antibody cross-match to minimise the risk of hyper-acute rejection (7). These immunological checks are a likely third delay.

The fourth potential delay is possibly the most important; the requirement for transportation of the organ to the recipient's hospital. In order to provide immunologically well-matched organs, and also to make sure that recipients with the greatest need are transplanted first, national and even supra-national matching schemes have been developed, Table 1.2. Organs must therefore be removed from the donor after death by a surgical team, and transported to the local transplant centre of the recipient.

The steps above may run in parallel, but in most healthcare systems they will result in a delay between organ retrieval from the donor and re-perfusion in the recipient (1).

Table 1.2. National transplant organisations and multi-national organ exchange systems co-ordinating between donor and recipient.

Organisation	Countries included	Web address
National Health Service, Blood and Transplant (NHSBT)	UK	www.organdonation.nhs.uk
Agence de la Biomédecine	France	www.agence-biomedecine.fr
Centro Nazionale Trapianti (CNT)	Italy	www.trapianti.salute.gov.it
National Organ Donation and Transplantation Office	Ireland	www.hse.ie
Poltransplant	Poland	www.poltransplant.org.pl
Instituto Português do Sangue e da Transplantação (IPST)	Portugal	Ipsangue.org
Organizacion Nacional de Transplantes (ONT)	Spain	www.ont.es
Organ Procurement and Transplantation Network (OPTN)	USA	Optn.transplant.hrsa.gov
OPTN is administered by the United Network for Organ Sharing (UNOS)		www.unos.org
Eurotransplant	Austria, Belgium, Croatia, Germany, The Netherlands, Luxembourg, Slovenia	www.eurotransplant.org
Scandiatransplant	Denmark, Finland, Norway, Sweden	www.scandiatransplant.org

1.2 Organ damage during death, retrieval and preservation

When an organ is removed from the human body, cellular metabolism continues within its tissues, using up nutrients and accumulating potentially harmful metabolic products. Without an adequate nutritional and oxidative support for this on-going metabolism, harmful pathways are initiated. It is theoretically attractive to keep organs in an environment as close to their

normal physiological state as possible, however this remains a complex challenge. In the face of this conundrum a simple method of organ preservation was first adopted, the rapid cooling and storage of the organ on ice to slow down metabolic processes (8). In the early 1960s Calne et al, of the Westminster Hospital, UK, were the first to demonstrate that simple ice-cooling could preserve kidneys with the conservation of life-sustaining function, and thus opened up transplantation to a wider population of patients (9). There are harmful side effects associated with this type of preservation, however it has persisted as the most common method, and counteracting these detrimental processes is the focus of current preservation techniques. Adequate organ preservation has become all the more important given the widening spectrum of donors we now require to meet the demands of transplant waiting lists.

1.2.1 **Cellular effects of hypothermia**

Despite the slowing of cellular metabolism at low temperatures, it does not completely stop, and the on-going processes lead to unwanted side-effects in the preserved organ, Figure 1.1. The harmful effects of hypothermic preservation are explained below and include: reduced cellular Adenosine Triphosphate (ATP) and impaired ATP-dependent processes, acidosis, cellular swelling, production of Reactive Oxygen Species (ROS), and altered enzyme activity. Nutrient precursors that support this on-going metabolism have therefore been an important consideration of hypothermic preservation techniques.

Hypoxic conditions during organ preservation cause cells to adopt anaerobic means for the generation of ATP. The metabolism of pyruvate in this situation leads to lactic acidosis. The compromised metabolic pathways utilise ATP but fail to replenish it as quickly as under normal circumstances, ATP-dependent processes slow down or halt altogether (10, 11). The severe acidosis arising from anaerobic metabolism ultimately leads to apoptosis (12, 13). Under normal

metabolic conditions, ATP-dependent pumps maintain a difference in calcium concentration between the intracellular and extracellular compartments (14). Under hypoxic conditions, the interruption of these pathways leads to increased intracellular calcium, which can activate proteases, increase protein kinase signalling and lead to cellular disruption (15, 16).

Under normal circumstances the interstitial fluid has a high sodium and low potassium concentration, the opposite of the intracellular composition. Cells rely on ATP-dependent pumps embedded in their membranes to maintain this balance by exporting sodium from inside cells.

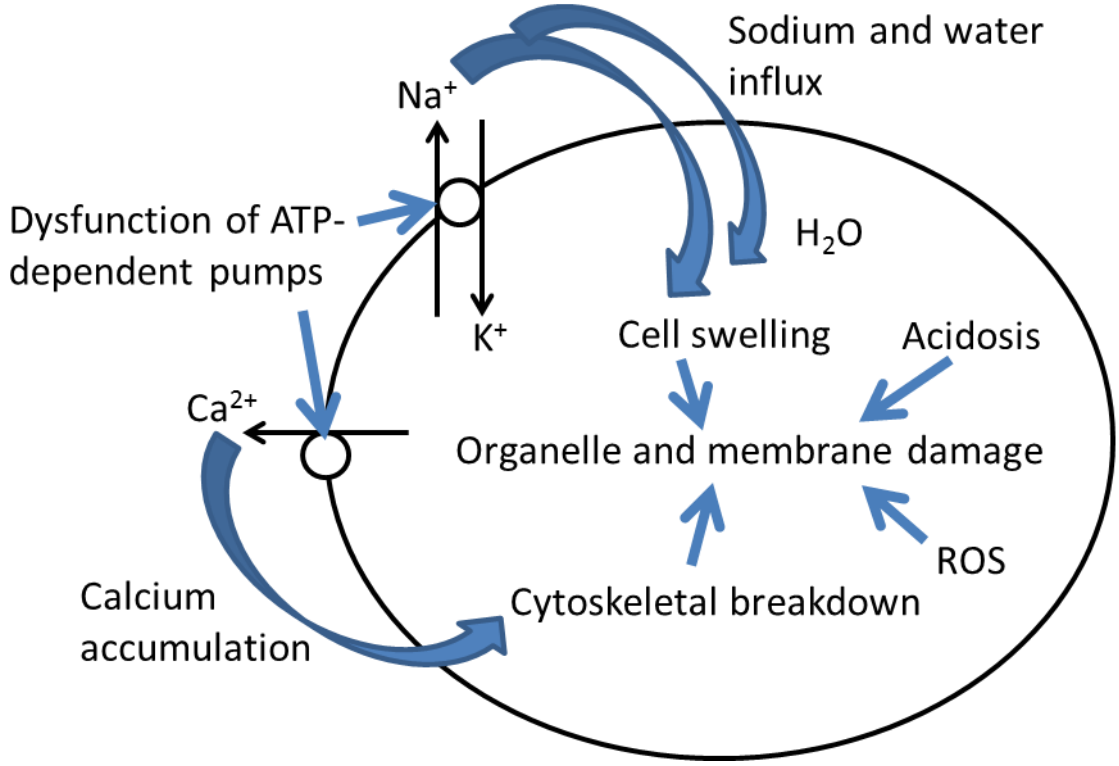
Under hypoxic conditions sodium excretion is reduced and sodium passively enters the cell down its concentration gradient, also attracted by the negatively charged intracellular proteins. The resulting intracellular environment attracts an inflow of water molecules, finally resulting in cell swelling and lysis (17). To prevent cellular swelling during hypothermic preservation, extracellular impermeant molecules and colloids that remain within the vascular and interstitial compartments are necessary components of preservation fluids. Large molecules which are unable to cross cellular membranes are most valuable in preventing cellular swelling (18-20). Negatively charged molecules can also act to retain positively charged sodium ions outside cells.

The potentially damaging ROS are generated in ischaemic tissues, and also in re-perfused organs that have recently been exposed to ischaemic conditions (21, 22). The enzyme xanthine oxidase is a key generator of ROS, as is free iron, released from Cytochrome P-450 during hypoxic conditions (23-27). These ROS react with key cytoplasmic molecules during reperfusion, causing severe damage to many intracellular structures (28, 29). The subsequent damage is known as the Ischaemia Reperfusion Injury (IRI). There is evidence that ROS contribute to cellular injury during both the cold and the reperfusion phases of organ preservation (13, 30). Inhibitors of ROS formation, as well as molecules

that are preferentially oxidised instead of cellular structures have therefore been added to fluids used for organ preservation.

Matrix Metalloproteinases (MMPs) may be activated during hypothermic conditions leading to uncoupling of endothelial cells from the supporting matrix through actin disassembly and cell rounding (31, 32). Components that inhibit MMPs may therefore have benefitted preservation fluids through an additional mechanism of action (32).

Figure 1.1. Summary of negative effects of cold ischaemia on cellular metabolism. Continued but slowed metabolism results in hypoxia, acidosis, slower ATP synthesis and reduced activity of sodium and calcium pumps. The resulting sodium influx to cells, followed by water, leads to cell swelling. ROS (reactive oxygen species) along with acidosis and lysosomal enzymes, damage cellular organelles and membranes.



1.2.2 **Changes to the donor pool**

Potential donors for solid organ transplantation currently include living and deceased human donors. Live donor transplants may be further sub-divided into related and un-related donor-recipient pairs. Deceased donors are categorised as either Donation after Brain-Death (DBD) or Donation after Circulatory Death (DCD) depending on the method used to establish death, either neurological or circulatory.

Mechanical ventilation permits the establishment of brainstem death using neurological criteria in potential donors that are kept haemodynamically stable. Countries with DBD transplant programs have specific requirements for tests to confirm brain-death (33). For the diagnosis of circulatory death, clinical examination documenting the absence of cardiac and respiratory function for an appropriate period of time is required (33). Legal changes in the 1970s recognising DBD allowed transplant programs to move from utilising organs from DCD only, to using more DBD, which eventually became the predominant type of deceased donor in many countries, including the UK.

In more recent years, in order to meet the demands of transplant waiting lists, organs from DCD have again been increasingly used (1, 3, 34), as have expanded criteria donors (ECD), Table 1.3 (35). Expanded criteria donors are defined by demographics which have a proven association with reduced graft survival (36). Expanded criteria donors include: all deceased donors over 60 years old, or aged 50-59 years with at least two of the following conditions: cerebrovascular cause of death, serum creatinine over 132 μmol/L or hypertension (36). The characteristics of donors contributing to the DBD organ pool has also changed over the years, with an increasing number and proportion of older donors of this type, Figure 1.2 (37, 38). Analysis of large databases shows that kidneys from ECD are more than four times as likely to be discarded than those from SCD, and DCD kidneys three times as likely (39).

Biopsy analysis is the most frequently cited reason for the discard of retrieved ECD kidneys (39).

Table 1.3. Numbers of deceased donors in the UK by financial year, split by donor type. There has been a gradual increase in absolute numbers of both donor types, but also a gradual increase in the proportion of donation after circulatory death. Percentages are of total deceased donors (1).

	2008-09	2009-10	2010-11	2011-12	2012-13
Donation after brain-death	611 (68%)	624 (65%)	637 (63%)	652 (60%)	705 (58%)
Donation after circulatory death	288 (32%)	335 (35%)	373 (37%)	436 (40%)	507 (42%)

1.2.3 **Organ damage in different donor types**

Organs from DCD undergo a period of warm ischemia before retrieval from the donor's body and may be categorized using the Maastricht Criteria, which broadly-speaking, takes the variable length of warm ischaemia into account, Table 1.4 (40, 41). In the case of Maastricht Category III donors, this warm ischaemic period follows the withdrawal of supportive treatment once a retrieval team is ready. In the case of the so-called "uncontrolled" donors of Maastricht Categories I, II and V, there may be an even longer warm ischaemic period after circulatory arrest before cold perfusion of the organs can be achieved (40). Kidneys from DCD have a higher risk of Delayed Graft Function (DGF) compared to DBD (34, 42, 43). They also have a higher risk of Primary Non-Function (PNF) and an increased risk of graft loss has been associated with DCD kidneys in some, but not all studies (34, 42, 43). Livers from DCD have a higher risk of biliary complications, a higher rate of re-transplantation (44) and worse patient survival compared to DBD (45).

Figure 1.2. Age of deceased donors in the USA 1988-2012. There is a noticeable increase in the proportion of donors over 50 years old. Based on OPTN data as of 19th June 2013, www.optn.transplant.hrsa.gov.

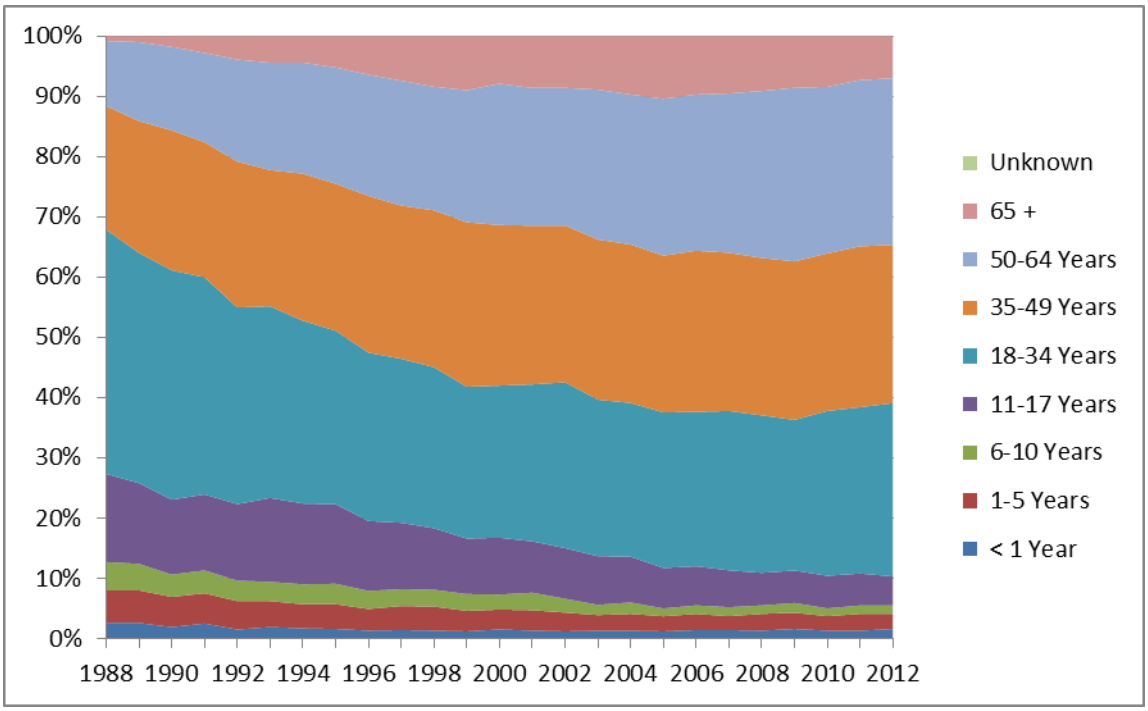


Table 1.4 Maastricht categories of donation after circulatory death.

Category	Description	Control
I	Dead on arrival at the hospital	Uncontrolled
II	Unsuccessful resuscitation at the hospital	Uncontrolled
III	Withdrawal of supportive treatment	Controlled
IV	Cardiac arrest following establishment of brain-death	Controlled
V	Cardiac arrest in hospital inpatient	Uncontrolled

Cerebral injury and brain-death are associated with the release of cytokines and the initiation of inflammatory processes that can directly injure organs and lead to further immune damage at reperfusion in the recipient (46-49). Organs from DBD therefore undergo cellular damage that is distinct from that experienced by organs from DCD. These changes in immunogenicity are characterized by increased expression of cytokines, adhesion molecules, and major histocompatibility complex class II antigens in the retrieved organ (48, 49). This

results in a more rapid influx of neutrophils, T-Cells and macrophages after reperfusion (49) and results in earlier acute rejection (48). Hormonal changes after brain-death include reduced levels of anti-diuretic hormone and thyroid hormones (50). Brain-death also causes myocardial suppression and decreased vascular tone, which in combination result in hypotension and insufficient perfusion of organs (51). Kidneys in this environment are exposed to perfusion dynamics and inflammatory cytokines that can lead to necrosis of renal tubules and fibrous proliferation of the arterial intima (52). The impact of brain-death on hepatic microcirculation is to reduce the perfusion of sinusoids, resulting in hepatocyte damage, reduced bile production and increased expression of adhesion molecules (53). Older donor age has negative implications for the outcomes of transplantation that are well established for both kidneys (34, 54) and livers (55). The cause of donor death is also important, irrespective of the type of donation (brain-death versus circulatory-death); stroke is associated with worse outcomes compared to trauma as cause of donor death (34).

Table 1.5. Outcomes of kidney and liver transplantation by donor type, donation after brain-death (DBD), versus donation after circulatory death (DCD). Kidney data (first grafts only, death censored graft survival) from Summers et al (34), liver data (any graft number, all cause graft loss) from Harring et al (45). P-Values are from the original papers.

Organ	Outcome	Donor type		P-value
		DBD	DCD	
Kidney	Primary non-function	3%	3%	0.89
	Delayed graft function	24%	39%	<0.01
	5 year graft survival	85.1%	83.2%	0.16
	5 year patient survival	88%	86.4%	0.31
Liver	5 year graft survival	66.5%	56.2%	<0.01
	5 year patient survival	72.5%	68.9%	<0.01

1.2.4 **Role of cold ischaemic time**

The period of time between the initial flush of cold preservation fluid through the organ's arteries in the donor and the reperfusion of an organ with blood in

the recipient is the Cold Ischaemic Time (CIT). The length of this potentially modifiable period is a very important factor in determining the outcome of solid organ transplantation. Analysis of the Collaborative Transplant Study (CTS), a voluntary, multinational database of transplant outcomes, found that CIT up to 18 hours made no difference to kidney allograft survival, however kidneys preserved for longer than 19 hours had worse graft survival (56). A longer CIT is also associated with increasing risk of DGF of kidneys (57, 58). Interestingly, kidneys from deceased donors that are preserved for very short amounts of time do not appear to have the same success rate as live donor kidneys (56). There are immunological implications of brain-death (48, 49), as well as hormonal imbalances (50) and episodes of poor end-organ perfusion in the last stages of life which may explain this phenomenon (51-53). Transplant professionals are currently willing to accept some degree of DGF in kidneys, as recipients can be maintained on dialysis. This is not the case with other organs however, where immediate function is essential to preserve life.

Liver allografts are also adversely affected by longer CIT, with higher rates of primary dysfunction and PNF at longer CIT (59, 60). Longer CIT is also a risk factor for the development of Ischaemic-Type Biliary Lesions (ITBL) (61) and has been associated with worse graft and patient survival (60).

Given the detrimental effect of long CIT, transplant programs have endeavoured to reduce preservation periods and have been successful in many instances; data from the CTS shows that the mean CIT for deceased donor kidneys has decreased steadily, from approximately 24 hours in 1990, to approximately 17 hours in 2004 (56).

1.3 Static cold storage

To preserve kidney allografts by static cold storage (SCS), the kidney is flushed through the renal artery with a chilled preservation solution both before and

after removal of the organ from the donor. Livers for transplantation are flushed through the hepatic artery and sometimes the portal vein depending on the retrieval surgeon's preference. Preservation fluid is run through under gravity or manual pressure until the effluent is clear. The kidney or liver is submerged in the preservation fluid in a sterile, sealed bag and is then surrounded by crushed ice in a cool box. This method is relatively cheap, easily transportable, and does not require input from the retrieval or implant team during the preservation period.

Static cold storage remains the most commonly used method of preservation for deceased donor kidneys and livers worldwide; data from CTS shows that SCS was used for approximately 98% of all kidney transplants recorded, with hypothermic machine preservation making up the remaining 2% (56). This picture is not necessarily repeated in all countries; in the USA for example, machine preservation is used for approximately 40% of deceased donor kidneys, and SCS for the remaining 60% (62).

The method of using a cold preservation fluid for an intravascular flush developed from the need to rapidly cool organs in the donor to slow the metabolism. Surface cooling with ice alone and subsequent hypothermia had been shown to preserve function in renal grafts (9). However, this method was too slow to achieve a low enough temperature and significantly reduced metabolism, so a move towards perfusion cooling was taken (8). Pre-existing, simple fluids, such as Ringer's lactate and albumin solution, which were not specifically designed for organ preservation, were initially used for SCS but none gave particularly good results (8). The first specifically designed organ preservation fluid was developed by Collins et al in the late 1960s (63). Collins et al tested four similar solutions based upon an intracellular electrolyte composition in canine experiments, and showed that SCS could be used to preserve kidneys for up to 72 hours with some degree of function retained (63). The results of these studies prompted a move away from the use of complex

and expensive continuous perfusion machines. The removal of magnesium from the Collins' Solution composition in the late 1970s (to prevent sedimentation) resulted in the Eurocollins Solution (EC) (64). Subsequently, several other preservation fluids have been developed. The composition of preservation solutions that are now commonly used all follow a similar pattern, they are described individually in Table 1.6.

Table 1.6. Composition of preservation solutions for static cold storage. Citrate, Histidine and Lactobionate also act as buffers. Histidine, Lactobionate and Mannitol also act as free radical scavengers. EC= Eurocollins, HEPES= 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HES= Hydroxyethylene Starch, HOC= Hyperosmolar Citrate, HTK= Histidine-Tryptophan-Ketoglutarate, IGL-1= Institut-George Lopez-1, MPS= Machine Perfusion Solution, PEG= Polyethylene Glycol, UW= University of Wisconsin Solution.

Component type	Component	Celsior	EC	HOC	HTK	IGL-1	UW	MPS
Colloids (mM)	**HES**	-	-	-	-	-	0.25	0.25
	PEG	-	-	-	-	0.03	-	-
Impermeants (mM)	**Citrate**	-	-	80	-	-	-	-
	Gluconate	-	-	-	-	-	-	85
	Glucose	-	195	-	-	-	-	10
	Histidine	30	-	-	198	-	-	-
	Lactobionate	80	-	-	-	100	100	-
	Mannitol	60	-	185	38	-	-	30
	Raffinose	-	-	-	-	30	30	-
	Ribose		-	-	-	-	-	5
Buffers (mM)	**HEPES**	-	-	-	-	-	-	10
	K2HPO4	-	15	-	-	-	-	-
	KH2PO4	-	43	-	-	25	25	25
	NaHCO3	-	10	10	-	-	-	-
Electrolytes (mM)	**Calcium**	0.25	-	-	0.0015	-	-	0.5
	Chloride	42	15	-	32	20	20	1
	Magnesium	13	-	40	4	5	5	5
	Potassium	15	115	84	9	25	120	25
	Sodium	100	10	84	15	120	30	100
ROS scavengers (mM)	**Allopurinol**	-	-	-	-	1	1	-
	Glutathione	3	-	-	-	3	3	-
	Tryptophan	-	-	-	2	-	-	-
Nutrients (mM)	**Adenine**		-	-	-	-	-	5
	Adenosine	-	-	-	-	5	5	-
	Glutamate	20	-	-	-	-	-	-
	Ketoglutarate	-	-	-	1	-	-	-
Osmolality (mOsm)		255	406	400	310	320	320	300

The history, effectiveness and current use of the most commonly used preservation solutions for kidneys and livers worldwide is described below.

1.3.1 **Eurocollins Solution**

Eurocollins Solution (EC, *Renograf®, Claris Lifesciences*) is a relatively simple preservation solution in its composition. Eurocollins Solution contains a relatively low sodium and high potassium concentration. Phosphates provide the buffering capacity of the fluid and glucose is the primary osmotic agent (63, 64). As glucose is able to cross the cellular membrane, it will enter cells and be metabolised, even in an anaerobic environment, reducing its effectiveness as an osmotic agent (65). Early studies demonstrated that kidney allograft preservation with EC was as effective as machine perfusion, prompting a move away from continuous perfusion to the cheaper and (at the time) less labour intensive SCS (66). A later study comparing EC with University of Wisconsin Solution (UW, see below) showed that DGF and one year graft survival were significantly better for kidneys preserved with UW (67). Eurocollins Solution was compared in another RCT to Histidine-Tryptophan-Ketoglutarate Solution (HTK, see below) and found to result in higher rates of DGF (68). Partly as a result of these studies, the use of EC for the preservation of kidneys fell dramatically from approximately 40% in 1990 to less than 4% by 2004 (56).

Eurocollins Solution was commonly used for liver allograft preservation in the 1970s and 1980s until UW Solution showed improved outcomes and the potential to extend preservation times (69-71). Glucose readily enters hepatocytes, as it does most cells (72), however hepatocytes have a much greater capability to metabolize glucose than renal cells under anaerobic conditions (73). Glucose is therefore a less effective impermeant for hepatocytes than it is for renal cells.

1.3.2 **Hyper-osmolar Citrate Solution**

Hyper-osmolar citrate Solution (HOC, Marshall's Solution, *Soltran®*, *Baxter*) has been used for kidney preservation since the 1970s (74-77). Hyper-osmolar citrate was initially developed at the University of Melbourne, Australia, by Vernon Marshall and colleagues. It was designed to extend preservation times compared to Collins' Solution and in canine experiments it showed the potential to conserve renal function in kidneys preserved for up to 72 hours (74). Hyper-osmolar citrate is a citrate and bicarbonate-buffered solution. Mannitol, a slightly larger monosaccharide than glucose, acts as the impermeant in the solution. Unlike glucose, mannitol cannot be metabolised and does not pass across the cell membrane easily, it also acts as a free-radical scavenger. Mannitol in HOC, along with its hypertonic osmolarity, prevents the entry of fluid into cells (78). Hyper-osmolar citrate has been shown to be effective in experimental (74) and clinical kidney preservation (79-81). There is some evidence from experimental studies however, that HOC may be associated with more tissue oedema than UW, as measured by weight gain and histological injury of the organ (82). A limited review of renal transplant studies found no differences in the outcome comparing HOC to both UW and Celsior Solutions (83). Hyper-osmolar citrate is used for the preservation of over 75% of kidneys in the UK (Chapter 4) but is not widely used elsewhere.

Some centres in the UK use HOC as an initial aortic flush for liver preservation before perfusion of the liver on the back-table and storage in UW (84). This approach was adopted to make use of the relatively low viscosity of HOC, which is perceived to help with the preservation of small peri-biliary vessels (84, 85). Hyper-osmolar citrate is currently ten-times cheaper than UW per litre in the UK and it is likely that clinical protocols have been influenced by this difference in cost.

1.3.3 **University of Wisconsin Solution**

Well into the 1980s transplantation programs were limited by the short cold ischaemic time that could be tolerated, which prevented the transport of organs over long distances and also meant that transplant surgery had to be undertaken on an emergency basis (8). The team lead by Belzer and Southard at the University of Wisconsin therefore set out to develop a more effective multi-organ preservation fluid. The result of this process, University of Wisconsin Solution (UW, *Viaspan®, Bristol-Meyers Squibb*, also supplied as *SPS-1®, Organ Recovery Systems* and *Belzer UW Cold Storage Solution®, Bridge to Life*) was designed to neutralise the effects of hypothermic preservation. Laboratory research led up to the clinical introduction of UW in 1987. Early results were good in the preservation of kidney, liver, and pancreas (17, 72, 86) and UW is now used for the preservation over 60% of deceased donor kidneys worldwide (56).

The impermeant components lactobionate, raffinose and Hydroxyethylene Starch (HES) provide the osmotic effect. Hydroxyethylene Starch is effective as an impermeant molecule, but being a large starch naturally makes UW Solution more viscous (67, 87). This quality could partially explain the slower washout of blood when UW is used (85, 88). Lactobionate is a relatively large anion that also acts as a buffer and ROS scavenger. Raffinose is a saccharide that adds complementary impermeant properties to those already offered by HES. Allopurinol, an inhibitor of xanthine oxidase, and glutathione, which is preferentially oxidised, also contribute to the antioxidant properties of the solution. Experimental studies have explored the effect of glutathione in reducing cell damage in models of cellular injury (89, 90). Later studies have also shown that glutathione is of particular importance in the longer term preservation of liver allografts (91). University of Wisconsin Solution has a relatively high potassium and low sodium concentration. The ATP precursor

adenosine provides for on-going metabolism. Following the results of numerous studies, UW is considered one of the preferred preservation fluids for kidney, liver, pancreas and small bowel storage (67, 92-97). Some studies have moved on to alter the basic composition of UW slightly to demonstrate that the sodium-to-potassium ratio could be reversed (98), and dextran could be substituted for HES (99). Historically insulin, dexamethasone and penicillin were added to UW, but this is now rarely done.

1.3.4 **Histidine-Tryptophan-Ketoglutarate Solution**

Histidine-Tryptophan-Ketoglutarate Solution (HTK, *Custodiol®, Dr Franz Köhler Chemie GmbH*) was originally used as a cardioplegia fluid for cardiac surgery (100). It has subsequently been used for the preservation of kidneys, livers and pancreas for transplantation (94-96). Histidine-Tryptophan-Ketoglutarate Solution has been used for the preservation of increasing numbers of kidneys since the early 1990s (56). Histidine-Tryptophan-Ketoglutarate Solution contains a relatively low concentration of both sodium and potassium. The amino acid histidine and the saccharide mannitol act as impermeant molecules. Histidine also acts as a buffer in the solution. Histidine-Tryptophan-Ketoglutarate Solution does not contain a colloid, such as the HES used in UW, and is therefore less viscous. Ketoglutarate acts as a nutrient to provide for ongoing metabolism, while tryptophan functions as an anti-oxidant and membrane stabilizer, by forming hydrogen bonds with membrane proteins (65, 101, 102). University of Wisconsin Solution and HTK have been compared in a large European RCT of renal preservation, which found equivalent rates of DGF and graft loss (68).

1.3.5 **Celsior Solution**

Celsior Solution (*Celsior®, Genzyme*), has been developed more recently and was designed specifically as a preservation fluid for cardiac allografts (103). It

had been intended to be suitable for use during initial cardiac arrest, the preservation period and reperfusion (103). Celsior has since proven to be effective in the preservation of kidneys, livers and pancreas as well as hearts (92, 104, 105). Celsior is somewhat similar to UW, however there are some key differences; Celsior has a relatively high sodium and low potassium concentration (the opposite of UW), Celsior includes mannitol as the main impermeant and does not contain HES, so is relatively low in viscosity as a result (103). Histidine is the main buffer and acts as an impermeant alongside lactobionate and mannitol. Reduced glutathione acts as a scavenger of ROS. Celsior has been compared to UW for kidney and liver preservation in a number of RCTs that have not proven superiority of either solution (104, 105).

1.3.6 **Institut Georges Lopez-1 Solution**

Institut George Lopez-1 Solution (*IGL-1®, IGL Group*) is similar to UW in its composition, including raffinose, lactobionate and potassium phosphate; however it incorporates Polyethylene Glycol (PEG) as an impermeant instead of HES. IGL-1 was developed in the early 1990s and has shown potential in the preservation of kidney, liver, pancreas and small bowel allografts (106-110). The tendency of HES to cause red cell aggregation prompted the testing of alternatives such as dextran and PEG (87, 111-115). Polyethylene Glycol binds water, which helps it to act like a colloid and also to arrange layers of water molecules around cell membranes (116). This mechanism has been shown to interfere with cellular interaction and it may potentially prevent identification by cells and antibodies of the immune system (117, 118). Several studies, both experimental and clinical, have assessed the effectiveness of PEG-containing fluids for liver, kidney, pancreas, small bowel and heart preservation, but as yet they have not been shown to be superior to any other preservation fluids in a clinical trial (106, 119-121).

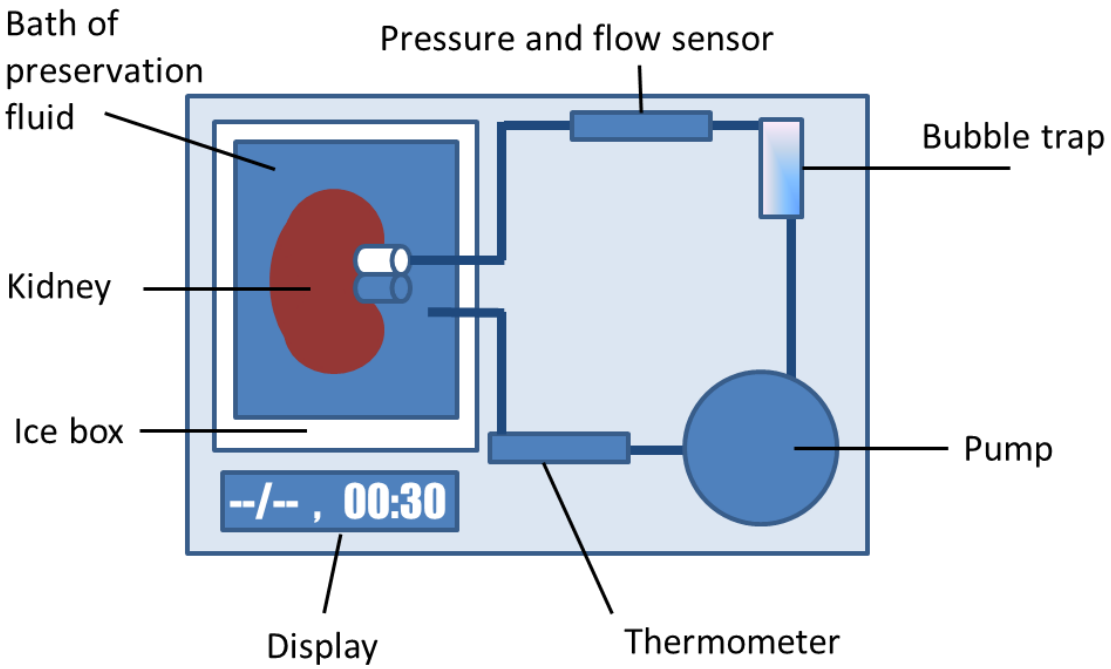
1.4 Hypothermic machine perfusion

Most of the clinical experience with Hypothermic Machine Perfusion (HMP) has been in kidney transplantation; only small case series in liver transplantation have been published (122, 123). For HMP of kidneys the retrieved kidney is placed within a chamber filled with chilled preservation solution surrounded by an ice box, Figure 1.3. The renal artery is cannulated by one end of a system of tubing, and a pump is used to generate a pulsatile or continuous flow of preservation solution through the renal vessels. The fluid pours from the renal vein into the reservoir from where the pump collects it again to recirculate it. The speculated benefits of HMP are the continual flush of the micro-circulation and the removal of waste products which may have knock-on effects on reducing ROS and IRI. Monitoring and assessment of the organ's vascular resistance during the perfusion is also a conjectured benefit. Currently available perfusion pumps are much smaller and technologically advanced than they were 40 years ago, although the basic principle is the same. Commercially available machines for non-oxygenated HMP of kidneys include the *RM3™ (IGL Group)* and the *Lifeport™ (Organ Recovery Systems).* Available machines that can deliver oxygenated perfusion include the *Waves™ (IGL Group),* the *Kidney Assist™ (Organ Assist)* and the *Airdrive™ (Portable Organ Perfusion).* Currently there is one machine available for the oxygenated HMP of livers, *Liver Assist™ (Organ Assist).*

Machine perfusion devices can be directed to deliver a pre-defined flow of fluid, or to maintain a desired pressure. Digital data collection during preservation allows the monitoring of the renal resistance to flow. Hypothermic Machine Perfusion was the original method of renal preservation during the early years of renal transplantation (124), and it was also tested for liver preservation around the same time (125). Following the development of preservation fluids that achieved equivalent results to HMP, with reduced

costs, SCS replaced HMP as the prevalent method of renal graft preservation (66). There is now particular interest in the use of HMP for the preservation of organs from DCD and ECD, where the potential to reduce DGF or improve graft survival is greater.

Figure 1.3. Basic components of a hypothermic machine perfusion device for kidney storage. The renal artery is cannulated and a pump is used to generate a pulsatile or continuous flow of preservation solution. The fluid exits the renal vein into the reservoir from where the pump collects it again to continue the process. An oxygenator may also be incorporated. The devices may be mains or battery powered.



1.4.1 Hypothermic machine perfusion of kidneys

Hypothermic Machine Perfusion has recently been compared to SCS in a large, European RCT using one of the new generation of pumps (126). Moers et al found that HMP could improve rates of DGF from approximately 27% to 21% (when assessing all donor types together). When DGF did occur, it also lasted

for a slightly shorter period following HMP than SCS. Graft survival up to three years after transplantation was also significantly better for kidneys preserved by HMP in this study (127). There was no significant difference in PNF rates between the groups, although it was too low to distinguish between the two arms (approximately 2% versus 5%, p=0.08). Analysis of the Scientific Registry of Transplant Recipients found that, historically at least, HMP had been used more for higher risk donors than SCS (128). Despite being used more commonly for kidneys with risk factors for DGF, HMP was associated with reduced rates of DGF in this registry (20% versus 28%) (128). Data from the CTS, on the other hand, found that HMP was associated with reduced graft survival compared to SCS (56). The most recent analysis of UNOS data has found a consistent reduction in DGF associated with HMP, but no difference in graft survival across multivariate analysis, paired kidney analysis, and propensity-matched comparisons (62). A recent meta-analysis of seven studies (published around the same time as work in this thesis), including all donor types, found a reduction in DGF with HMP, but no difference in graft survival or PNF (129).

For DCD kidneys, where expected DGF rates are higher to begin with, there is a potential for a greater reduction in absolute risk. Results from a recent RCT of DCD kidneys in Europe show a large reduction in DGF with HMP (70% versus 54%, p=0.03) (130). A smaller RCT from the UK did not find any benefit, with DGF rates equally low at approximately 56% and 58% in both groups (p=0.99) (131). Both of these studies found no difference in one year graft survival and PNF rates between groups (130, 131). A recent meta-analysis of four studies of DCD kidneys (published around the same time as work in this thesis) found a reduced risk of DGF with HMP, but no difference in graft survival (132).

Hypothermic Machine Perfusion has also been tested for the preservation of ECD kidneys. In a recent multicentre RCT of kidneys from ECD, HMP reduced PNF rates, and improved one year graft survival rates, although surprisingly DGF rates were not improved (133). Analyses of large databases have shown a

significant reduction in DGF with the use of HMP for ECD kidneys, but without such an impact on graft survival (128, 134, 135).

1.4.2 Hypothermic machine perfusion of livers

Hypothermic Machine Perfusion of liver allografts with a new generation of portable pumps remains in the early stages of clinical development, despite experience with similar systems as long ago as the 1960s (136). So far liver HMP has been tested in one case-control study of 20 DBD livers (123). This study showed that up to seven hours dual portal and arterial perfusion reduced early dysfunction (ED), serum liver enzymes post-operatively, and hospital stay compared to SCS (123). Tissue analysis from samples collected during this study suggests that HMP reduces the overall cellular stress response, potentially by as simple a mechanism as diluting pro-inflammatory cytokines (137). Liver HMP has demonstrated the potential for longer term preservation in animal studies (138, 139).

Given the longer warm ischaemic time associated with DCD, there is more potential to reverse ischaemic damage in livers from DCD than DBD. Hypothermic Machine Perfusion has been tested for the preservation of livers from DCD in animal models, reducing cellular damage and improving recovery of ATP (140, 141). More marginal steatotic livers are also a potential target for improving transplant outcomes. HMP has shown some promise in the preservation of steatotic livers for long preservation times (142). In one study bile production, ammonia clearance and ATP levels were significantly higher after HMP compared to SCS, suggesting better recovery of function (142).

1.4.3 Hypothermic machine perfusion fluid

During the early stages of development, HMP was associated with tissue oedema due to the relatively high hydrostatic pressures involved, and this prompted the development of perfusion fluids with strong oncotic effects to

retain fluid within the vascular compartment. Human plasma was used initially, allowing the first successful machine preservation of a human kidney for 17 hours (124). Plasma however contains lipids that aggregate and can potentially block vessels during perfusion. The freezing and thawing out of plasma will remove the lipids, producing Cryoprecipitated plasma (CPP) (8). Cryoprecipitated plasma has previously been used for both kidney and liver preservation (8). An early randomized study comparing albumin solution to CPP for machine perfusion of kidneys found equivalent transplant outcomes, although graft survival was only 50% in both arms (143). An effective pump fluid that did not carry the risk of disease transmission was therefore still required. Plasma Protein Fraction (PPF) was also tested against CPP for renal perfusion, with acceptable results in small studies (144). A prospective trial comparing the three available perfusates at the time (PPF, CPP and albumin solution) demonstrated similar results with all three; one year graft survival was 58-68% (145). In the 1980s a machine perfusion solution was developed that contained HES and gave very good results even at long preservation times, Belzer Machine Perfusion Solution (MPS, *KPS-1®, Lifeline Scientific Inc.*) (146). Belzer MPS is somewhat similar to UW Solution in its composition, for example it includes HES. However, the sodium-to-potassium ratio is reversed and Belzer MPS contains gluconate instead of lactobionate as the main impermeant molecule. Belzer MPS is the standard perfusion fluid used worldwide for HMP of kidneys.

The addition of glutathione to Belzer MPS in a small study was not found to improve rates of immediate function, which were already high (147). The addition of a combination of metabolic substrates, antioxidants and vasodilators in combination, however (*Vasosol®, Procent Technologies LLC.*) has shown the potential to further reduce DGF following kidney perfusion, although graft survival was unchanged (148). Polysol, a colloid based solution (*Polysol®, Doorzand Medical Innovations*), has been tested in animal experiments for the

preservation of livers and has shown reduced tissue oedema and transaminase levels compared to Belzer MPS (142).

1.4.4 Oxygenated hypothermic machine perfusion

The provision of oxygen during hypothermic preservation may theoretically provide for any on-going metabolism. Hypothermic machine perfusion systems may be configured to deliver oxygen, which has been shown experimentally to be beneficial for kidney and liver transplantation (149-151). Membrane oxygenators within machine perfusion systems have been shown to promote ATP synthesis (152). Oxygenation of machine perfusates with pure oxygen has also been tested as an adjunct to machine perfusion, and can recondition kidneys to some extent, showing improved creatinine clearance after transplantation (153).

Oxygenated HMP has been tested in animal models of DCD. Using oxygenated HMP at the end of a period of cold storage to precondition the organs leads to a reduction in cellular damage compared to SCS alone and increased cellular ATP recovery (154-157). Livers preserved by oxygenated HMP also have improved graft survival in some animal models (155). However, a recent animal study in a model of DCD (90 minutes warm ischaemia) reported a significant ischaemic endothelial damage that could not be reversed by oxygenated perfusion (158).

1.4.5 Vascular resistance during hypothermic machine perfusion

One hypothesized benefit of HMP is that it permits the monitoring of renal resistance and this can be used to predict future kidney function and survival (39, 159-162). In large registry analyses it is clear that pumped kidneys with high resistance values are more likely to be discarded (39, 163). Interestingly, the same analyses also demonstrated that some transplant centres are using ECD kidneys that would have been discarded, had they not been pumped (39).

The discard of large numbers of kidneys with poor perfusion statistics, and the perception of better outcomes with HMP makes it difficult to accurately assess the relationship between renal resistance and transplant outcome. A large analysis of vascular resistance values from kidneys that were all transplanted, and which were not disclosed to transplant surgeons pre-operatively, was possible following the European multicentre MP trial. These results showed that renal resistance at the end of the perfusion period was an independent risk factor for DGF as well as graft loss (160). Despite this association, the renal resistance had a poor predictive value in this study and the authors concluded that there was no renal resistance at which an absolute boundary could be set (160). In an analysis of DCD kidneys in which teams were blinded to perfusion data, vascular resistance was associated with higher rates of PNF and DGF; again however vascular resistance was of low predictive value (163). As yet there are no clinical studies in liver transplantation evaluating the relevance of flow and pressure data.

1.4.6 **Biomarker assay during hypothermic machine perfusion**

The measurement of biomarkers in the perfusate during HMP could potentially allow assessment of organ quality, decrease the risk of discarding viable organs, and avoid transplanting organs with a high risk of failure or poor function. Samples of the fluid perfusate may be collected during HMP and analysed for potential biomarkers of cellular injury, without the risk and delay associated with an invasive tissue biopsy. As with renal resistance during HMP, the exact relevance of perfusate biomarkers has been clouded by the discard of kidneys in studies and case series. A systematic review of kidney perfusate and urine biomarkers concluded that injury biomarkers have yet to be systematically evaluated (164). The review concluded that perfusate levels of lactate dehydrogenase, glutathione-S-transferase and aspartate transaminase were all significantly associated with DGF in the majority of the studies in which they

were evaluated (164). However, additional validation was recommended before implementation of these factors as predictors of allograft outcomes, particularly long term outcomes beyond one year after transplantation (164).

Using samples from kidneys that were all transplanted, several injury biomarkers were recently assessed during the European MP trial (165). Analysis of perfusate samples collected at the end of the preservation period showed that glutathione-S-transferase, heart-type fatty acid binding protein and N-acetyl-beta-D-glucosamine were all predictors of DGF (165). None of the biomarkers were independently associated with PNF or graft survival (165). An analysis of machine perfused DCD kidneys found that lactate dehydrogenase and IL-18 were independent risk factors for PNF, and along with redox active iron, were independent risk factors for DGF (166). Predictive values however, were also poor in this analysis, and biomarker concentrations were not associated with the risk of graft failure in the first year after transplantation (166). Overall it seems that the diagnostic accuracy of perfusate biomarkers in predicting viability of kidneys is still poor.

The only clinical case series of hypothermic liver perfusion found an increase in albumin concentration in the effluent during the preservation period (123). Potentially this may prove to be a marker for the recovery of hepatocyte function. Experimental studies continue to evaluate markers of ischaemic injury that may be assessed during liver perfusion, such as AST and pH (167).

1.5 Normothermic perfusion

Technological advances and increased scientific understanding have allowed the development of systems that can perfuse retrieved organs at body temperature during the preservation period. The supposed advantages of normothermic perfusion include the avoidance of cellular damage caused by cooling and the facilitation of on-going normal cellular functions. Oxygen

delivery and physiological temperature allow ATP regeneration to support these functions and reduce the risk of IRI. Normothermic perfusion of the donor organ can be conducted in one or more of the following three methods: Normothermic Regional Perfusion (NRP) in the donor prior to retrieval, Normothermic Machine Perfusion (NMP) during the preservation period after removal from the donor body and normothermic reconditioning immediately prior to transplantation.

For NRP the donor is attached to a cardiopulmonary bypass machine after circulatory arrest to restart blood circulation in the abdominal vessels only, also known as normothermic recirculation or Extra-Corporeal Membrane Oxygenation (ECMO). This technique allows regional perfusion immediately before retrieval of DCD organs, for example. Normothermic Machine Perfusion (NMP) requires an extra-corporeal system in which to maintain the organ, consisting of a pump, oxygenator and heat exchanger. This technique could theoretically be used throughout the preservation period and minimises exposure of the organ to hypothermia. Normothermic reconditioning can be achieved with a similar system and may be used for a short period immediately prior to implantation. Clinical testing of these relatively new technologies is still in the very early stages, particularly for NMP.

Published clinical studies describing the results of kidney and liver transplantation following the use of normothermic preservation techniques are limited. The largest experience has been with the use of NRP in uncontrolled DCD in France and Spain. Kidney transplant programs that make use of this donor type report high rates of organ discard and high rates of DGF, although this method does open up the potential use of this donor type, which would not be possible otherwise (41, 168-170). One very small series has compared NRP to both hypothermic regional perfusion and to *in situ* cold perfusion with EC solution, showing improved DGF rates with NRP (171). The first case-control studies of NRP for uncontrolled DCD liver preservation have relied upon strict

selection criteria to develop experience with this potential donor base (172, 173). There is little published experience of the use of NRP for controlled DCD kidneys. One very small case series (15 donors) reports low rates of DGF and PNF in this setting (174) and a larger cohort study demonstrates five-year graft survival rates comparable with kidneys from DBD (175).

There are as yet no published clinical studies of NMP in either kidney or liver transplantation. The *Metra*® (*Organox)* normothermic liver perfusion machine is currently in testing in a Phase I study in the UK and will be compared to SCS in a multinational RCT starting in 2014 (176).

The use of NMP for a short period immediately prior to transplantation (normothermic reconditioning) is logistically and technically much simpler than normothermic perfusion for the whole preservation period. The Leicester group lead the way in this approach, publishing the first clinical case in renal transplantation (177). The same group went on to do the first case series using a short period of normothermic recirculation, which showed only 5% DGF rate in a series of 20 ECD kidneys (178).

1.6 Aims and objectives

The aim of this thesis is to provide a thorough analysis of hypothermic preservation of deceased donor liver and kidney allografts that is relevant to current clinical practice. Work for this thesis will take the form of four studies:

1. *Which preservation fluid is best for the static and hypothermic preservation of deceased donor kidney allografts? Systematic review and meta-analysis.*
2. *What are the outcomes of renal allografts preserved with Hyper-osmolar Citrate in the United Kingdom? How do these outcomes compare to kidneys preserved with UW Solution? Analysis of national data.*
3. *Does hypothermic machine perfusion of deceased donor kidneys result in better transplant outcomes than static cold storage? Systematic review and meta-analysis.*
4. *Which preservation fluid is best for the static and hypothermic preservation of deceased donor liver allografts? Systematic review and meta-analysis.*

Themes that will permeate through all four studies include:

1. *An assessment of the evidence base in terms of quality and risk of bias.*
2. *Providing a higher level of evidence than is currently available.*
3. *Highlighting pitfalls in methods of studies of organ preservation.*
4. *Providing recommendations for future studies, trials and clinical practice.*

1.7 Thesis structure

The main body of this thesis consists of seven chapters. The introduction is the first chapter and describes the importance of adequate organ preservation along with a summary of the currently available evidence. Chapter 2 describes the methods used in the later chapters. Chapters 3-6 are results-based chapters for the four studies introduced above. Chapter 7 summarises the findings and provides an overall discussion of the results and implications of the studies in combination.

2. Methods

2.1 Systematic review

Systematic review is the process of identifying, assessing and summarizing all relevant evidence for a well-defined research question. Meta-analysis is the statistical method used to combine results from multiple studies. The systematic review of published and unpublished studies has a role in both assessing the quality of current evidence, as well as bringing together all the relevant information to draw new conclusions. The review of evidence in this way can also provide recommendations for future studies and guidance for methodology. Overall the quality of reporting of RCTs in transplantation has been poor and it cannot be taken for granted that RCTs will be without bias (179). The conduct of systematic review is transparent because the process is specified beforehand. The method of systematic review can be broken down into five main steps: framing the research question, searching the literature, assessing quality, summarising the evidence, interpretation and conclusions (180, 181).

2.1.1 Framing the research question

The research question to be answered by the review should be precise and clearly defined. It may start as a free form question, but should then be framed along the following lines as the reviewers specify each of the following domains: Populations, Interventions, Comparators, Outcomes, Study designs (PICOS). For example: *"What is the evidence for antibiotic treatment of ear-ache?"* becomes "*In randomised controlled trials, including children under five years old with acute otitis media, without perforated tympanic membrane, does 3 days of oral co-amoxiclav reduce hospital admissions compared to no antibiotics?"* Review questions

should be planned in advance of the study to prevent the review process being driven by presumed findings. However, unexpected issues should be explored and sometimes it is necessary to amend the review protocol. It is now recommended that review protocols are registered with an open-access database in advance (182). The Centre for Evidence in Transplantation currently uses the PROSPERO system from the Centre for Reviews and Dissemination at the University of York (183).

2.1.2 Searching the literature

The literature search should be thorough and comprehensive in its nature in order to identify all relevant studies. A balance is struck between capturing all relevant papers in the search and not having so many to review that the relevant papers get lost. It should be noted that relevant data may be published, unpublished, or available in abstract form only. It is therefore necessary to search a number of different databases that cover these different types of information source.

Databases

The Centre for Evidence in Transplantation maintains a library of all RCTs in transplantation which is easy to search but naturally does not include other study types. The databases Medline and EMBASE overlap to a considerable extent but appropriate studies may only be included in one or the other database (184). Unpublished studies may be identified by searching research registries such as the International Clinical Trials Registry Platform (185). The Cochrane Collaboration has databases of reviews and controlled trials that can also be searched relatively easily (186).

Search terms

The terms used for searching should be broad, using synonyms and spelling variations, so that all relevant studies are identified. It is advisable not to use

language limits which may bias against non-English publications. Search terms may be "free-text" or use the keywords that the database has assigned to the studies. It is advisable to use both "free-text" and keywords when searching in order to account for mislabelling with the wrong keyword and language translations.

Selecting references

Relevant studies can be selected from the retrieved abstracts based upon the exclusion and inclusion criteria specified in the review protocol. The reasons for exclusion of studies are documented for later reference. Multiple reports of the same study may be included but data from the most complete report with the longest follow-up should be prioritised.

Publication bias

Publication bias refers to the tendency for studies with unfavourable or non-significant results to remain unpublished. A visual method for assessing for publication bias is the funnel plot (187). The standard error of each study is plotted against the intervention effect estimate in each study (181). The studies should form a funnel shape with the larger studies nearer the apex; smaller studies should appear either side of the effects observed in larger studies. In the presence of publication bias, a gap will appear leading to an asymmetrical appearance of the funnel plot. If studies with non-significant results remain unpublished then meta-analysis will tend to over-estimate the treatment effect (187). Statistical tests can be used to assess for missing studies or a skewed funnel, both of which may indicate publication bias, location bias (not finding appropriate studies), language bias, or database bias (181).

2.1.3 **Assessing quality**

Quality assessment of included studies is important in order to assess the validity of the study findings. In short each study must be assessed for the risk

of bias and this can be done in a number of ways; The Centre for Evidence in Transplantation now uses several methods in combination to assess the quality of trials and to judge risk of bias, described below. At least two reviewers should independently assess the quality of a study.

Cochrane Collaboration risk of bias tool

The Cochrane Collaboration recommends assessing several domains and declaring explicitly the reasons for the reviewers' conclusions. This system is referred to as the Cochrane Collaboration risk of bias tool (188). Six areas of potential bias are individually assessed by the reviewers and judged to be of high, low or unclear risk of bias (188).

Selection bias

The comparison groups should be similar from the start of the study, otherwise confounding factors will be unevenly spread and interfere with the conclusions. Random allocation to groups may account for this. Whether or not the arms of the study are similar in terms of known and unknown factors should be considered.

Performance bias

This is largely to do with blinding and whether or not participants and personnel were prevented from determining group allocation.

Detection bias

Outcome assessors may be blinded, in order to prevent them knowing group allocations.

Attrition bias

How complete is the outcome data? What dropouts and exclusions were made?

Reporting bias

What outcomes are reported and how?

Other bias

Any important concerns about bias not covered in the other domains.

Each domain should be individually assessed and presented. An overall judgement about the risk of bias across studies in a review can be made. The relative importance of different domains must be considered.

Jadad Score

The Jadad score is a scoring system in which up to 5 points are awarded to a study. It is only applicable to randomised controlled trials (189). A score of 3 or more is generally accepted as indicating good quality. The points to be assessed are:

Was the study described as randomised?

An extra point is awarded if the method of randomisation is described and would give an equal chance for each participant to receive each intervention. The first point is withdrawn if the randomisation method is inappropriate.

Was the study described as blinded?

An extra point is awarded if the method of blinding would prevent both the study participant and the assessor from identifying the intervention. The first point is withdrawn if the blinding method is inappropriate or ineffective.

Was there a description of withdrawals and dropouts?

The number and reasons for any dropouts must be described for both groups. If there were no withdrawals it should be stated in the report.

Allocation concealment

For randomised studies the benefit of randomisation is not maintained without allocation concealment. Effective allocation concealment prevents participants and personnel foreseeing assignment. Potential methods include a central allocation system or sealed, opaque envelopes. A truly random sequence may not result in equal distribution between treatment groups if there is, for example, an open random allocation schedule, unsealed, transparent envelopes,

or alternation. These scenarios permit personnel or participants to introduce selection bias at the enrolment stage.

Intention-to-treat analysis

Analysing outcomes by the original group allocation, known as intention-to-treat analysis (ITT), maintains the benefit of randomisation, namely an equal distribution of known and unknown prognostic factors in each study arm. Participants who drop out of the study, or switch treatment groups, for example, should be analysed as if they had stayed in their original allocation group. Difficulties may arise when data is not available for some participants. The Centre for Evidence in Transplantation now advocates a four strategy assessment of outcome analysis, see below (190). The field of transplantation can throw up particular difficulties in analysis strategy when considering preservation methods as the randomisation may occur long before an organ is assessed for transplantation.

Strict intention-to-treat analysis

All patients are included in the analysis. Missing data are imputed in case of dropouts.

Available case analysis

Data are analysed according to the assigned intervention for every participant for whom the outcome was obtained. Missing data are not imputed.

Modified intention-to-treat analysis

Data are analysed according to the assigned intervention. The analysis excludes participants who did not adhere to the protocol. For example, patients not transplanted or patients not receiving the study drug.

Per protocol analysis

Only patients who sufficiently complied with the protocol are included in the analysis.

2.1.4 Summarising the evidence

Data extraction by two authors independently, with cross-checking, is done to minimise the risk of errors. The results and demographics of participants from individual studies can be presented in tabular form to demonstrate how each study has contributed to the overall conclusion of the review. The results can then be described in a narrative review whereby the results of individual studies and the merit of each are discussed and put into context. If possible this may be supplemented by summary statistics from meta-analysis of individual study results.

2.1.5 Interpretation and conclusions

The combination and analysis of the available data in a narrative review or a meta-analysis can be used to answer the specified research question. In doing so, the strength and quality of the evidence must be made explicit. It may not be possible to draw a firm conclusion and in this situation the review process can be used to identify unanswered questions and inform future research.

2.2 Meta-analysis

Meta-analysis is the statistical method used to combine results from multiple studies. It has the potential to increase the power for detecting small treatment effects by combining the results from multiple studies and helps understand the results of a study in the context of the other related studies (191). If the effect size in individual studies is consistent across the data, then we may assume that there is a true treatment effect that would be seen if the treatment were applied to the whole population. Individual studies are samples of the population and thereby only estimate the effect of the intervention. The smaller a study is, the more likely its individual result will lie far from the true population effect. By combining the effects seen in individual studies in meta-analysis we aim to

estimate the true-population effect of the intervention, the summary effect (191). If the effect sizes in individual studies vary then we should quantify the variance and consider the implications.

The summary effect is the weighted mean of the individual study effects, each study in the meta-analysis is weighted (192). This ensures intervention groups are only compared with control groups in their own study, maintaining the benefits of randomisation.

A forest plot may be used to depict the studies included in a meta-analysis and the summary effect. Usually a log scale is used if the summary statistic is relative risk (RR) or odds ratio (OR). The line of no effect sits at RR=1 or OR=1. Confidence Intervals overlapping 1 indicate a lack of statistically significant effect at the pre-specified significance level.

2.2.1 **Assessing heterogeneity**

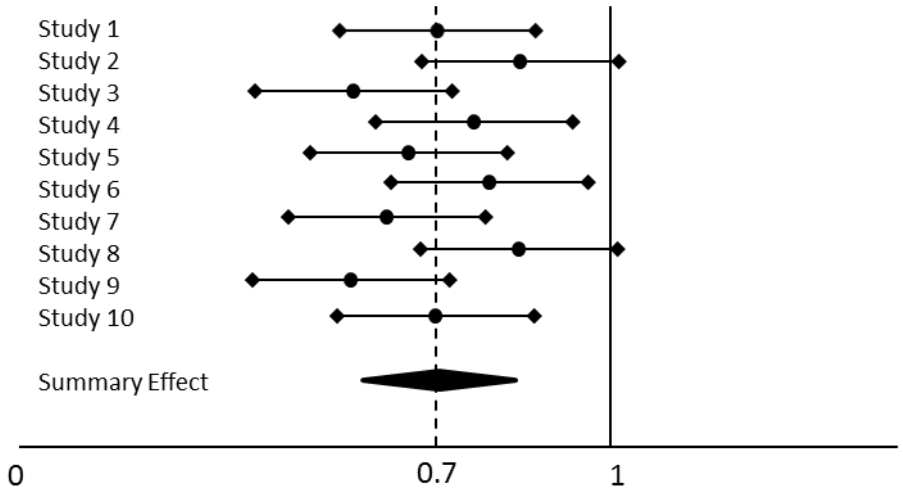
It is important that inconsistency or heterogeneity of the included studies is assessed. The variation in observed effects may be due to real differences in effect as well as random variation (193). The first stage in assessing heterogeneity is simply done by the reviewer who must assess whether or not the clinical characteristics and methodology of the studies are homogeneous. Potential sources for heterogeneity include, for example: different study populations, interventions, outcomes or study quality. Statistical methods for quantifying heterogeneity include the Cochran Q Test which assesses whether or not the differences between studies are likely to be due to chance. Typically a value of $p<0.1$ is taken to indicate significant heterogeneity, however this test lacks power when a small numbers of studies is included (193). The I^2 Test describes the percentage of variability in effect estimates that is due to heterogeneity rather than chance (194). The I^2 statistic is not affected by the number of studies in the analysis. If heterogeneity is statistically apparent then a cause should be sought. It may still be possible to use a random effects meta-

analysis in the presence of unexplained heterogeneity, see below. If differences in study quality prove to be an explanation for heterogeneity, summary effects will be biased due to undue weighting of studies with inferior quality. Most meta-analyses are based upon one of two proposed statistical models, fixed effect and random effects.

2.2.2 **Fixed effect meta-analysis**

The fixed effect model (FEM) assumes there is a single true effect size of an intervention and the variation in the observed effects between studies can be explained entirely by sampling error. If each study had an infinite sample size, the observed effect for each study would be the same as the true effect (192). In real life this is not possible, sample sizes for studies are finite and therefore sampling error results in the observed effect of an intervention in a study not being the same as the true effect (Figure 2.1). The summary effect of a fixed effect meta-analysis is an estimate of the common effect size. It should be noted that a fixed effect model may give undue precision to a summary effect if there is significant unexplained heterogeneity.

Figure 2.1 Forest plot to demonstrate fixed effect model and distribution of sampling error between studies. The diagram depicts the results of a study conducted 10 times in a row. The true effect of the intervention is a relative risk of 0.7. Each time the study is conducted, random sampling error produces a slightly different observed effect and confidence interval, represented by the dot and whiskers. Each study was large enough for 80% power, hence 2 times out of 10, a non-significant result is observed and the confidence interval crosses 1. The summary effect is depicted by the diamond. Studies with smaller sample sizes are more likely to have an observed effect that is further from the true effect.

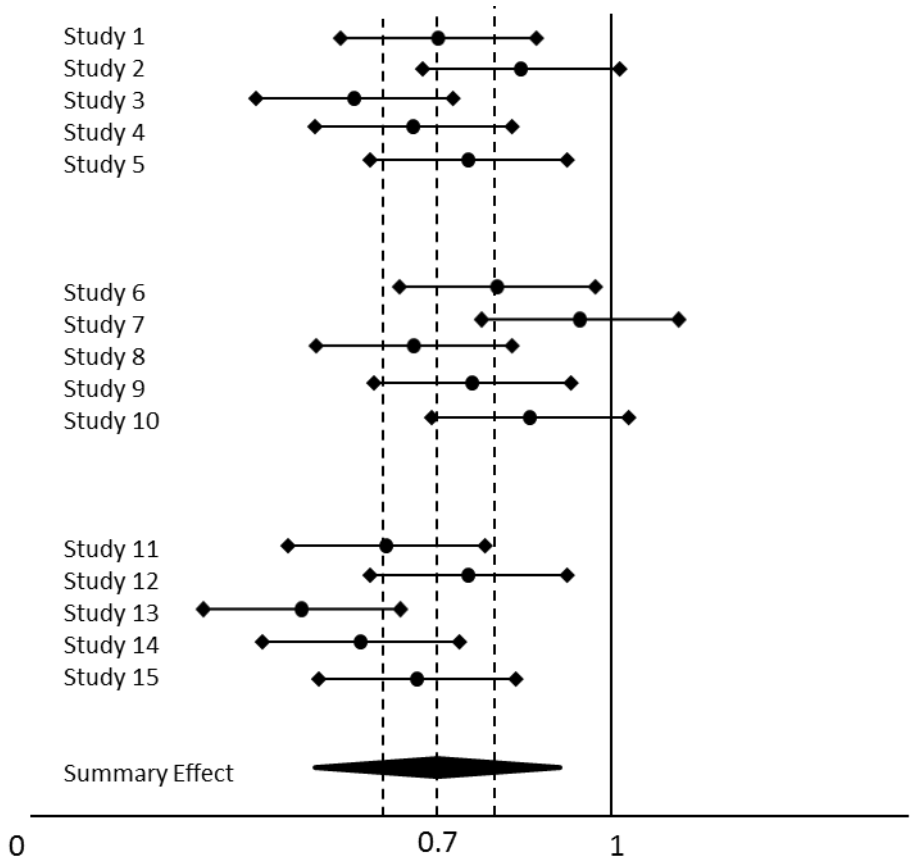


2.2.3 **Random effects meta-analysis**

Random effects models (REM) assume no single treatment effect, but a distribution of effects depending on study and participant characteristics (192). There may be different effect sizes underlying different studies. A random effects analysis assumes that the true effects are normally distributed about a mean treatment effect (Figure 2.2). The distance between the overall mean and the observed effect in a given study is composed of two parts: true variation in effect sizes and sampling error. These two components can also be described as the deviation of the study's true treatment effect from the mean treatment effect, and the deviation of the observed effect from the true treatment effect (192). The summary effect of a random effects meta-analysis is an estimate of the mean of multiple true effect sizes. The weighting of individual studies in a random effects meta-analysis therefore takes account of both the within-study and between-study variance. Since each study provides information about a

different effect size, a smaller range of weights is assigned to the individual studies than would be in a fixed effect analysis. This may exaggerate the impact of publication bias and poor quality in smaller studies (192). A random effects meta-analysis will also produce wider confidence intervals for the summary effect when there is heterogeneity compared to a fixed effect meta-analysis.

Figure 2.2. Forest plot to demonstrate random effects model, between-study and within-study variance. The diagram depicts a study carried out 15 times in a row, but in slightly different populations between each five studies. The mean of the true effects of the intervention is a relative risk of 0.7. For the first five studies the overall observed effect is a relative risk of 0.7 but for the second five it is slightly higher, and for the last five slightly lower. Each time the study is conducted random sampling error produces a slightly different observed effect and confidence interval, represented by the dot and whiskers. The distance between the overall mean of true effects and the observed effect for any given study consists of two parts: true variation in effect sizes and sampling error. The summary effect is depicted by the diamond. The confidence interval for the summary effect is wider than for fixed effect meta-analysis.



2.2.4 **"R"**

"R" is a free software environment for statistical computing and graphics (195). The core of "R" is a computing language with some integral statistical functionality that can be augmented by packages of functions. The add-on package "metafor" is used to conduct meta-analysis in the program "R" (196). The package provides functions that implement calculations for many different potential meta-analysis models. Fixed-effect, random-effects and mixed methods models can be applied through this package. Forest and funnel plots can be produced. Functions used in the conduct of this thesis are described below:

```
R>rma.uni
```

The function "rma.uni" provides the framework for meta-analytical models. For example using binary data:

```
R>rma.uni(ai=event.s, n1i=n.s, ci=event.c, n2i=n.c,
measure="RR", data=MPSCS.data, method="FE")
```

Where:
event.s= number of events in the study arm
n.s= total observations in the study arm
event.c= number of events in the control arm
n.c= total observations in the control arm
MPSCS.data= the data file to be used
RR= relative risk as summary effect measure
FE= fixed effect method. Random effects method is the default, but can be specified by method="REML". The results of the model include I^2 and Cochran Q tests for heterogeneity as well as the log relative risk (and log 95% confidence

interval), standard error and p-value. This function may be used with any of the usual effect size or outcome measures: relative risk ("RR"), odds ratio ("OR"), risk differences ("RD") and mean differences ("MD" or "SMD") for example.

So for example, using continuous data:

```
R>rma.uni(m1i=mean.s, sd1i=sd.s, n1i=n.s, m2i=mean.c,
sd2i=sd.c, n2i=n.c, data=MPSCS.data, measure="MD",
method="FE")
```

Where:
mean.s= mean value in the study arm
sd.s= standard deviation in the study arm
n.s= total number of observations in the study arm
mean.c= mean value in the control arm
sd.c= standard deviation in the control arm
n.c= total number of observations in the control arm

The subset command can be used to select a subset of studies for meta-analysis. This is useful for analysing categorical variables that may influence the effect size. For example include the "subset" command with the rma.uni function to select studies with a Jadad score of 3 or more:

```
R>rma.uni(ai=event.s, n1i=n.s, ci=event.c, n2i=n.c,
data=MPSCS.data, measure="RR", method="FE",
subset=(jadad>3))
```

Continuous moderator variables can be incorporated in the meta-analytical model to perform "mixed effects" analysis. This method has the ability to demonstrate the influence of moderators on the effect size. For example include

the "mods" command with the rma.uni function to include the moderators "year" and "ischaemictime" in the model:

```
R>rma.uni(ai=event.s, n1i=n.s, ci=event.c, n2i=n.c,
data=MPSCS.data, measure="RR", method="FE",
mods=cbind(year, ischaemictime)
```

The "metafor" package will produce basic forest plots. In order to produce more appealing and customizable forest plots, I have used a series of custom functions written by Simon Knight. These custom functions do not alter the statistical calculations of the underlying function and are primarily cosmetic.

2.3 Multivariate analysis

The description of statistical analysis in the following sections regarding linear regression, logistic regression, survival analysis and selection of variables has been summarised from several chapters in the book *Essential Medical Statistics,* by Kirkwood and Sterne (197). It is intended to provide an overview relevant to interpretation of Chapter 4 and is by no means exhaustive.

The analysis in Chapter 4 is based upon the analysis of national registry data maintained by NHS Blood & Transplant. Registry data are not designed primarily for research purposes and researchers using such datasets should explore the data in order to prepare it for analysis. Retrospective analysis is open to the influence of confounding variables. To adjust for possible confounding variables, multivariate analysis was chosen as the method for this study.

2.3.1 **Linear regression**

Simple linear regression is used to estimate the best-fitting straight line to describe the association between a numerical outcome and a numerical exposure. Multiple regression models are used for the effect of more than one exposure on a numerical outcome.

The line of best fit is derived by the method of "least squares". This method finds the parameters describing the constant and the coefficient for the regression line that minimise the sum of the squared vertical distances of the points from the line.

The first assumption necessary for linear regression is that for any value of x, y is normally distributed. The second is that the magnitude of the scatter of points is the same along the length of the line. The vertical deviations of each point from the line of best fit are known as residuals.

In multiple linear regression, the inclusion of multiple variables estimates an exposure effect after accounting for the effects of other variables. Binary exposure variables (indicator variables) can be incorporated in multiple regression as values 0 or 1. The coefficient of indicator variables is the difference between the mean in one group and the mean in the other. The effects of categorical exposures with more than two levels are estimated by introducing a series of indicator binary variables that are compared to a baseline group.

Non-linear exposure variables may be incorporated in multiple regression in a number of ways. Firstly they may be divided into subgroups and treated as a categorical variable. They may also be transformed (for example with natural log or square), and the transformation used for the analysis. Lastly, an algebraic description of the relationship may be used (quadratic, for example).

2.3.2 **Logistic regression**

Logistic regression is a method for assessing the effect of exposure variables on binary outcomes. Baseline odds are calculated for the odds of the outcome for the group against which all others will be compared. The exposure odds ratio is then calculated to express the effect of the exposure on the odds of the outcome. The regression coefficient is the log of the odds ratio. Logistic regression can incorporate binary exposure variables, categorical exposure groups, and continuous exposure variables. Categorical variables may be assessed by comparing the risk in each category to a baseline category, or by assessing change in odds per exposure group, if they can be put into numerical order. Multivariate logistic regression is also possible.

2.3.3 **Survival analysis**

In longitudinal studies, individuals are usually followed for different lengths of time for various reasons, and methods that take this into account are used when comparing survival outcomes. The proportion of patients remaining free of the adverse outcome changes over time.

Methods for comparative survival analysis make allowance for the fact that the event rate is also not constant over time. The Kaplan-Meier method is one way of estimating the survival curve using exact failure and censoring times. For Kaplan-Meier estimates, risk sets of individuals being studied at each time point are used to estimate a survival probability at each time. This is displayed as a survival curve that is horizontal at all times at which there is no outcome event, and drops vertically corresponding to the change in the survivor function at each time an event occurs. By comparing the hazards in two groups over the duration of follow up, we can account for differences in survival time. Rather than assume that the hazard of the event is constant over the duration of

a study, we assume that the Hazard Ratio between the two groups remains the same. This is known as the proportional hazards assumption.

The Mantel-Cox Chi^2 test (also known as the log rank test) can be used to compare hazards between two groups. The test is based upon a comparison of the number of exposed individuals at each time point experiencing the event, with the expected number if there were no difference in the hazards between the groups. These methods can also be adjusted for different demographics of the two groups being compared, such as age or sex, using Cox regression analysis.

2.3.4 **Selection of variables**

An important consideration in multivariate regression models is which variables to include when data on a large number of exposure variables is available. However, the considerations change depending on the purpose of the model. For the purposes of the work conducted for this thesis, regression was conducted to estimate the effect of preservation methods accounting for potential confounding variables, not to produce a predictive model. Backwards stepwise regression was used to narrow the variables down from the initial entries. A p-value of <0.1 was set as a limit for inclusion, as a compromise between the traditional threshold of 0.05 and the desire to produce a useful picture of factors that may be realistically linked to transplant outcomes.

Thinking about the model, and including variables that have been shown in previous work to be important, or have a suggested association in univariate analysis, is more valuable than plugging all available variables into a stepwise regression. The higher the number of original variables included, the higher the chance of selecting variables with chance associations. I have therefore explored univariate associations as well as considered the scientific rationale for associations, as well as the results of other studies.

2.3.5 **"Stata®"**

Stata® (*Statacorp LP, Texas, USA*) is an integrated statistical software package. It encompasses a vast array of statistical tests and graphical functions. It does not require any additional modules or packages. The commands for Stata® are well documented online and in handbooks.

2.4 Other statistical methods used

Three other key methods were necessary for the conduct of the statistical analyses in this thesis and warrant further description here.

2.4.1 **Combining mean and standard deviation of two groups**

When provided with a continuous outcome or variable related to two groups separately, it is possible to estimate the mean and standard deviation of the two groups combined (197). See below, where N1 and N2 are group sizes, M1 and M2 are means of each group, M3 is the overall mean, SD1 and SD2 are the standard deviations of each group, SD3 is the combined standard deviation.

$$M3 = \frac{(N1 \times M1) + (N2 \times M2)}{(N1 + N2)}$$

$$SD3 = \sqrt{\frac{((N1-1) \times SD1^2) + ((N2-1) \times SD2^2)}{(N1 + N2 - 2)}}$$

2.4.2 **Estimation of relative hazard**

Variability in the length of follow up between studies can prove problematic for meta-analysis; as the length of follow up increases, odds ratios increase, whilst relative risks decrease, and the underlying hazard ratio remains the same (198). Variation in odds ratio or relative risk due to different lengths of follow up can

introduce heterogeneity into a meta-analysis. When the hazard ratio is not provided by an individual study, but a two-by-two table of exposure and outcome is available, a method for estimating the relative hazard has been proposed (198). The relative hazard can be expressed as a ratio of the logarithms of survival proportions, the Relative Log Survival (RLS) (198), see below, where the values *a-d* represent the two-by-two table of exposure and outcome.

$$RLS = \frac{\ln(a/(a+b))}{\ln(c/(c+d))}$$

This method was validated by Perneger et al (198) using Richard Doll's work on cigarette smoking and survival in British doctors (199) and Feigl and Zelen's work on leukaemia survival (200). The method requires that hazards are proportionate. The use of RLS in meta-analysis has since been validated with studies weighted by inverse of variance (201) using results of chemotherapy trials in colorectal cancer (202). The sampling variance of the log of the RLS can be obtained by the equation below (198).

$$Variance = \frac{a}{b(a+b)\times \ln(b/(a+b))^2} + \frac{c}{d(c+d)\times \ln(d/(c+d))^2}$$

Regardless of the sample size, as long as event rates are below 75%, the equation above can be simplified to the equation below (198).

$$Variance = \frac{1}{a} + \frac{1}{c}$$

The 95% confidence interval for the RLS is obtained by the exponent of the equation below (198).

$$\ln(RLS) \pm 1.96 \times \sqrt{\frac{1}{a} + \frac{1}{c}}$$

2.4.3 **Test of Interaction**

A test of interaction is the comparison between the treatment effect in two subgroups, the test can be applied to odds ratios and relative risks. The two estimates should be independent and not obtained from the same individuals (203). It is necessary to obtain the logs of the relative risks and their confidence intervals before estimating the ratio of the relative risks (or odds ratios for that matter). Even when the estimates from each group and the related p-values seem very different, the test of interaction may not be significant. It is also not correct to assume that when two confidence intervals overlap, that the two estimates are not significantly different (203). The z-statistic for the test of interaction may be calculated with the equation below (203).

$$z = \frac{(\ln RR1 - \ln RR2)}{\sqrt{(SE1^2 + SE2^2)}}$$

Where SE1=the standard error of the lnRR of group 1 and SE2= the standard error of the lnRR of group 2. As 95% confidence intervals are obtained as 1.96 standard errors either side of the estimate, the standard error in each group may be calculated by the following equation (203).

$$SE = \frac{\ln(Upper95\%CI) - \ln(Lower95\%CI)}{2 \times 1.96}$$

3. Preservation solutions for static storage of kidneys

3.1 Introduction

The preservation of kidney allografts allows the transport and sharing of organs between centres to improve histocompatibility matches and also allows transplantation to take place on a less urgent basis. Immune induction and maintenance immunosuppressive regimens have advanced considerably in recent years, as has the understanding of allograft rejection, yet worldwide the preservation of kidneys persists in its most simple form; static cold storage (56). Numerous preservation solutions have been developed to counteract the detrimental effects of the recovery process, graft cooling and reperfusion. They have been designed to specifically target the biochemical and structural changes that occur during this process, yet they vary considerably in the exact nature and concentration of their constituents (204).

Registry data from national and international databases of deceased-donor kidney transplants suggests that the choice of preservation solution can affect both the short and long-term outcomes of the graft (56, 205). Of particular interest is the time lag before the kidney provides adequate renal function, removing the need for dialysis, the primary purpose of the transplant. This has implications for the risk of acute rejection, long term graft survival and the cost-effectiveness of the transplant procedure (83, 206).

A number of prospective trials have investigated the effect that the choice of preservation solution has on renal transplant outcomes over many years, with variable results. Such trials are often underpowered to identify differences in

important outcomes, such as DGF. Two previous systematic reviews have explored these differences but did not include studies of all preservation solutions. One review included only comparisons of Celsior, UW, and Marshall's Solution (HOC) (83) whilst the other considered only comparisons of UW with HTK (207).

Hypothermic machine perfusion for renal allografts using a new generation of pumps has been the subject of recent studies (126, 131), but the evidence for the superiority of machine preservation over static cold storage remains uncertain. The aim of this study was to systematically appraise the evidence comparing available preservation solutions for the static cold storage of deceased donor kidneys.

3.2 Methods

3.2.1 Research question

Which preservation fluid is best for the static and hypothermic preservation of deceased donor kidney allografts?

3.2.2 Inclusion and exclusion criteria

Inclusion criteria specified any prospective, comparative study of preservation solution for static cold storage of deceased-donor renal allografts from any class of adult or paediatric donor. First and subsequent transplants were included. All kidneys were stored by SCS alone. Retrospective, animal, non-comparative and/or live donor studies were excluded. I reviewed abstracts for inclusion independently from, but in duplicate with, Robert Morgan. Differences were agreed by discussion with Simon Knight and/or Peter Morris.

3.2.3 Literature search

A systematic literature search was performed using MEDLINE and EMBASE, the Cochrane Library, the Transplant Library of RCTs from the Centre for Evidence in Transplantation and the International Clinical Trials Registry Platform. Searches were conducted using MeSH and EMTREE keywords with free-text aliases for preservation solutions in order to capture all relevant references. MEDLINE was searched from 1948 and EMBASE from 1980 to the current date. No language limits were applied. References of included studies, citing articles of included studies and reviews were studied for further potentially relevant references. The final date for literature searches was 20th July 2011.

3.2.4 Data extraction

Studies are referred to throughout by the first author and year of the earliest peer-reviewed publication. Demographic, quality and outcome data were independently extracted from the included studies into a pre-designed Microsoft Excel spreadsheet. Data was taken from all papers describing the study; in the case of discrepancies the most comprehensive paper was used. I extracted data independently from, but in duplicate with, Robert Morgan and any discrepancies in data extraction were settled by discussion with Simon Knight and/or Peter Morris.

3.2.5 Outcomes

The primary outcome was the rate of DGF. The definition of DGF varies between studies. Despite such differences, the underlying effect should be the same and we have used the definition provided in the original paper. Secondary outcomes were PNF, graft survival, renal function (study defined), biopsy proven acute rejection and patient survival.

3.2.6 **Quality Assessment**

Randomised controlled trials were assessed firstly using the Jadad score, a 0-5 scale dependent upon adequate descriptions of randomisation method, blinding and withdrawals (189). A score of 3 or more on this scale indicates good quality. Secondly, both RCTs and Non-Randomised Controlled Trials (Non-RCTs) were assessed by an adequate description of allocation concealment, intention-to-treat analysis, sample size calculation, description of withdrawals and use of appropriate statistical tests. Similarity between study group and control group was assessed on the basis of demographic data provided in the manuscript. All reports from each trial were utilised in assessing study quality. All corresponding authors were contacted by email with a standardised letter detailing our assessment, requesting clarification of areas that were unclear from the publication and requesting a response to our quality assessment. First or senior authors were also contacted with the same letter if no reply was received from the corresponding author within four weeks. Supplementary information was returned for four studies and affected our initial assessment in one case where details of the randomisation method were provided (208).

3.2.7 **Data Synthesis**

The statistical package "R" (195) was used for statistical analysis in combination with the metafor package available for "R" (196). Meta-analysis was conducted using data from RCTs alone. Heterogeneity was analysed using Cochran's Q test, with $P < 0.10$ indicating significant heterogeneity, and the I^2 test, which describes the proportion of variation that is due to heterogeneity beyond chance. One meta-analysis was conducted for this study, and heterogeneity was low, so a fixed-effect meta-analysis was used (209). Binary outcomes were

analysed using the Pearson Chi^2 test, or Fisher's exact test for samples of less than ten.

3.3 Results

Initial literature searches identified 3,725 references across all databases Figure 3.1. Fifteen studies (10 RCTs and 5 Non-RCTs) described in 28 references met the full inclusion criteria. Details of the included studies are shown in Table 3.1.

Figure 3.1. Flow chart of inclusions and exclusions. RCT= Randomised Controlled Trial, ICTRP=International Clinical Trials Registry Platform, Transplant Library= Library of RCTs from the Centre for Evidence in Transplantation.

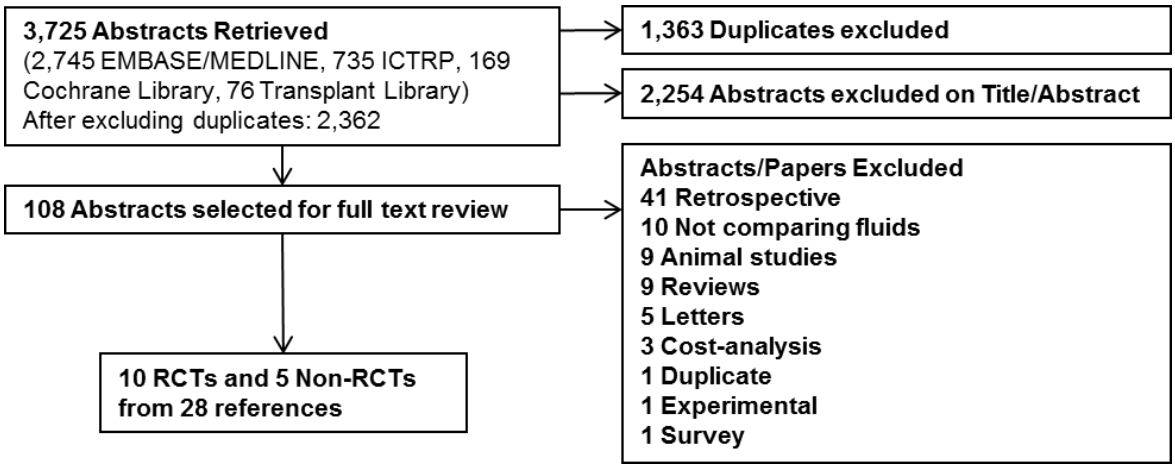


Table 3.1. Details of included studies comparing two or more preservation solutions, grouped by solutions compared. DBD= Donor after Brain-death, DCD= Donor after Circulatory Death, EC= Eurocollins, HOC= Hyperosmolar Citrate, HTK= Histidine Tryptophan Ketoglutarate, IGL-1= Institut Georges Lopez-1, PBS= Phosphate Buffered Sucrose, Solns= Preservation Solutions studied, UW= University of Wisconsin, UWMod= University of Wisconsin Modified.

Solns.	Study	Number of Kidneys	Study Type	Donor Types Included	Country	Refs.
EC UW	Ploeg 1990	795 (343/352)	RCT	Not multi-organ	Multinational[1]	(67, 210-214)
	Hefty 1991	40 (20/20)	RCT	All	USA	(215, 216)
	Ishibashi 1994	90 (44/46)	RCT	DCD	Japan	(217)
EC HTK	Isemer 1988	18 (9/9)	NonRCT	All	Germany	(218)
	Moisiuk 1996	108 (52/56)	NonRCT	DCD	Russia	(219)
	Trushkov 2003	88 (54/34)	RCT	All	Latvia	(220)
	Groenewoud 1990[2]	569 (292/277)	RCT	DBD, not multi-organ	Eurotransplant[3]	(68, 94, 221-224)
HTK UW	Groenewoud 1990[2]	611 (314/297)	RCT	DBD, not multi-organ	Eurotransplant[3]	
	Klaus 2007	51 (24/27)	RCT	All	Brazil	(225)
Celsior UW	Faenza 2001	187 (99/88)	RCT	All	Italy	(226)
	Pedotti 2004	441 (172/269)	RCT	Multiorgan	Italy	(105)
	Montalti 2005	50 (25/25)	RCT	>60 years old	Italy	(208)
HOC PBS	Lam 1989	184 (92/92)	NonRCT	DBD	UK	(81, 227)
HOC Perfudex	Slapak 1979	47 (31/16)	NonRCT	DCD	UK	(80)
UW UW Mod	Baatard 1993	82 (41/41)	RCT	All	France	(99)
IGL-1 UW	Badet 2005	223 (121/102)	NonRCT	Multi-organ	France	(109, 228)

[1] This multinational study included centres within the following countries: Austria, Belgium, France, Germany, The Netherlands, Luxembourg and Portugal.
[2] Parallel study of four interventional arms.
[3] The Eurotransplant International Foundation coordinates transplantation within the following countries: Austria, Belgium, Croatia, Germany, Luxembourg, The Netherlands and Slovenia.

Table 3.2. Quality assessment of included studies.

Study	Jadad Score[4]	Allocation Concealment[5]	Groups Similar[6]	Sample Calculation	Withdrawals Accounted For	Statistical Tests Described[7]
Ploeg 1990 (210)	3	Yes	Yes	Yes	Yes	Yes
Hefty 1991 (215)	2	No	Yes	No	Yes	Yes
Ishibashi 1994 (217)	2	Yes	Yes	No	No	Yes
Isemer 1988 (218)	NA	No	Unclear	No	Yes	None
Moisiuk 1996 (219)	NA	No	Yes	No	No	Unclear
Trushkov 2003 (220)	1	Unclear	Unclear	No	No	Unclear
Groenewoud 1990 (221)	2	Yes	Yes	No	Yes	Yes
Klaus 2007 (225)	2	No	Yes	No	Yes	Unclear
Faenza 2001 (226)	2	Unclear	Yes	No	Yes	Yes
Pedotti 2004 (105)	1	No	Yes	No	No	Yes
Montalti 2005 (208)	3	Yes	Yes	No	Yes	Yes
Lam 1989 (81)	NA	No	Yes	No	No	Yes
Slapak 1979 (80)	NA	No	Yes	No	Yes	Unclear
Baatard 1993 (99)	2	Unclear	Yes	No	Yes	Yes
Badet 2005 (109)	NA	No	Yes	No	Yes	Yes

[4] NA= Not Applicable to Non-RCTs.
[5] Unclear if no description of randomisation method .
[6] Unclear if no description of group demographics.
[7] Unclear if insufficient information provided regarding statistical tests to make a judgement.

3.3.1 Quality of included studies

Methodological quality of the included studies was variable with included RCTs achieving between 0 and 3 points out of 5 on the Jadad scale, Table 3.2. No studies described any form of blinding. Four studies described a randomisation method that was consistent with allocation concealment (67, 208, 217, 221), eight described a method that was not (80, 81, 105, 109, 215, 218, 219, 225) and it was unclear in three studies (99, 220, 226). It was uncertain whether or not any of the studies performed an intention-to-treat analysis because descriptions of group switching post-randomisation and pre-transplantation were rarely provided. Withdrawals and dropouts were adequately accounted for in 10 studies (67, 80, 99, 109, 208, 215, 218, 221, 225, 226). Only one study described a sample-size calculation (67). Appropriate statistical tests were described in 10 studies (67, 81, 99, 105, 109, 208, 215, 217, 221, 226).

3.3.2 Delayed graft function

A wide range of overall rates of DGF were reported by the included studies (13-73%), Table 3.3. This could be partly explained by the different definitions used for DGF. The most common definition was a requirement for dialysis within the first week after transplantation, used by seven studies (81, 105, 109, 208, 215, 217, 226). Three studies defined DGF as at least two dialysis sessions within the first week (67, 221, 225) and one study defined it as at least three dialysis sessions within the first week (220). Two studies defined DGF by a lack of immediate urine production (218, 219). Two studies did not report DGF as an outcome (80, 99).

Table 3.3. Overall rates of delayed graft function (DGF) by solution studied. Relative Risk (RR) of DGF is Solution 1 vs Solution 2, >1 favours Solution 2. Studies grouped by comparisons made. CIT= Cold Ischaemic Time, EC= Eurocollins, HOC= Hyperosmolar Citrate, HTK= Histidine Tryptophan Ketoglutarate, IGL-1= Institut George Lopez-1, NR= Not Reported, PBS= Phosphate Buffered Sucrose, UW= University of Wisconsin.

Study	DGF/Kidneys in Study (%)	Mean CIT (hours)	Solution 1 DGF/Kidneys in Group (%)		Solution 2 DGF/Kidneys in Group (%)		RR of DGF (95%CI)	P-value
Ploeg 1990 (67)	194/695 (28%)	24	EC	114/343 (33%)	UW	80/352 (23%)	1.46 (1.15-1.87)	<0.01
Hefty 1991 (215)	6/40 (15%)	23	EC	3/20 (15%)	UW	3/20 (15%)	1.00 (0.23-4.37)	1
Ishibashi 1994 (217)	66/90 (73%)	11	EC	34/44 (77%)	UW	32/46 (70%)	1.11 (0.87-1.43)	0.41
Isemer 1988 (218)	10/18 (56%)	NR	EC	6/9 (67%)	HTK	4/9 (44%)	1.50 (0.63-3.56)	0.64
Moisiuk 1996 (219)	38/108 (35%)	NR	EC	27/52 (52%)	HTK	11/56 (20%)	2.64 (1.46-4.77)	<0.01
Trushkov 2003 (220)	18/88 (20%)	NR	EC	18/54 (33%)	HTK	0/34 (0%)	23.55 (1.47-378.34)	<0.01
Groenewoud 1990 (221)	204/569 (36%)	24	EC	119/277 (43%)	HTK	85/292 (29%)	1.48 (1.18-1.85)	<0.01
Groenewoud 1990 (221)	204/611 (33%)	24	UW	99/297 (33%)	HTK	105/314 (33%)	1.00 (0.8-1.25)	1
Klaus 2007 (225)	29/51 (57%)	20	UW	17/27 (63%)	HTK	12/24 (50%)	1.26 (0.77-2.06)	0.35
Faenza 2001 (226)	61/187 (33%)	17	Celsior	31/99 (31%)	UW	30/88 (34%)	0.92 (0.61-1.39)	0.69
Pedotti 2004 (105)	101/441 (23%)	15	Celsior	40/172 (23%)	UW	61/269 (23%)	1.03 (0.72-1.46)	0.89
Montalti 2005 (208)	25/50 (50%)	19	Celsior	12/25 (48%)	UW	13/25 (52%)	0.92 (0.53-1.61)	0.78
Lam 1989 (81)	39/160 (24%)	22	PBS	16/78 (21%)	HOC	23/82 (28%)	0.73 (0.42-1.28)	0.27
Badet (109)	29/223 (13%)	17	IGL-1	16/121 (13%)	UW	13/102 (13%)	1.04 (0.52-2.05)	0.92

University of Wisconsin Solution versus Eurocollins Solution

Three RCTs compared UW with EC including a total of 825 kidneys (67, 215, 217). These trials had a large variation in the overall rate of DGF (range 15-73%) and significant differences in their donor populations (one study used DCD only (217)). It was therefore inappropriate to combine the studies in a meta-analysis. Ploeg et al conducted a large, good quality, multi-centre RCT using a central randomisation list to allocate groups (n=695 kidneys) (67). They found that EC preserved kidneys had a significantly higher rate of DGF than UW preserved kidneys (RR=1.46, 95%CI=1.15-1.87, p<0.01). Two smaller RCTs made the same comparison; Ishibashi et al conducted a multi-centre RCT (n=90 kidneys) which found the risk of DGF to be equal with the two solutions (RR=1.11, 95%CI=0.87-1.43, p=0.41) (217). There were a large number of unexplained withdrawals post-allocation in this study. A central randomisation table was used to allocate kidneys to preservation groups. Hefty et al conducted a smaller, single centre RCT (n=40 kidneys) finding no difference in the rate of DGF between the 2 groups (RR=1, 95%CI=0.23-4.37, p=1.00) (215). The method of randomisation was unclear.

Hisitidine-Tryptophan-Ketoglutarate versus Eurocollins Solution

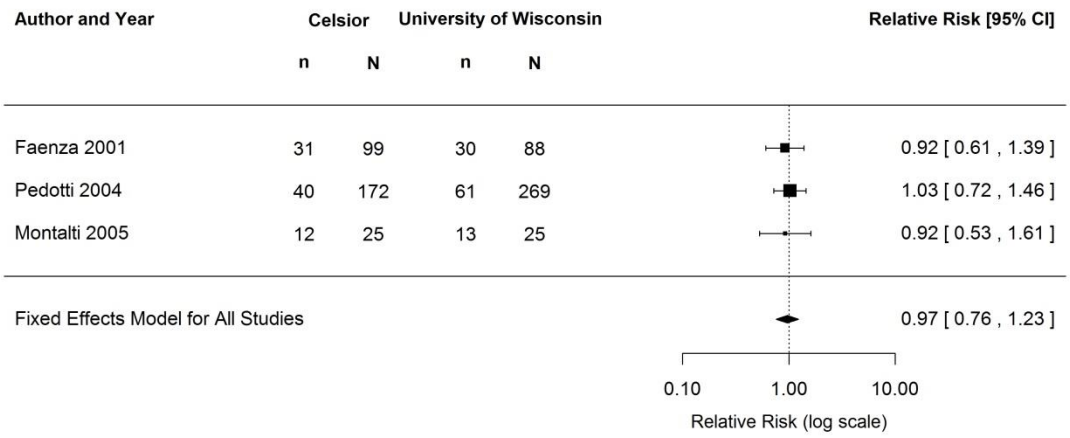
Two RCTs (220, 221) and two Non-RCTs (218, 219) compared HTK with EC, including a total of 783 kidneys. Significant differences between these studies in terms of the donor populations (DCD only (219), DBD only (221)), the overall rate of DGF (range 20-56%) and the definition of DGF, prevented meta-analysis. Groenewoud et al conducted the largest, multi-centre RCT using kidney only donors (n=569 kidneys) (221). DGF was defined as a requirement for two or more dialysis sessions in the first week post-operatively. The group found that the rate of DGF was significantly higher with EC than HTK stored kidneys (RR=1.48, 95%CI=1.18-1.85, p<0.01). Trushkov et al conducted a smaller, single-centre RCT (n= 88 kidneys) (220). DGF was defined as a requirement for three or more dialysis sessions in the first

week post-operatively. The rate of DGF was much higher with EC than HTK stored kidneys (RR=23.55, 95%CI=1.47-378.34, p<0.01). It is unclear how many organs were eligible for this study and the group sizes differ considerably. In both RCTs the method of randomisation was unclear. Two Non-RCTs defined DGF as a lack of immediate urine production. Moisiuk et al (n=108 kidneys) found the risk of DGF to be higher with EC than HTK stored kidneys (RR=2.64, 95%CI=1.46-4.77, p<0.01) (219). Isemer et al (n=18 kidneys) found the risk of DGF to be equivalent with EC and HTK stored kidneys (RR=1.5, 95%CI=0.63-3.56, p=0.64)(218). The method of allocation was unclear for both Non-RCTs.

University of Wisconsion Solution versus Celsior Solution

Three multi-centre RCTs compared UW with Celsior Solution, including a total of 678 kidneys (105, 208, 226). Pedotti et al conducted the largest trial making this comparison (n=441 kidneys) using a randomisation list to allocate patients (105). The group found the risk of DGF to be equal for UW and Celsior preserved kidneys (RR= 1.03, 95%CI 0.72-1.46, p=0.89). Faenza et al conducted a large trial (n=187 kidneys) for which the method of randomisation was unclear (226). The group found the risk of DGF to be equal for UW and Celsior preserved kidneys (RR=0.92, 95%CI=0.61-1.39, p=0.69). Montalti et al conducted a smaller trial (n=50 kidneys) using only donors over 60 years old (208). A computerised random number generator was used to allocate groups. This group also found the risk of DGF to be equal for UW and Celsior preserved kidneys (RR=0.92, 95%CI=0.53-1.61, p=0.78). All 3 studies defined DGF as a requirement for dialysis in the first week post-operatively. The overall risk of DGF in our meta-analysis of these studies was equal for both solutions (FEM: RR=0.97, 95%CI=0.76-1.23, p=0.79, Figure 3.2. There was no heterogeneity, I^2=0%, Cochrane Q Test for heterogeneity: Q=0.19, p=0.91.

Figure 3.2. Forest plot to show the Relative Risk (RR) of Delayed Graft Function (DGF) comparing Celsior with University of Wisconsin Solution. N= total patients in study arm, n= number of patients with DGF. Summary RR calculated by fixed effect meta-analysis, >1 favours University of Wisconsin Solution. Squares represent individual study effects, diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =0%, Cochran Q Test for heterogeneity Q=0.19, p=0.91.



University of Wisconsin Solution versus Histidine-Tryptophan-Ketoglutarate

Two RCTs compared UW with HTK including a total of 662 kidneys. Groenewoud et al compared UW to HTK (n=611 kidneys), parallel to their EC versus HTK study (described previously) (221). The group found the risk of DGF to be 33% for both HTK and UW stored kidneys (RR=1.00, 95%CI=0.80-1.25, p=1). Klaus et al conducted a smaller, single-centre RCT (n=51 kidneys) defining DGF as a requirement for 2 or more dialysis sessions in the first week post-operatively (225). They found no difference in the risk of DGF (RR=1.26, 95%CI=0.77-2.06, p=0.87). The method of randomisation was unclear. As only two studies made this comparison, meta-analysis was not appropriate.

Other comparisons

Slapak et al compared Perfudex with HOC in a Non-RCT (n=47 kidneys) (80). Kidneys stored in Perfudex on average required more dialysis sessions post-operatively than kidneys stored in HOC (4.4 vs 1.7, p<0.01). The outcome DGF as purely a requirement for dialysis was not presented. The method of allocation was unclear.

Lam et al compared HOC with Phosphate Buffered Sucrose (PBS) in a Non-RCT (n=104 kidneys) (81). Post-operative requirement for dialysis was not significantly different for HOC and PBS stored kidneys (RR=0.73, 95%CI=0.42-1.28, p=0.35). Alternation was used to allocate kidneys to each group.

Two studies compared UW to two slight modifications of its composition, UW modified (UWMod) and Institut George Lopez-1 solution (IGL-1). Baatard et al conducted a single-centre RCT comparing UW with UWMod, which has Hydroxyethylene starch, allopurinol, and adenosine removed (n=82 kidneys) (99). Results were provided for the average number of dialysis sessions post-operatively; there was no statistically significant difference between the groups (0.65±5 sessions versus 0.85±5 sessions, p=NS). The method of randomisation was unclear. Badet et al compared UW to IGL-1 (which is UW with polyethylene glycol substituted for Hydroxyethylene starch) in a Non-RCT (n=223 kidneys) (109). The risk of DGF was equivalent for IGL-1 and UW stored kidneys (RR=1.04, 95%CI=0.52-2.05, p=0.92). The method of allocation was unclear.

3.3.3 **Primary non-function**

Five studies reported rates of PNF, although it was not defined by any study (67, 105, 109, 219, 225), Table 3.4. Four studies had PNF rates under 5%, while one study reported PNF rates of 10% (219); this was a study of DCD only, in which 90% of the donors had cardiac arrest after head trauma. The average duration of cold ischaemia was not reported by this study. Given the low PNF rates, all included trials were underpowered to demonstrate a significant difference in this outcome between the preservation solutions examined.

Table 3.4. Overall rate of primary non-function (PNF) rate by solution studied. EC= Eurocollins, HTK= Histidine Tryptophan Ketoglutarate, IGL-1= Institut George Lopez-1, UW= University of Wisconsin.

Study	Overall Rate of PNF (%)	Solution 1 Rate of PNF (%)		Solution 2 Rate of PNF (%)	
Ploeg 1990	35/695 (5%)	EC	22/345 (6.4%)	UW	22/343 (3.7%)
Moisiuk 1996	11/108 (10.2%)	EC	8/52 (15.4%)	HTK	3/56 (5.4%)
Pedotti 2004	8/441 (1.8%)	UW	4/269 (1.5%)	Celsior	4/172 (2.3%)
Badet 2005	1/223 (0.5%)	UW	0/102 (0%)	IGL-1	1/121 (0.8%)
Klaus 2007	1/51 (2%)	HTK	1/24 (4.2%)	UW	0/27 (0%)

3.3.4 **Graft survival**

Twelve studies reported graft survival rates at a variety of follow-up time-points, Table 3.5. Only one study reported log-rank p-values for graft survival (67). This study found there was an increased rate of graft loss for kidneys stored with EC compared to UW (82% vs. 88% survival at 12 months, log-rank p=0.04). This finding was, in part, supported by the 12 month cumulative graft survival from the other RCT making the same comparison, (84% for EC and 90% for UW stored kidneys) (217). Unfortunately, there was insufficient data from this study to calculate the Hazard Ratio. Overall the reporting of numbers followed up at the declared time-points was poor, making Hazard Ratio calculation, and therefore meta-analysis, impossible. One study found an improved graft survival for Celsior stored kidneys compared to UW stored kidneys (226), although the other two studies making this comparison found no statistically significant difference (105, 208). Three studies reported graft survival for comparisons of HTK with EC (219-221) and two compared HTK with UW (221, 225), finding no significant difference in graft survival. Equal graft survival was also reported for comparisons of HOC with PBS (81) and UW with IGL-1 (109).

The two largest studies, which examined EC, HTK and UW, compared long-term graft survival for kidneys with and without DGF, demonstrating that DGF was associated with worse long-term graft survival for kidneys preserved with any one of these solutions (67, 221).

Table 3.5. Graft survival rates at reported follow up time points. Studies grouped by solutions compared. Where numbers followed up were not stated in the papers, the reported percentage graft survival is given. Blank spaces indicate no figures provided for that time-point in the study report. EC= Eurocollins, HOC= Hyperosmolar Citrate, HTK= Histidine Tryptophan Ketoglutarate, IGL-1= Institut Georges Lopez-1, PBS= Phosphate Buffered Sucrose.

Study	Soln.	1 month	3 months	12 months	24 months	36 months	48 months	60 months
Hefty 1991 (215)	UW	20/20 (100%)						
	EC	20/20 (100%)						
Ploeg 1990 (67)	UW		316/343 (92%)	265/300 (88%)				
	EC		295/335 (88%)	233/282 (82%)				
Ishibashi 1994 (217)	UW		96%	90%				
	EC		88%	84%				
Groenewoud 1990 (221)	HTK			80%	76%	177/253 (70%)		
	EC			78%	71%	170/254 (67%)		
Moisiuk 1996 (219)	HTK			83%				
	EC			65%				
Trushkov 2003 (220)	HTK			88%				
	EC			79%				
Faenza 2001 (226)	UW				75%			
	Celsior				84%			
Pedotti 2004 (105)	UW	96%		91%				
	Celsior	96%		94%				
Montalti 2005 (208)	UW			96%				87%
	Celsior			92%				79%
Groenewoud 1990 (221)	UW			81%	73%	191/281 (68%)		
	HTK			83%	77%	212/291 (73%)		
Klaus 2007 (225)	UW		26/27 (96%)	21/27 (78%)				
	HTK		23/24 (95%)	19/24 (79%)				
Lam 1989 (81)	PBS			85%			61/82 (74%)	
	HOC			85%			55/78 (71%)	
Badet 2005 (109)	UW			101/102 (99%)				
	IGL-1			118/121 (98%)				

3.3.5 **Renal function**

Renal graft function post-operatively was reported at a variety of time-points and different studies used different measurements. One study comparing EC to UW found a more rapid fall in serum creatinine within the first week with UW (67), although at 3-4 months follow-up there was no significant difference, and this was supported by the other two studies making this comparison (215, 217). One study comparing EC with HTK reported faster normalisation of serum creatinine and lower levels up to two weeks post-operatively with HTK (220). Another study making the same comparison found a faster normalisation of serum creatinine with HTK stored kidneys as well, although from a limited subset of its included patients (221). Levels remained lower until 30 days after surgery. Two smaller studies found the time to normalisation of serum creatinine was equal for both HTK and EC (218, 219). All three studies comparing UW to Celsior found no significant difference in post-operative serum creatinine before discharge (208, 226) or up to two weeks follow-up (105). One study reported comparable serum creatinine for kidneys stored in UW and HTK from one month up to one year post-operatively (225). The other study making this comparison also found no significant difference in levels up to one month in a subset of its patients (221).

Patients receiving UW and UWMod stored kidneys were found to have similar serum creatinine from one month up to one year post-operatively (99). IGL-1 stored kidneys were associated with lower serum creatinine than UW stored kidneys from six days to one year post-operatively (109). Patients receiving HOC-stored kidneys had significantly lower serum creatinine than those receiving Perfudex stored kidneys at day 10 post-operatively (80).

3.3.6 **Acute rejection**

Eight studies reported rates of acute rejection (99, 105, 109, 208, 217, 218, 225, 226), Table 3.6. Acute rejection was only defined as proven on biopsy by one study, Klaus

et al (225) . This group found that acute rejection episodes during the first 12 months were equal for UW and HTK stored kidneys (0.38 and 0.33 episodes per patient, p=0.78). Acute rejection was not defined in the remainder of the studies. The preservation solution was not related to acute rejection in any study that reported this as an outcome. One study reported the percentage of patients experiencing rejection (226), the others declared total episodes of rejection (i.e. some patients in these studies had multiple episodes). Graft loss of 3.3% due to hyper-acute rejection was reported by one study (221), and graft loss due to acute rejection was reported by three studies (67, 208, 219). The number of grafts lost due to acute rejection was very small (range 1.9-4%) and no study reported that the preservation solution was related to graft loss from acute rejection.

Table 3.6. Episodes of acute rejection at declared follow up time-points. Information regarding immune suppression is given where available from the original paper. ATG= Antithymocyte Globulin, Aza= Azathioprine, CsA= Cyclosporine, MMF= Mycophenolate mofetil, OKT3=Muromonab-CD3, Pred= Prednisolone, Rap= Rapamycin, Tac=Tacrolimus.

Study	Follow-up	Solution	Episodes/ Patients	Episodes per Patient	Immune Suppression	
					Induction	Maintenance
Isemer 1988	Unclear	EC HTK	10/9 4/9	1.11 0.44	Not Reported	
Baatard 1993	3 months	UW UW Mod	7 /41 6/41	0.17 0.15	ATG	CsA+Aza+Pred
Ishibashi 1994	3 months	UW EC	21/46 25/44	0.46 0.57	+/-ATG	CsA/Tac+MMF/Aza
Pedotti 2004	1 month	UW Celsior	59/269 31/172	0.22 0.18	ATG/OKT3/ Basiliximab	CsA/Tac +MMF+Pred
Montalti 2005	Inpatient	UW Celsior	2/25 2/25	0.08 0.08		CsA/Tac
Badet 2005	Unclear	UW IGL-1	13/102 15/121	0.13 0.12		CsA/Tac/Rap +Pred+ MMF/FTY740
Klaus 2007	12 months	UW HTK	10/27 8/24	0.38 0.33	Not Reported	
Patients with acute rejection						
Faenza 2001	Inpatient	UW Celsior	13% 12%			CsA/Tac

3.3.7 **Patient survival**

Limited data was available for long-term patient survival; it was only reported by five studies (67, 105, 109, 208, 225), Table 3.7. The choice of preservation solution was not related to worse patient survival in any of these studies. Limited information on numbers followed up prevented meta-analysis.

Table 3.7. Patient survival rates at declared follow-up time-points. EC= Eurocollins, HTK= Histidine Tryptophan Ketoglutarate, IGL-1= Institut Georges Lopez-1. Blank spaces indicated no data provided for that time point in the study report.

Study	Fluid	Post-operative	1 month	3 months	12 months
Ploeg 1990	EC			96%	94%
	UW			98%	95%
Pedotti 2004	Celsior		100%		99%
	UW		100%		98%
Badet 2005	IGL-1				98%
	UW				100%
Montalti 2005	Celsior	100%			
	UW	100%			
Klaus 2007	HTK				86%
	UW				84%

3.3.8 **Implications of longer CIT and donor type**

Subgroup analysis by type of donor and length of CIT was presented by four studies. The mean length of CIT, where reported, was no longer than 24 hours in any study, Table 3.3. Ploeg et al compared DGF rates after <24 hours CIT with 25-35 hours and >35 hours. They did not find that the risk of DGF increased at longer CIT with either EC or UW preservation (67). This study also found that kidneys from so-called "bad" donors (n=294, cardiac arrest, severe hypotension, serum creatinine >175 micromol/l, oliguria) did not have a significantly higher risk of DGF compared to so-called "good" donors, regardless of the preservation solution used. Ishibashi et al reported DGF rates for donors >60 years old as 100% for both EC and UW (217). Too few donors >60 years old were included for a statistical analysis (n=10 in each study arm). Both Pedotti et al and Faenza et al found that longer CIT (>17 hours and >18 hours respectively) did not help to distinguish between Celsior and UW, with

both solutions being associated with an equal increase in the rate of DGF (105, 226). Pedotti et al also performed a subgroup analysis of donors >60 years old (n=102), finding no difference between UW and Celsior. Montalti et al only included donors >60 years old, finding that the risk of DGF was equally high with UW and Celsior Solutions (208).

3.4 Discussion

This systematic review examined the evidence for the commonly used static cold preservation solutions in renal transplantation. The risk of DGF is influenced by the choice of preservation solution for static cold storage. However, the evidence assessed here cannot provide a specific relative risk for DGF when using one preservation solution over another. The risk of DGF is increased with EC stored kidneys when compared to both UW and HTK in the largest, best quality RCTs. This is congruent with the findings of the smaller studies that made the same comparisons, and registry data (56). On the basis of the three RCTs that compared UW with Celsior and two that compared UW with HTK, it is fair to conclude that these three solutions are associated with a comparable risk of DGF and this is partly supported by analysis of registry data (205). Previous systematic reviews have included an analysis of studies comparing UW with Celsior (83), and UW with HTK (207), also concluding that the risk of DGF was equivalent.

Long-term graft survival was worse for EC than UW stored kidneys in the one good quality RCT that provided follow up data for this comparison. Absolute graft survival was also worse for EC than HTK stored kidneys in the three studies with long-term follow up. However, not enough information was provided to aggregate the data. The association between renal grafts with DGF and worse long-term graft survival has been described previously (206).

Analysis of the Collaborative Transplant Study data suggests UW stored kidneys, with up to 24 hours of CIT, have a lower rate of graft loss than EC stored kidneys

and equivalent rate of graft loss compared to HTK and HOC stored kidneys. With CIT beyond 24 hours, the risk of graft loss increases more with EC, HTK and HOC stored kidneys than it does with UW stored kidneys (56). Interestingly, the two RCTs comparing HTK to UW had mean CIT of 23 and 20 hours (221, 225); CTS data analysis suggests that no difference in graft loss would be seen at this length of CIT. At odds with the CTS analysis, UNOS data shows that HTK storage for long periods is not associated with increased graft loss when compared to UW (205). Overall, however, UNOS data suggests that HTK is associated with an increased risk of graft loss compared to UW from one year post-transplant, regardless of CIT.

Data regarding the secondary outcomes (primary non-function, acute rejection, patient survival and post-transplant renal function) was insufficient in the included studies to draw strong conclusions. Given the overall low rates of acute rejection, primary non-function and patient death, much larger studies would be required to reveal any differences. There is a suggestion that post-transplant serum creatinine may fall more quickly with UW or HTK than EC stored kidneys, although the exact clinical significance of this is unclear. Similarly, IGL-1 storage appeared to result in improved serum creatinine compared to UW storage even at 12 months post-operatively in one study; again exactly what effect this would have is unclear.

A unique finding of this systematic review is the lack of RCTs comparing HOC for renal preservation to any of the solutions in use before its development, or to newer solutions such as UW and HTK. Despite this it was used for the preservation of large numbers of kidneys on a background of animal experiments (74, 229) and trials involving machine perfusion (75). Moreover, it is still used for the static cold storage of 2-3% of kidneys included in the CTS registry and approximately 80% of kidneys in the UK (Chapter 4).

Overall the methodological quality of the included studies was poor. Randomisation was not always adequate and the method of allocation to groups was not often concealed, allowing clinicians to predict which kidneys would be allocated to each study group. Participants were excluded from analysis post-allocation in many

studies and the reporting of numbers followed up was lacking. Blinding of participants or investigators was not declared in any study, although this is to some extent mitigated by the measurement of objective outcomes such as graft loss. Furthermore, this study has highlighted the need to establish a universal definition for DGF that is both validated and objective. Given that the decision to dialyse is subjective, a lack of allocation concealment can lead to the introduction of bias through the manipulation of this outcome.

For this review, randomised and prospective comparative studies were included to present the best quality evidence. There is also a large body of evidence available from retrospective studies and registries. Retrospective studies were deliberately excluded to avoid the biases inherent in registry data, such as selection bias and performance bias. Although RCTs are not without bias, these can be mitigated when the body of evidence is substantial. The volume of clinical trial evidence is small and, given the various comparisons made, the amount of data had to be further divided. Despite this, the outcomes reported by the studies included here are largely in agreement with the available registry data. This study was conducted in accordance with the PRISMA statement but did not achieve all recommended reporting items (182); the review protocol could not be prospectively registered and published, as the study was too far advanced to be accepted by the UK National Institute for Health Research's Prospective Register of On-going Systematic Reviews (NIHR-PROSPERO) when this system became available in February 2011 (183). The assessment of reporting bias could also not be completed as the small number of studies making each comparison precluded funnel plots.

There is no difference in the incidence of DGF with the use of Celsior, HTK and UW. A preference for one solution over another could theoretically be based upon the relative cost of each solution, in combination with the recommended flush volume. However, these three solutions do not vary greatly in price; Celsior costs US$250 per litre, HTK costs US$195 per litre and UW costs US$190 per litre. These differences in price are small when contrasted with the much cheaper HOC, which costs

approximately US$15 per litre. Even with the small difference in recommended aortic flush volumes (Celsior, 3-5 litres; HTK, 5-6 litres; and UW, 2-4 litres), there does not appear to be support for the use of one of these three solutions for kidney preservation over another on the basis of cost. Intra-aortic flush volumes and back-table flush volumes may have affected outcomes in the included studies via an increased rate of graft cooling with larger volumes; however this information was not adequately reported in the included studies. Another consideration is the efficacy of each solution in the preservation of non-renal organs perfused in-situ at the same time as the kidneys.

Conclusion

The choice of preservation solution has an effect upon short-term outcomes for renal allografts, which may in turn affect long-term outcomes. From the limited evidence identified, Celsior, HTK and UW are associated with a comparable risk of DGF and graft loss.

4. Kidney preservation with hyper-osmolar citrate

4.1 Introduction

Over 1,700 kidneys from deceased donors are transplanted in the United Kingdom every year (1) and the majority are preserved by static cold storage (34). Hypothermic machine perfusion has not grown in popularity in the UK as much as it has in other countries, perhaps because results of renal transplantation have remained acceptable (34) and analyses of the cost-effectiveness of machine perfusion had been unconvincing (83). In the UK, the most commonly used preservation fluids for static cold storage are UW Solution and Hyper-osmolar Citrate (HOC, Marshall's Solution).

University of Wisconsin Solution was developed through systematic research by Belzer and Southard in the 1980s (8). It has since been used successfully for the preservation of kidneys, liver, pancreas and small bowel allografts. Hyper-osmolar citrate, on the other hand, was developed by Marshall, Escott and Ross in Melbourne, Australia, and has been used since the late 1970s, predominantly in Australia and the UK, but with few detailed, published comparisons to other preservation fluids (76, 77, 79, 230). Hyper-osmolar citrate is by far the cheaper of the two fluids, costing approximately US$15 per litre, whereas UW costs approximately US$190 per litre. The two fluids differ considerably in their composition, Table 1.6 (230).

University of Wisconsin Solution and Hyper-osmolar citrate have never been compared in an RCT, and in fact there is limited evidence comparing HOC to any other currently used preservation solution (230). The perception in the UK

is that outcomes with the relatively cheap HOC are good enough to justify its continued use for kidney preservation. The changing donor population now required to meet the demands of waiting lists means that it may now be wise to re-examine the effectiveness of HOC preservation. The national transplant registry in the UK is maintained by National Health Service Blood and Transplant (NHSBT). It is a legal requirement for all transplant centres in the UK to report all kidney transplants to this registry. The aim of this study was to assess the outcomes of renal allografts preserved with HOC in comparison to those preserved with UW in the UK.

4.2 Methods

The proposal for this study was reviewed by the Kidney Advisory Group of NHSBT and approved by them before providing anonymised data. The transplant registry did not contain all relevant information regarding preservation protocols, so this information was requested from each retrieval and renal transplant team in the UK. Responses were subsequently returned for all renal transplant centres. Centres were asked to clarify the fluid used for the initial aortic flush in the donor, flushing of the kidney on the back table before packing, and the fluid used to store the kidney. Information regarding differences in perfusion protocol for kidney-only and multi-organ donors was requested. The primary purpose of the study was to assess the relationship between the preservation fluid and transplant outcomes when adjusted for confounding variables.

4.2.1 Research question

What are the outcomes from renal allografts preserved with Hyper-osmolar citrate in the United Kingdom? How do these outcomes compare to kidneys preserved with UW Solution?

4.2.2 **Study population**

Data regarding deceased donor kidney transplants performed during the period January 1st 2005 to December 31st 2008 was requested. Information for transplants carried out during this period was selected to allow for at least three years follow up for all recipients.

4.2.3 **Outcomes**

The primary outcomes were PNF (never-functioning graft) and DGF (the requirement for dialysis within the first week after transplantation) as reported to NHSBT by each transplant centre. Survival outcomes assessed were: graft survival (death censored), patient survival and transplant survival (transplant loss includes death with a functioning graft). Renal function was assessed by serum creatinine at one year after transplantation. Acute rejection episodes were reported to NHSBT by each transplant centre.

4.2.4 **Statistical analysis**

Data was analysed using the statistical programme Stata® version 12 (*Statacorp LP, Texas, USA*). In univariate analysis, binary variables were assessed with Chi-Squared Test, continuous variables with Student's T-Test and survival outcomes with log rank test. Relationships between two continuous variables were assessed with linear regression. Multivariate logistic and linear regression models were fitted in a stepwise fashion to analyse relationships between donor, recipient and transplant factors and transplant outcomes. Cox's proportional hazards regression models were fitted to analyse the combined effect of variables on survival outcomes. The multivariate analysis included only patients with complete data on variables that were selected on the basis of univariate analysis, or prior analyses which have indicated associations.

Kaplan-Meier curves are used to demonstrate survival outcomes. A p-value<0.05 was taken to indicate statistical significance.

4.3 Results

5,027 kidney transplants were performed during the study period, of whom 3,838 (76.35%) received kidneys from DBD and 1,189 from DCD (23.65%), Table 4.1. The number of kidney transplants performed each year showed a steady increase during the inclusion period, from 1,187 in 2005, to 1,369 in 2008.

4.3.1 Donor factors

Mean age of donors was 45.48 years and the age range of donors was wide (range 1-82 years, Figure 4.1). There were more male than female donors (approximately 53% versus 46%). Donor ethnicity was homogenous, with over 96% white ethnicity; the next largest group was Asian, making up approximately 1%, Table 4.2. For the purposes of data analysis, both donor and recipient ethnicity were therefore assessed as either "white" or "non-white". A liver was donated as well as kidneys in approximately 77% of cases and a pancreas also in approximately 29% of cases, Table 4.1. The most common cause of death was intracranial haemorrhage (approximately 60%), Table 4.3. Trauma of several types made up the next largest group (approximately 15%). Hypertension was a pre-existing comorbidity in 973 donors (approximately 22%).

Table 4.1. Donor demographics. % is of all transplants with available data for each characteristic. Continuous outcomes are presented as mean+/-standard deviation (range in brackets). DBD= donation after brain-death, DCD= donation after circulatory death.

Donor Factor		
Age (years)	45.27+/- 15.85 (1-82)	
Terminal creatinine (µmol/l)	87.62+/-46.16 (5-702)	
Gender	**Male**	2,705 (53.81%)
	Female	2,322 (46.19%)
Liver donated	**Yes**	3,880 (77.18%)
	No	1,147 (22.81%)
Pancreas donated	**Yes**	1,465 (29.14%)
	No	3,562 (70.86%)
Hypertension	**Yes**	977 (19.44%)
	No	3,452 (68.67%)
	Unknown	598 (11.9%)
Donor type	**DBD**	3,838 (76.35%)
	DCD	1,189 (23.65%)

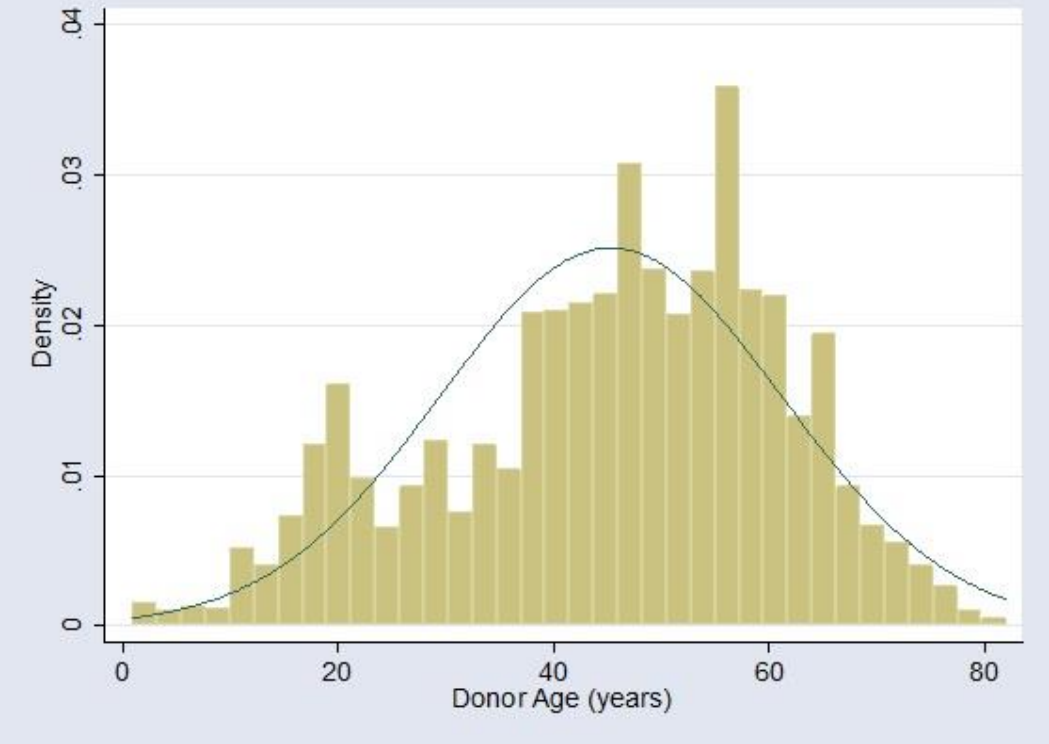
Table 4.2. Donor and recipient ethnicity. % is of all donors or all recipients with information on ethnicity.

Ethnicity	**Donor**		**Recipient**	
	Number	**%**	**Number**	**%**
White	4,869	96.9	4,061	80.78
Asian	68	1.35	553	11
Black	44	0.88	306	6.09
Mixed	24	0.48	9	0.18
Oriental	11	0.22	43	0.86
Other	9	0.18	49	0.97
Unknown	2	0.04	6	0.12%

Table 4.3. Cause of donor death for included transplants. % is of all transplants included. RTA= road traffic accident.

Cause of donor death	Number	%
Intracranial haemorrhage	3,011	59.9
Intracranial thrombosis	75	1.49
Brain tumour	68	1.35
Hypoxic brain damage	472	9.39
Intracranial unclassified	125	2.49
Trauma-RTA-car	206	4.1
Trauma-RTA-motorbike	69	1.37
Trauma-RTA-pushbike	48	0.95
Trauma-RTA-pedestrian	127	2.53
Trauma-RTA-unknown	33	0.66
Trauma-suicide	30	0.6
Trauma-accident	193	3.84
Trauma-unknown	39	0.78
Cardiac arrest	49	0.97
Myocardial infarction	9	0.18
Aneurysm	7	0.14
Congestive cardiac failure	2	0.04
Pulmonary embolism	3	0.06
Cardiovascular-unclassified	9	0.18
Pneumonia	4	0.08
Asthma	21	0.42
Carbon monoxide poisoning	3	0.06
Respiratory-unclassified	2	0.04
Meningitis	105	2.09
Septicaemia	5	0.1
Infections-unclassified	5	0.1
Liver failure- not self-poisoning	2	0.04
Multi-organ failure	6	0.12
Paracetamol overdose	1	0.02
Other drug overdose	10	0.2
Self-poisoning-unclassified	2	0.04
Not reported	24	0.48
Other	17	0.34
Unknown	245	4.83

Figure 4.1. Histogram of donor age (years) with superimposed normal distribution. Mean 45 years, standard deviation 16 years, range 1-82 years.



4.3.2 **Retrieval and transplant centres**

Retrieval and transplant activity was spread across all renal transplant centres in the UK during the inclusion period, Table 4.4. The eight most active retrieval teams were responsible for the retrieval of approximately 80% of the included kidneys. The study inclusion period started before the initiation of the multi-organ National Organ Retrieval Service (NORS) in 2010, which requires dedicated organ retrieval teams to be on call 24 hours per day. This has drastically cut the number of teams involved in abdominal organ retrieval to 10 (Birmingham, Cambridge, Cardiff, King's College London, Leeds, Manchester, Newcastle, Oxford, Royal Free, Scotland).

Table 4.4. Numbers of kidneys retrieved and transplanted by each renal transplant centre in the United Kingdom during the inclusion period January 1st 2005 to December 31st 2008. Order by number of kidneys retrieved during the inclusion period. Data on kidneys retrieved in the United Kingdom, but transplanted outside the United Kingdom, is not available (Dublin and Overseas).

Hospital/Renal Unit	Kidneys Retrieved	Kidneys Transplanted
Guy's-Kings-St Thomas'	775	229
Birmingham	665	310
Cambridge	580	294
Oxford	466	223
Leeds	450	431
Newcastle	394	277
Royal Free	345	196
Manchester	344	411
Edinburgh	303	179
Cardiff	125	222
Plymouth	103	173
Bristol	98	274
Glasgow	80	223
Portsmouth	64	166
Liverpool	48	191
West London	46	213
St George's	22	201
Leicester	21	103
Sheffield	18	140
Royal London	17	183
Dublin	15	-
Nottingham	8	128
Belfast	8	120
Overseas	2	-
Coventry	0	62
Great Ormond Street	0	48

4.3.3 **Preservation and transplant factors**

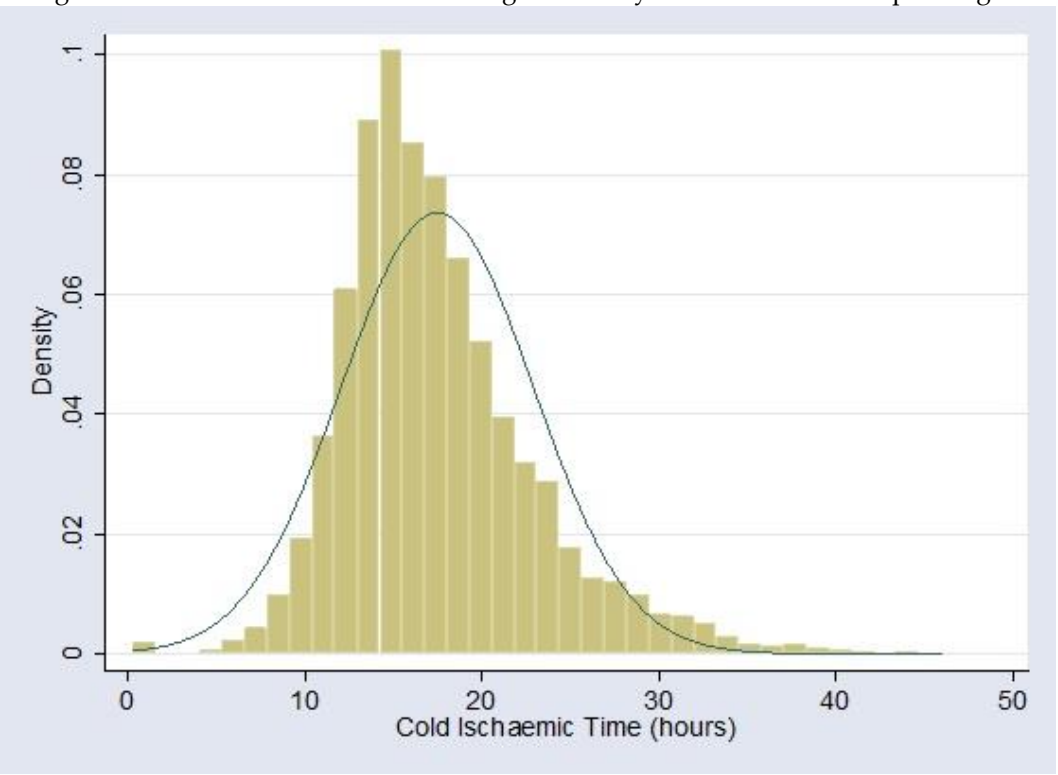
The mean CIT was 17.59 hours (range 0.3-166.7 hours), Table 4.5, Figure 4.2. Cold ischaemic time is calculated by the transplanting centre from the time of initial cold flush in the donor until removing the kidney from ice in the transplanting theatre; NHSBT receive a calculated value from the transplanting centre. Thirty kidneys (0.6%) had cold ischaemic time recorded as 166 hours; this was treated as missing data and an entry in error, so these kidneys were removed from the analysis. The majority of kidneys were static cold stored (approximately 85%) and a small number (approximately 6%) were machine

perfused. If the preservation method was unknown (machine perfusion versus static cold storage) kidneys were treated as having had static cold storage, this being the predominant method of preservation in the UK at the time. Kidneys known to be machine perfused were excluded from the analysis. The majority of transplants were first grafts (approximately 85%) and some were second grafts (approximately 12%). Very few were third or fourth grafts (approximately 2% and <1% respectively). Calculated Reaction Frequency (CRF) is the percentage of potential donors against which the recipient is sensitized. Currently in the UK, CRF is calculated using the last 10,000 blood group identical donors. CRF was split into quintiles for this analysis. All graft numbers were used for univariate analysis, but first grafts only were used for multivariate analysis.

Table 4.5. Preservation and transplant factors. % is of all transplants with available data for each demographic. HLA=human leucocyte antigen. HLA mismatch level as described by Johnson et al 2010 (231). Continuous outcomes are presented as mean+/-standard deviation (range in brackets).

Factor		Number (%)
Cold ischaemic time (hours)		17.49+/-5.41 (0.3-46)
Machine perfusion	**Yes**	317 (6.31%)
	No	4,257 (84.68%)
	Unknown	453 (9.01%)
HLA mismatch level	**1 (000)**	817 (16.25%)
	2 (0 DR and 0/1B)	1,665 (33.11%)
	3 (0 DR and 2 B or 1 DR and 0/1 B	2,017 (40.02%)
	4 (1 DR and 2 B or 2 DR)	528 (10.50%)
Graft number	**1st**	4,278 (85.10%)
	2nd	623 (12.39%)
	3rd	110 (2.19%)
	4th	16 (0.32%)

Figure 4.2. Histogram of cold ischaemic time (hours) for included kidneys, with superimposed normal distribution. Cold ischaemic time presented is as reported to NHSBT by the transplanting centre. The transplanting centre calculates this from the time between initiating cold flush in the donor and removing the kidney from ice in the transplanting theatre.



4.3.4 **Preservation protocols**

Each transplant centre reported on their retrieval practice during the period of interest. Responses were received from every renal transplant centre in the United Kingdom, Table 4.6. Preservation protocols were then matched to kidneys in the database according to the retrieval team. All teams reported that they used either HOC and/or UW for static cold storage of kidneys during the inclusion period. Slightly more kidneys had initial aortic flush in the donor with HOC than with UW (approximately 52% versus 41%). A small number (n=299, 6.34%) had a mixture of UW and HOC in aortic flush due to a perfusion protocol for pancreas retrieval used when King's College Hospital was the multi-organ retrieval team, Table 4.6, Table 4.7. This protocol used one litre of UW solution initially, then the superior mesenteric artery was clamped and further aortic perfusion was done with HOC. For flush on the back-table and

storage of kidneys, HOC was used far more than UW Solution (approximately 79% versus approximately 20%). For the vast majority of kidneys (over 96%) the fluid used for the back-table and storage were the same. The only protocol describing a mixture of different back-table flush and storage fluids was the Plymouth protocol, which used UW for kidney flushing of multi-organ donors, before storage in a bag of HOC. Given mixing of aortic flush fluids, and mixing where the aortic flush differed from the packing fluid, a total of 1,263 kidneys (approximately 28%) had mixing of fluids at some stage during the preservation period, Table 4.7.

Table 4.6. Preservation protocols for renal transplant and retrieval teams in the United Kingdom 2005-2008. For centres that did not do multi-organ retrieval, the protocol for the relevant multi-organ team has been used. HOC=Hyper-osmolar Citrate, Marshall's Solution, NA= Not Applicable, UW= University of Wisconsin Solution.

		Aortic flush		Kidney storage	
Team	**Donor**	**Kidney only**	**Multi-organ**	**Kidney only**	**Multi-organ**
Belfast		HOC	UW	HOC	HOC
Birmingham		HOC	HOC	HOC	HOC
Bristol		HOC	HOC[8]	HOC	HOC
Cambridge	**DBD**	HOC	HOC[9]	HOC	HOC[9]
	DCD	UW	UW	UW	UW
Cardiff	**DBD**	HOC	HOC[8]	HOC	HOC
	DCD	UW	HOC[8]	UW	HOC
Coventry		HOC	HOC	HOC	HOC
Edinburgh		UW	UW	UW	UW
Glasgow		HOC	NA	HOC	NA
Guys		HOC	HOC[8]	HOC	HOC
Imperial		UW	UW	UW	UW
King's		HOC	HOC[8]	HOC	HOC
Leeds	**DBD**	HOC	HOC[9]	HOC	HOC
	DCD	UW	UW	UW	UW
Leicester		HOC	HOC	HOC	HOC
Liverpool		HOC	HOC	HOC	HOC
Manchester	**DBD**	HOC	HOC[9]	HOC	HOC[9]
	DCD	UW	UW	UW	UW
Newcastle		HOC	UW	HOC	HOC
Nottingham		HOC	NA	HOC	NA
Oxford		HOC	UW	HOC	HOC
Plymouth		UW	HOC[8]	HOC	HOC
Portsmouth	**DBD**	HOC	HOC[8]	HOC	HOC
	DCD	HOC	HOC[8]	UW	UW
Royal Free		HOC	UW[10]	HOC	HOC
Royal London		HOC	UW[10]	HOC	HOC
Sheffield		HOC	NA	HOC	NA
St Georges	**DBD**	HOC	HOC	HOC	HOC

[8] If a pancreas donor then 1 litre aortic flush using UW, then the SMA was clamped and further flush with HOC continued.
[9] UW if pancreas donor.
[10] If dual flush of liver, would give aortic HOC and portal UW, if single flushing just UW aortic.

Table 4.7. Summary of preservation fluids used for kidneys during the inclusion period.

	Hyper-osmolar Citrate Solution	University of Wisconsin Solution	Mixture of both Solutions
Aortic flush	2,466 (52.30%)	1,950 (41.36%)	299 (6.34%)
Back-Table flush pre-storage	3,941 (79.15%)	1,038 (20.85%)	-
Kidney storage	3,788 (79.61%)	970 (20.39%)	-

4.3.5 **Recipient factors**

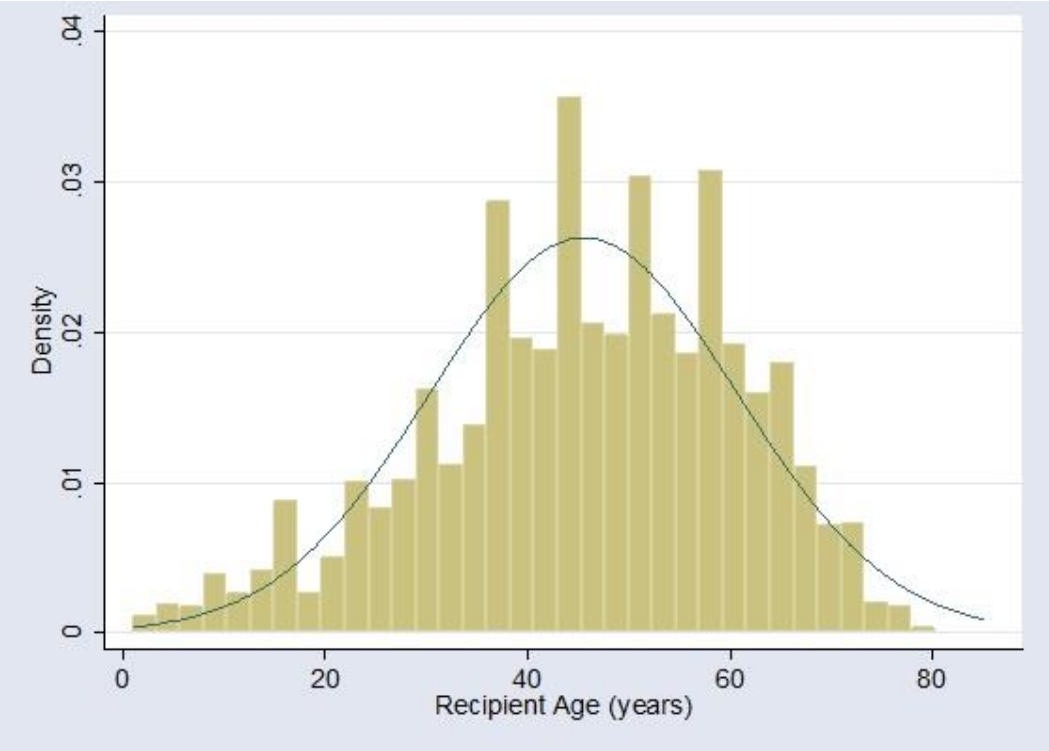
Average recipient age was the same as the average donor age, approximately 45 years (range 1-85 years). There were more male recipients than female recipients (approximately 61% versus 39%). Approximately 80% of recipients were white; the next largest group was Asian, approximately 11%, Table 4.2. For the purposes of data analysis, recipient ethnicity was assessed as either "White" or "Non-white".

Immune suppression regimens were most commonly combinations of tacrolimus and mycophenolate mofetil. Prednisolone was prescribed for the majority of patients at the time of transplantation (82% approximately) but the withdrawal of steroids was not assessed. Azathioprine was not prescribed for the majority of patients at the time of transplantation (18%). The available data did not have good information regarding induction antibodies as only anti-thymocyte globulin (2%) and muromonab (<1%) were recorded, Table 4.8.

Table 4.8. Recipient demographics and initial immune suppression. Initial immune suppression is the immune supressing medication prescribed at the time of transplantation. Continuous outcomes are presented as mean+/-standard deviation (range in brackets).

Recipient Factor		
Age (years)		45.53+/-15.19 (1-85)
Gender	**Male**	3,063 (60.93%)
	Female	1,964 (39.07%)
Use of induction antibodies		
Anti-thymocyte globulin	**Yes**	96 (1.91%)
	No	4,902 (97.51%)
	Unknown	29 (0.58%)
Muromonab-CD3 (OKT3)	**Yes**	8 (0.16%)
	No	4,987 (99.20%)
	Unknown	32 (0.64%)
Initial immune suppression		
Prednisolone	**Yes**	4,101 (81.57%)
	No	906 (18.02%)
	Unknown	20 (0.99%)
Cyclosporine	**Yes**	1,103 (21.94%)
	No	3,901 (77.76%)
	Unknown	23 (0.46%)
Tacrolimus	**Yes**	3,739 (74.38%)
	No	1,267 (25.20%)
	Unknown	21 (0.42%)
Azathioprine	**Yes**	905 (18.00%)
	No	4,098 (81.54%)
	Unknown	24 (0.48%)
Mycophenolate mofetil	**Yes**	3,357 (66.77%)
	No	1,648 (32.78%)
	Unknown	22 (0.44%)

Figure 4.3. Histogram of recipient age (years) with superimposed normal distribution. Mean 45 years, standard deviation 15 years, range 1-85 years.



4.3.6 **Overall unadjusted outcomes**

Overall event rates for adverse outcomes and survival outcomes were within expected ranges (Table 4.9). Primary non-function rate was approximately 3% and DGF rate was approximately 28%. Overall one year graft survival (death censored), patient survival and transplant survival (transplant loss includes death with a functioning graft) were 96%, 97% and 94% respectively. Further exploration of the data in univariate analysis is described in the next section.

Table 4.9. Outcomes and event rates, overall and split by donor type. Univariate analysis of data. Graft survival is death censored. Transplant Survival includes death with a functioning graft as a graft loss. Primary non-function, delayed graft function and acute rejection are as reported to the NHSBT by local centres. P-value is chi squared test for binary outcomes comparing donor types, t-test for continuous outcomes, and logrank test for survival analysis (using full length of follow up available). Follow up time for acute rejection episodes is mean 47+/-18 months. Values for serum creatinine are mean+/-standard deviation.

Outcome	Overall	DBD	DCD	P-Value
Primary non-function	146 (3.14%)	104 (2.92%)	42 (3.87%)	0.113
Delayed graft function	1,299 (27.94%)	798 (22.38%)	501 (46.22%)	<0.001
Acute rejection	874 (17.99%)	712 (19.13%)	162 (14.25%)	<0.001
Serum Creatinine 12 months post-transplant (μmol/L)	143+/- 61	141+/-57	148+/-71	0.006
Graft survival				
12 months	95.81%	95.45%	97.18%	0.151
24 months	93.88%	93.46%	95.44%	
36 months	91.96%	91.61%	93.24%	
Patient survival				
12 months	96.75%	96.73%	96.81%	0.925
24 months	94.84%	94.89%	94.69%	
36 months	92.77%	92.71%	90.78%	
Transplant survival				
12 months	93.71%	93.37%	94.97%	0.461
24 months	90.68%	90.39%	91.75%	
36 months	87.43%	87.14%	88.44%	

4.3.7 **Univariate analysis**

Hazard Ratios for recipient age and donor age are presented as the Hazard Ratio between two individuals one year apart in age. For a comparison between more distant age groups, the HR presented must be multiplied to the power of the number of year's difference. For example: if HR=1.01, the HR comparing an individual 40 years old to one 20 years old would be 1.01^{20}, or HR=1.23.

Variables and outcomes associated with preservation protocols

Compared to UW Solution, the use of HOC as aortic flush was associated with longer CIT (17.85+/-5.46 hours versus 16.97+/-5.36 hours, p<0.001), older donors (47.67+/-15.63 years versus 43.03+/-15.77 years, p<0.001), older recipients

(46.77+/-14.82 years versus 44.79+/-15.43 years, p<0.001), non-liver donors (p<0.001), non-pancreas donors (p<0.001), donor hypertension (25.55% versus 17.59%, p<0.001) and donation after brain-death (p<0.001).

The use of HOC as kidney storage fluid was also associated with longer CIT (17.63+/-5.46 hours versus 16.91+/- 5.31 hours, p<0.001), older donors (45.72+/-15.90 years versus 43.31+/-15.65 years, p<0.001), non-liver donors (p<0.001), non-pancreas donors (p<0.001), donor hypertension (23% versus 16%, p<0.001) and donation after brain-death (p<0.001). The use of HOC as kidney storage fluid was not associated with older recipients (p=0.134).

Mixing of preservation fluids at some stage during the preservation period was associated with younger donors (42.04+/-15.69 years versus 46.36+/-15.79 years, p<0.001), younger recipients (42.81+/-15.56 years versus 46.35+/-14.91 years, p<0.001), liver donors (p<0.001), pancreas donors (p<0.001), less donor hypertension (17% versus 26%, p<0.001), donation after brain-death (p<0.001) and non-white recipient ethnicity (23% versus 18%, p=0.001). Mixing preservation fluids was not associated with longer CIT (p=0.914).

Primary non-function rates and delayed graft function rates associated with the two preservation fluids in the included data are presented in Table 4.10. The use of UW for kidney storage was associated with a higher risk of DGF, potentially due to its greater use for DCD (37% versus 25%, p<0.001), and the use of HOC for aortic flush was associated with a higher risk of PNF, potentially due to a number of confounding factors (3% versus 2%, p=0.027), Table 4.10.

Table 4.10. Delayed graft function and primary non-function rates associated with different preservation fluids, univariate analysis. Graft survival is death censored, HOC=Hyper-osmolar citrate, UW=University of Wisconsin Solution. P-value from Chi Squared test for binary outcomes and logrank test for survival outcomes.

	Outcome	HOC	UW	P-Value
Aortic flush fluid	**DGF**	28.34%	29.88%	0.424
	PNF	3.54%	2.31%	0.027
	Graft survival			
	12 months	95.28%	96.63%	0.156
	24 months	93.53%	94.76%	
	36 months	91.64%	92.80%	
Kidney storage fluid	**DGF**	25.04%	36.87%	<0.001
	PNF	3.22%	2.41%	0.219
	Graft survival			
	12 months	95.34%	97.34%	0.007
	24 months	93.38%	95.52%	
	36 months	91.29%	94.25%	

Primary Non-function

Older donors (p<0.001) and older recipients (p=0.007) were associated with higher rates of PNF. Donor hypertension was associated with higher PNF rate (4.6% versus 2.72%, p=0.003), as were male recipients (3.6% versus 2.4%, p=0.030) and non-white recipient ethnicity (4.3% versus 2.9%, p=0.030). Cold ischaemic time over 18 hours was associated with an increasing risk of PNF (3.76% versus 2.7%, p=0.049), however longer CIT was not associated with increasing PNF in a linear fashion (p=0.362). There was no association between donor sex (p=0.332), donor ethnicity (p=0.245), donor type (p=0.113) or donor terminal creatinine with PNF (p=0.360).

Delayed graft function

Older donors (p<0.001) and older recipients (p<0.001) were associated with higher rates of DGF. Longer cold ischaemic time was associated with an increasing risk of DGF (p=0.045), however CIT over 18 hours was not associated with an increased risk compared to less than 18 hours (p=0.189). Donation after circulatory death was associated with higher DGF rate (46% versus 22%,

p<0.001). Male donors (31% versus 24%, p<0.001) and male recipients were also associated with higher risk of DGF (30% versus 25%, p=0.002). Donor hypertension (p<0.001) and higher donor terminal creatinine were associated with higher risk of DGF (p<0.001). Donor and recipient ethnicity were not associated with DGF (p=0.277 and p=0.658 respectively).

Serum creatinine at 12 months

Serum creatinine at 12 months after transplantation displayed a positively skewed distribution, Figure 4.7, so this data was log transformed for analysis, as this showed the most normal distribution. Older donors and older recipients were associated with higher serum creatinine at 12 months (both P<0.001, Figure 4.4, Figure 4.5, Table 4.11). Increasing donor age was more important than increasing recipient age, but both factors showed very low coefficients that are not clinically relevant across the current age spectra (Coefficient 1.008 versus 1.004). Female donors were associated with higher serum creatinine at 12 months (p<0.001), as were male recipients (p<0.001) and donor hypertension (p<0.001) White recipient ethnicity was associated with higher serum creatinine at 12 months (p<0.001), but donor ethnicity was not significantly associated with serum creatinine at 12 months (p=0.115). Higher donor terminal creatinine was associated with higher serum creatinine at 12 months, although it was very weakly predictive (Coefficient 1.029, p=0.037, Figure 4.6). Donation after circulatory death was associated with higher serum creatinine at 12 months (p<0.001). There was no significant relationship between cold ischaemic time and serum creatinine at 12 months (p=0.302), Table 4.11. Average serum creatinine at 12 months was much higher than donor terminal creatinine on average (143 μmol/L versus 88 μmol/L).

Figure 4.4. Relationship between log of serum creatinine at 12 months (log(serum12) or lserum12) and donor age (years). Red line is line of best fit by method of least squares.

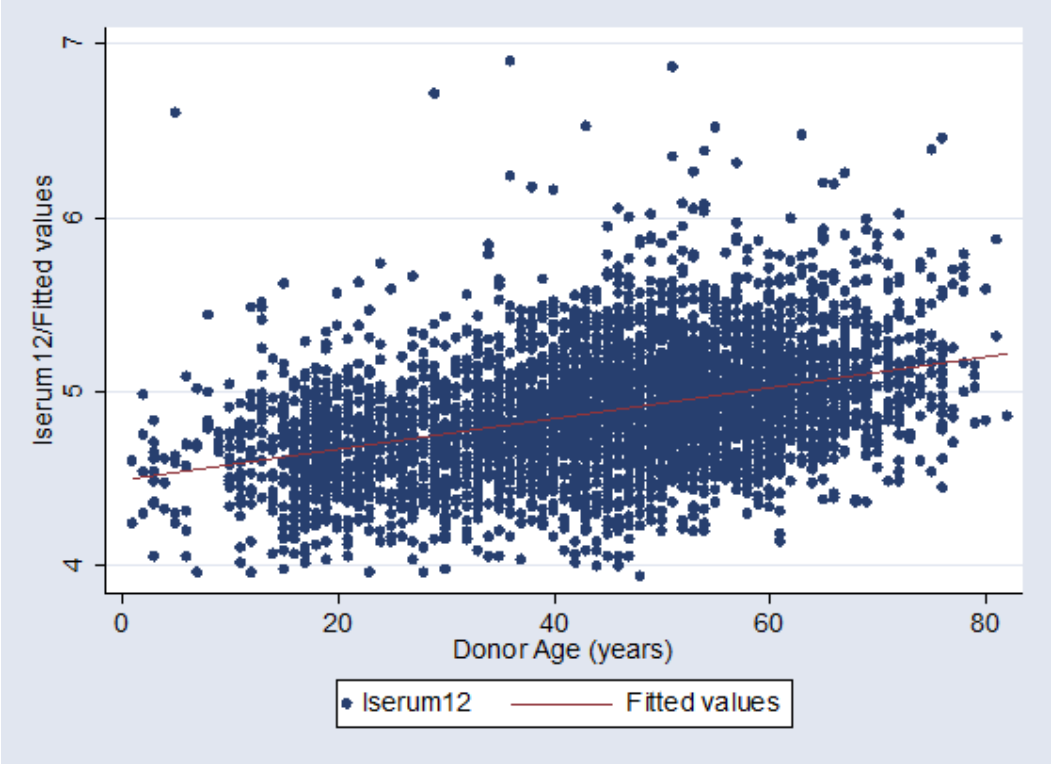


Figure 4.5. Relationship between log of serum creatinine at 12 months (log(serum12) or lserum12) and recipient age (years). Paediatric recipients demonstrated a non-linear relationship, and were removed from the analysis. Red line is line of best fit by method of least squares.

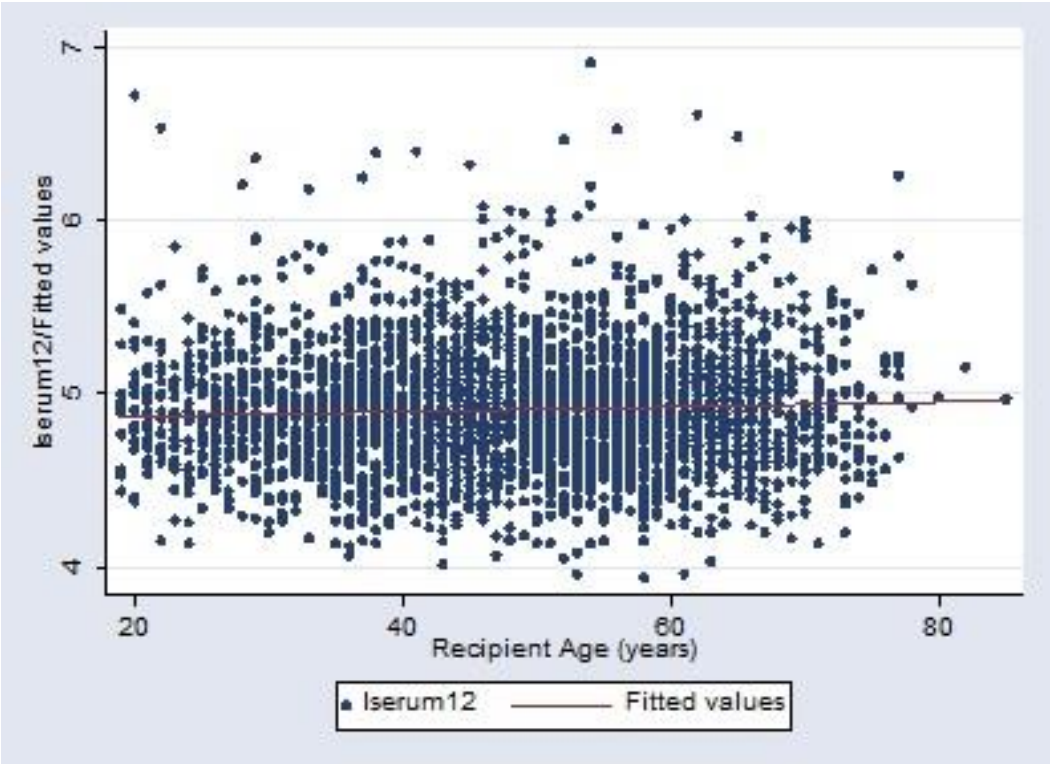


Figure 4.6. Relationship between log serum creatinine at 12 months after transplantation (lserum12) and log donor terminal creatinine (ltercreat). Red line is line of best fit by method of least squares.

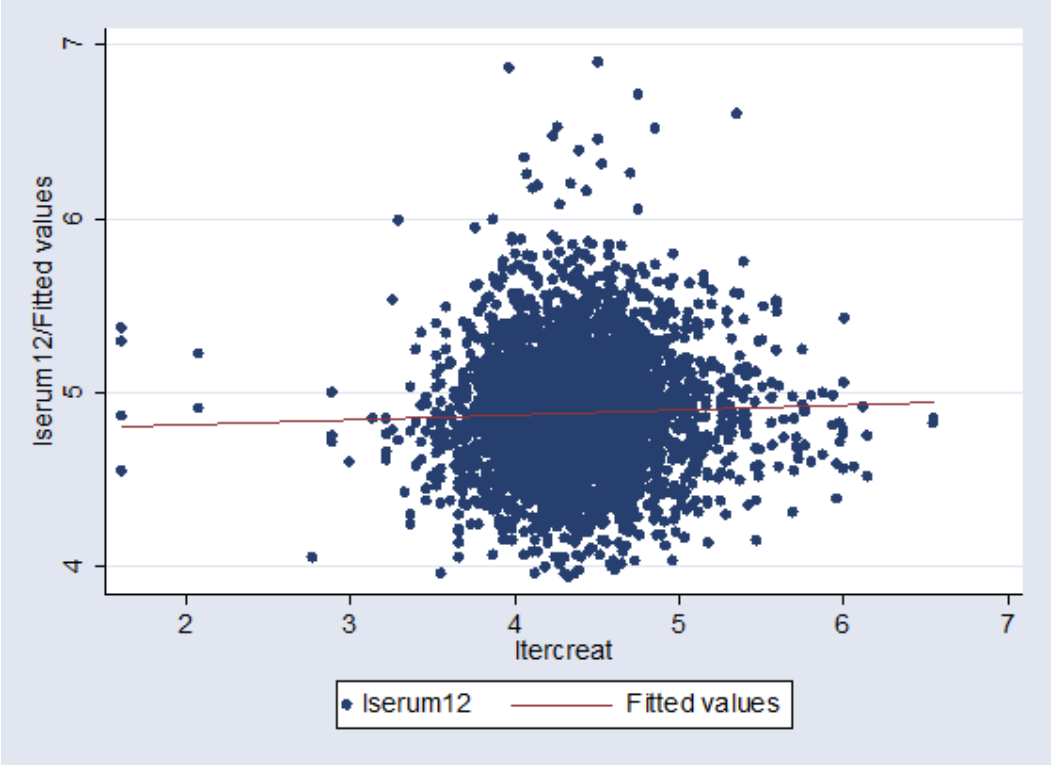
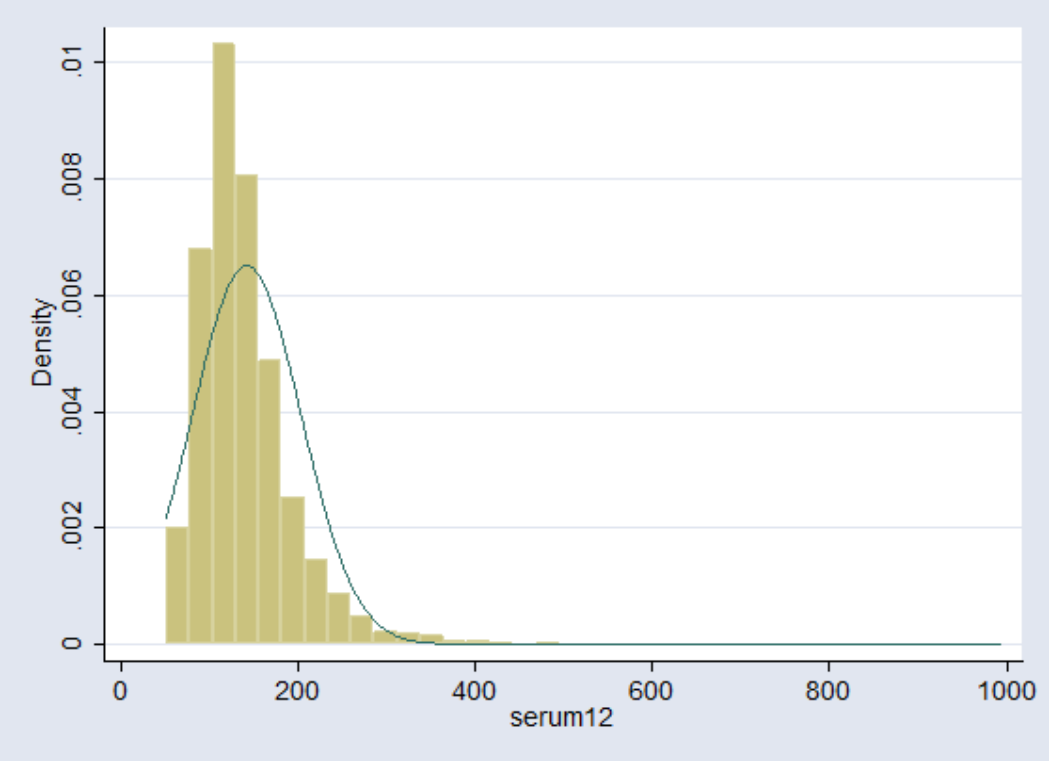


Table 4.11. Factors significantly associated with higher serum creatinine at 12 months after transplantation. Presented as mean+/- standard deviation, units are μmol/l. P-Value from Student's T-test for two-group comparisons and linear regression for coefficients.

Factor	Factor present	Factor not present	P-value
Female donor	149+/-67	139+/-56	<0.001
Male recipient	155+/-65	126+/-51	<0.001
White recipient	146+/-63	136+/-55	<0.001
Donation after circulatory death	148+/-71	141+/-57	0.006
Donor hypertension	157+/-59	139+/-63	<0.001
Higher donor terminal creatinine	Coefficient=1.029		0.037
Older donor	Coefficient=1.008		<0.001
Older recipient	Coefficient=1.004		<0.001

Figure 4.7. Serum creatinine at 12 months as reported to NHSBT. Superimposed normal distribution demonstrates this outcome is positively skewed.



Acute rejection

Acute rejection episodes throughout the follow up period were reported to NHSBT by local centres. The mean follow up was 47 months (standard deviation 18 months). Younger recipients (p<0.001), donation after brain-death (19% versus 14%, p<0.001) and donor hypertension (21% versus 18%, p=0.031) were all associated with higher acute rejection rate. Higher HLA mismatch level was associated with increasing risk of acute rejection. Acute rejection rates at different levels were: Level 1, 15.29%; Level 2, 18.02%; Level 3, 18.41%; Level 4, 25.87% (p<0.001). Higher levels of CRF did not show a significant increase in acute rejection rate, although CRF>80% had the highest rate; Acute rejection rates: 16.48% if CRF 0-20%, 17.46% if CRF 21-40%, 17.86% if CRF 41-60%, 20.04% if CRF 61-80% and 21.79% if CRF >80% (p=0.365).

There was no significant association between donor age and acute rejection (p=0.060). There was no significant association between longer cold ischaemic

time and acute rejection rate (p=0.437). Donor sex and recipient sex were not associated with risk of acute rejection (p=0.230 and p=0.343 respectively). Donor and recipient ethnicity were not significantly associated with acute rejection (p=0.152 and p=0.798 respectively). Donor terminal creatinine was not associated with risk of acute rejection (p=0.685).

Patient survival

Overall patient survival was 97% at one year, 95% at two years and 93% at three years. In univariate analysis, patient survival was worse if associated with DGF (HR=1.30, 95%CI=1.03-1.64, p=0.049, Figure 4.8, Table 4.12), donor hypertension (HR=1.70, 95%CI=1.34-2.15, p<0.001), older donors (HR=1.02, 95%CI=1.02-1.03, p<0.001), or non-pancreas donors (HR=1.69, 95%CI=1.28-2.23, p<0.001). Worse patient survival was also associated with the use of azathioprine as initial therapy (HR=1.28, 95%CI=1.00-2.64, p=0.029, Figure 5.3), and older recipients (HR=1.07, 1.06-1.08, p<0.001).

Patient survival was not affected by donor sex (p=0.786), donor ethnicity (p=0.214), recipient sex (p=0.238), or recipient ethnicity (p=0.834). Patient survival was also not affected by donor type (p=0.925) or whether or not the liver was donated (p=0.157). Acute rejection episodes (p=0.492) and increasing CRF (p=0.342) were not associated with patient survival, however HLA mismatch level showed a trend in its association with patient survival (p=0.093). Donor terminal creatinine showed a trend in its association with patient survival in univariate analysis (p=0.068). Patient survival was not associated with prednisolone (p=0.119), cyclosporine (p=0.629), tacrolimus (p=0.722), or mycophenolate mofetil (p=0.925) as initial therapy. Patient survival was not affected by longer CIT (p=0.823). There was no association between patient survival and the choice of preservation fluid used for aortic flush (p=0.144) or kidney storage (p=0.988). There was also no association between patient survival and the mixing of preservation fluids (p=0.598).

Figure 4.8. Kaplan-Meier survival curve for patient survival, univariate analysis. Patients with delayed graft function are compared to those without delayed graft function (Hazard Ratio=1.30, 95% confidence interval=1.03-1.64, p=0.049). Analysis time in days post-transplant.

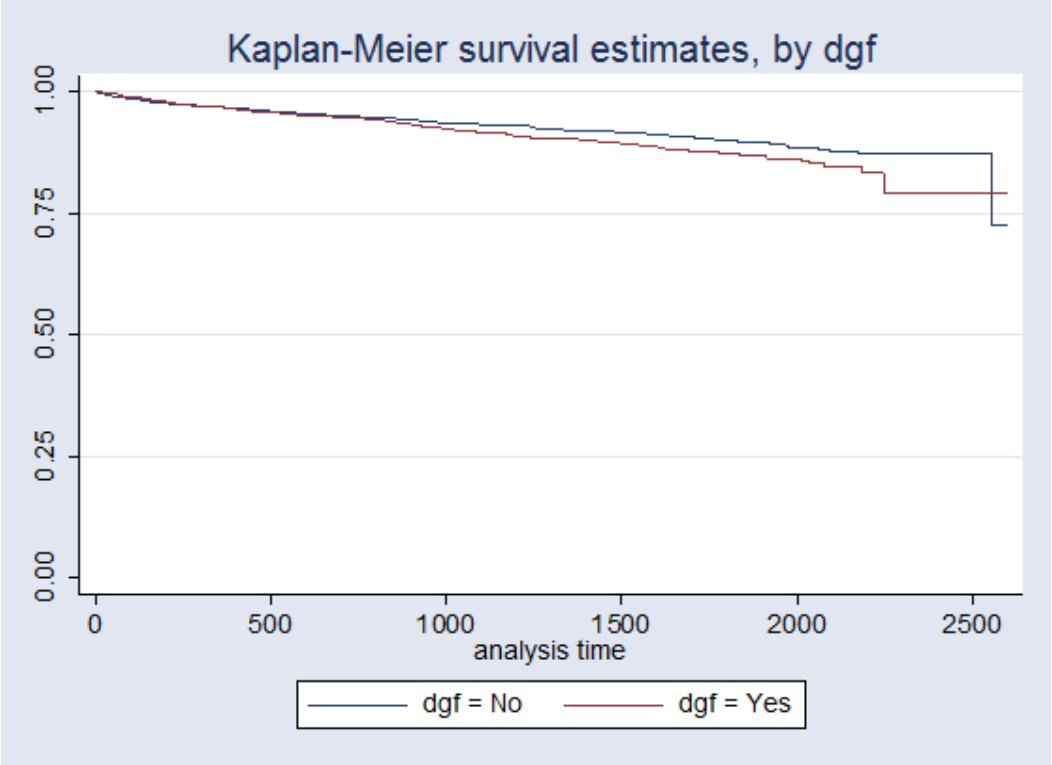
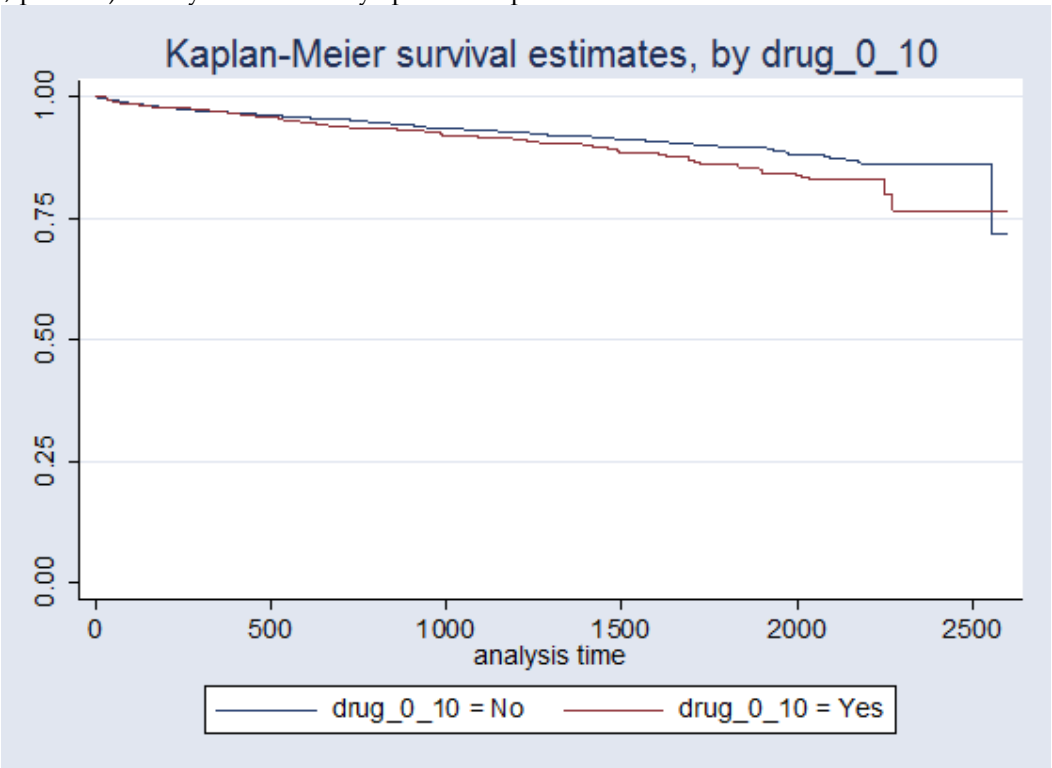


Figure 4.9. Kaplan-Meier survival curve for patient survival, univariate analysis. Patients receiving azathioprine are compared to those not receiving azathioprine as initial immune suppressive therapy after transplantation (Hazard Ratio=1.28, 95% confidence interval=1.00-1.64, p=0.029). Analysis time in days post-transplant.



Graft survival

Overall graft survival was 95% at one year, 94% at two years and 91% at three years. In univariate analysis worse graft survival was associated with DGF (HR=1.53, 95%CI=1.22-1.91, p<0.001, Figure 4.10, Table 4.12) and donor hypertension (HR=1.51, 95%CI=1.22-1.91, p<0.001). Worse graft survival was associated with increasing donor age (HR=1.02, 95%CI=1.01-1.02, p<0.001) but not increasing recipient age (p=0.272). Acute rejection was associated with worse graft survival (HR=1.88, p<0.001). HLA mismatch level was not significantly associated with worse graft survival in a linear fashion, although grafts with HLA mismatch level 4 displayed the worst survival figures (p=0.113, Figure 4.11).

Figure 4.10. Kaplan-Meier survival curve for graft survival, univariate analysis. Kidneys with delayed graft function are compared to those without delayed graft function (Hazard Ratio=1.53, 95% confidence interval=1.22-1.91, p<0.001). Analysis time in days post-transplant.

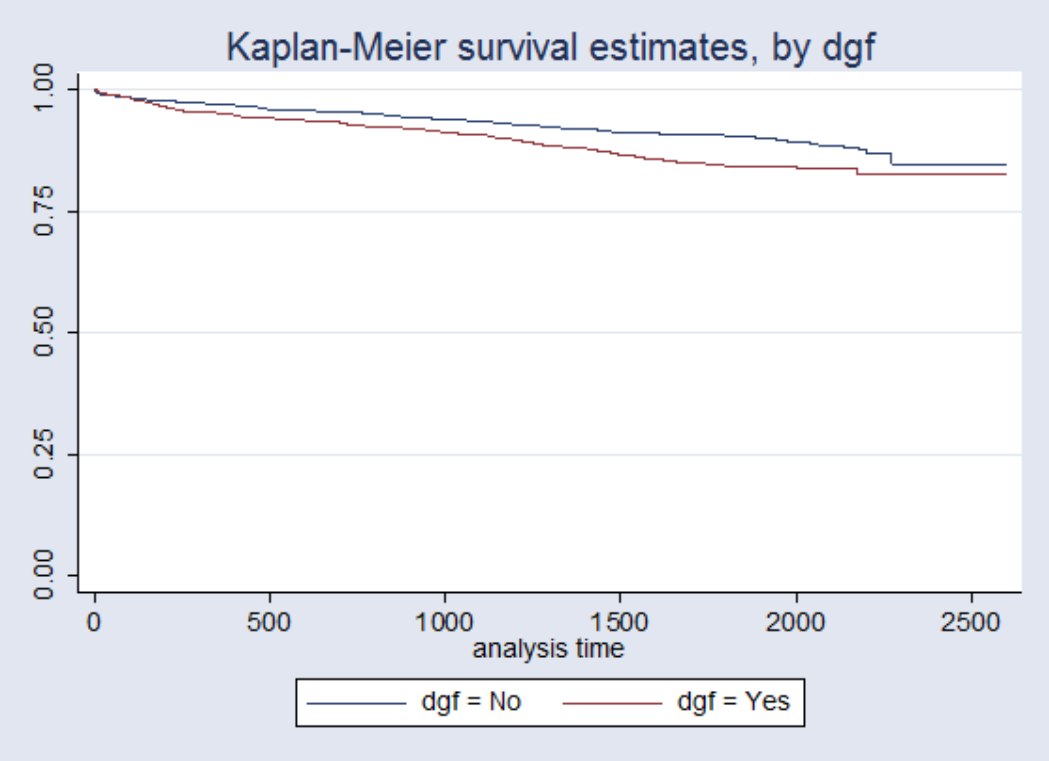
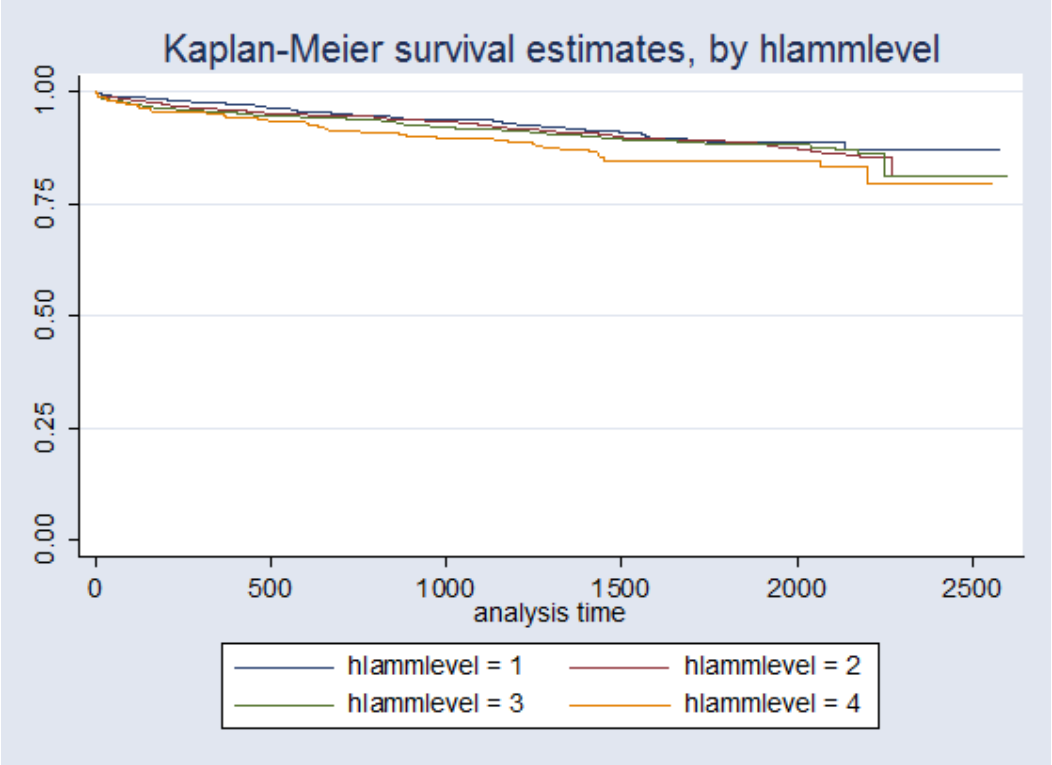


Figure 4.11. Kaplan-Meier survival curve for graft survival by different HLA mismatch level, univariate analysis. HLA mismatch levels as described by Johnson et al: Level 1= 000, Level 2= 0DR+0/1B mismatch, Level 3=0DR+2B mismatch or 1DR+0/1B mismatch and Level 4=2B+1DR mismatch or 2DR mismatch (231). Analysis time in days post-transplant (logrank p=0.113).



Graft survival was similar between kidneys from DBD and DCD (p=0.151). Graft survival was not associated with donor sex (p=0.595), recipient sex (p=0.088), donor ethnicity (p=0.145), or recipient ethnicity (p=0.905). Graft survival was not associated with pancreas donors (p=0.178) or liver donors (p=0.643). Cold ischaemic time over 18 hours was not associated with worse graft survival (p=0.303). Cold ischaemic time as a linear variable was not associated with worse graft survival (p=0.703). Graft survival was not related to donor terminal creatinine (p=0.843).

Graft survival was not associated with the use of azathioprine (p=0.818), prednisolone (p=0.796), cyclosporine (p=0.080), tacrolimus (p=0.265) or mycophenolate mofetil (p=0.500) as initial immune suppression.

There was no association between graft survival and the choice of preservation fluid used for aortic flush (p=0.156). In univariate analysis, the use of HOC for

kidney storage was associated with worse graft loss than UW (HR=1.50, 95%CI=1.12-2.02, p=0.007, Figure 4.12). There was no association between graft survival and the mixing of preservation fluids (p=0.384).

Graft survival data were then split by donor type for analysis. Factors which were associated with worse graft survival in DBD but not in DCD were: DGF (p<0.001 versus p=0.185, Figure 4.13 and Figure 4.14), donor hypertension (p<0.001 versus p=0.406), increasing HLA mismatch level (p=0.027 versus p=0.853), cyclosporine (p=0.046 versus p=0.723) and HOC as kidney storage fluid (p=0.018 versus p=0.360).

Figure 4.12. Kaplan-Meier survival curve for graft survival with different preservation fluids used for kidney storage, univariate analysis. Kidneys stored with Marshall's Solution (HOC) are compared to those stored with University of Wisconsin Solution (UW) (Hazard Ratio=1.50 95% confidence interval=1.12-2.02, p=0.007). Analysis time in days post-transplant.

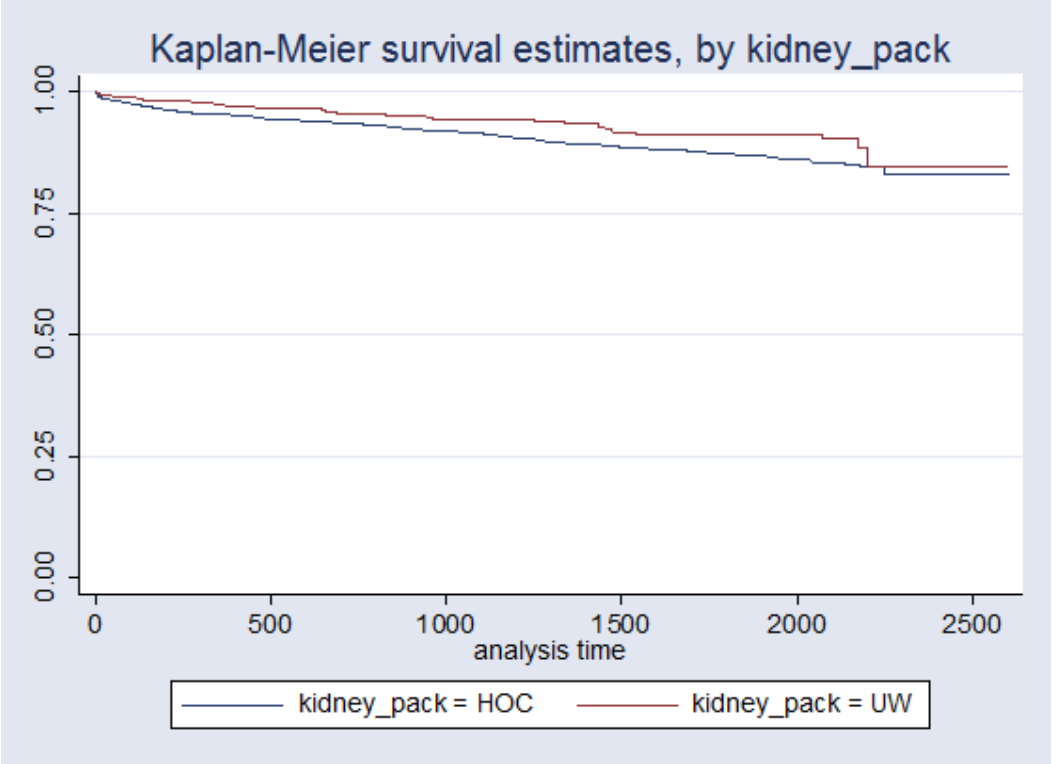


Figure 4.13. Kaplan-Meier survival curve for graft survival of DBD kidneys. Kidneys with DGF are compared to those without DGF (HR=2.01, 95% confidence interval=1.57-2.56, p<0.001). Analysis time in days post-transplant.

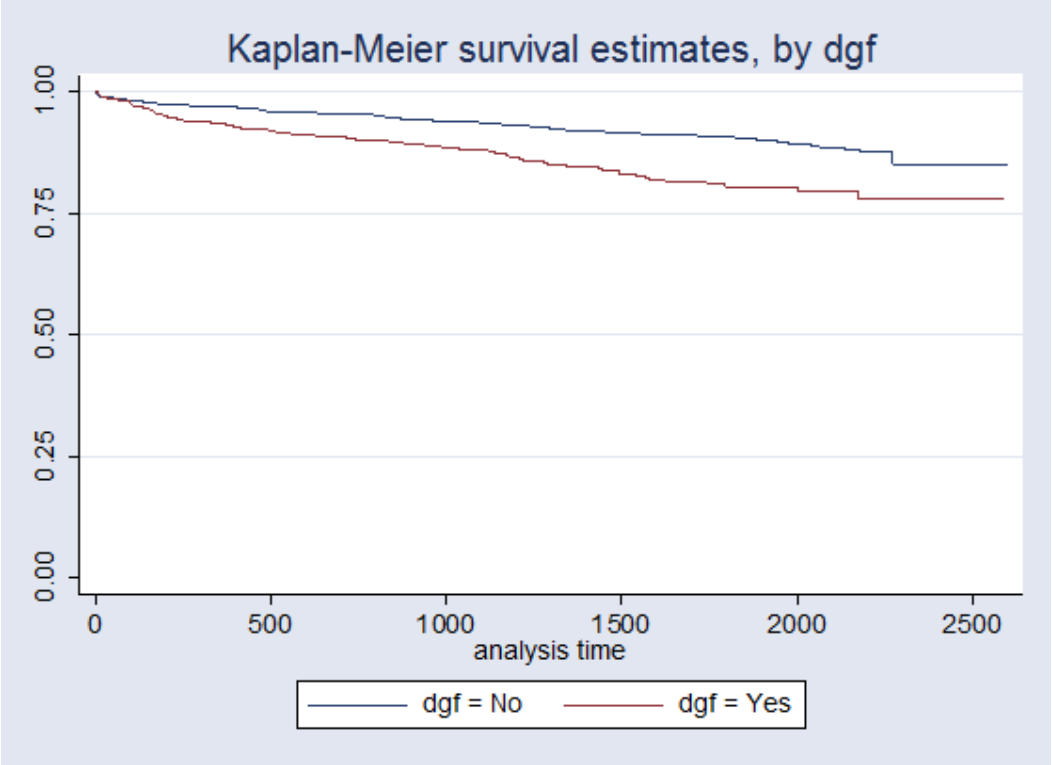
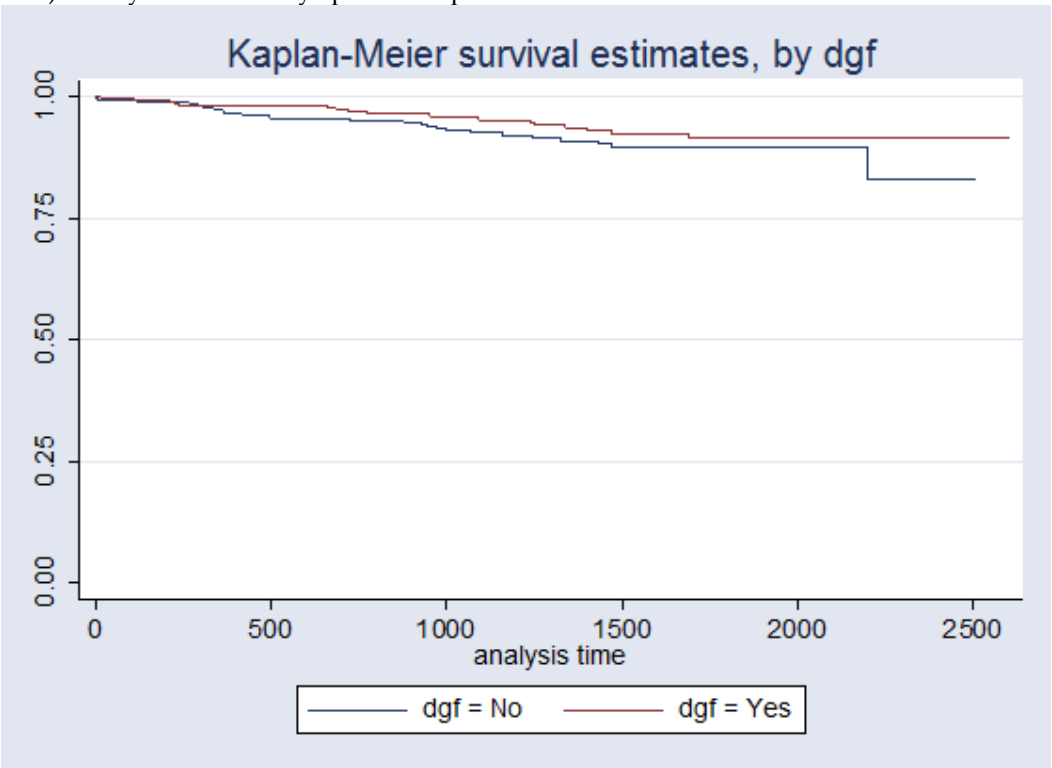


Figure 4.14. Kaplan-Meier survival curve for graft survival of DCD kidneys. Kidneys with DGF are compared to kidneys without DGF (HR=0.69, 95% confidence interval=0.39-1.16, p=0.154). Analysis time in days post-transplant.



Transplant survival

Overall transplant survival was at 94% one year, 91% at two years and 87% at three years. In univariate analysis worse transplant survival was associated with DGF (HR=1.47 95%CI=1.23-1.75, p<0.001, Table 4.12), donor hypertension (HR=1.49, 95%CI=1.24-1.79, p<0.001), non-pancreas donors (HR=1.24, 95%CI=1.03-1.49, p=0.020), acute rejection (HR=1.62, 95%CI=1.34-1.95, p<0.001), increasing donor age (HR=1.02, 95%CI=1.01-1.02, p<0.001) and increasing recipient age (HR=1.02, 95%CI=1.01-1.02, p<0.001). Worse transplant survival was associated with cyclosporine as initial immune suppression (HR=1.24, 95%CI=1.04-1.47, p=0.017). Transplant survival was not associated with the use of azathioprine (p=0.380), prednisolone (p=0.478), tacrolimus (p=0.083) or mycophenolate mofetil (p=0.445) as initial immune suppression.

Transplant survival was not associated with donor sex (p=0.561), recipient sex (p=0.439), donor ethnicity (p=0.650), recipient ethnicity (p=0.643), liver donors (p=0.509), HLA mismatch level (p=0.172), CIT (p=0.790) or donor type (p=0.461)

There was no association between graft survival and the choice of preservation fluid used for aortic flush (p=0.093) or kidney packing (p=0.165). There was no association between transplant survival and the mixing of preservation fluids (p=0.903).

Transplant survival data were then split by donor type for analysis. Factors which were associated with worse transplant survival in DBD but not in DCD were: DGF (p<0.001 versus p=0.454), donor hypertension (p<0.001 versus p=0.538) and non-pancreas donors (p<0.001 versus p=0.772).

Table 4.12. Factors significantly associated with worse survival outcomes in univariate analysis. 95%CI= 95% confidence interval, HOC= Marshall's solution. P-values from logrank test.

Survival outcome	Factor	Hazard Ratio	95% CI	P-Value
Patient survival	Donor hypertension	1.70	1.34-2.15	<0.001
	Non-pancreas donor	1.69	1.28-2.23	<0.001
	Delayed graft function	1.30	1.03-1.64	0.049
	Azathioprine	1.28	1.00-1.64	0.029
	Older recipient[11]	1.07	1.06-1.08	<0.001
	Older donor[11]	1.02	1.02-1.03	<0.001
Graft survival	Acute rejection	1.88	1.49-2.37	<0.001
	Delayed graft function	1.53	1.22-191	<0.001
	Donor hypertension	1.51	1.22-1.91	<0.001
	HOC kidney packing	1.50	1.12-2.02	0.007
	Older donor[11]	1.02	1.01-1.02	<0.001
Transplant survival	Acute rejection	1.62	1.34-1.95	<0.001
	Donor hypertension	1.49	1.24-1.79	<0.001
	Delayed graft function	1.47	1.23-1.75	<0.001
	Non-pancreas donor	1.24	1.03-1.49	0.020
	Cyclosporine	1.24	1.04-1.47	0.017
	Older recipient[11]	1.02	1.01-1.02	<0.001
	Older donor[11]	1.02	1.01-1.02	<0.001

[11] Hazard Ratios for recipient age and donor age are presented as the Hazard Ratio between two individuals one year apart in age. For a comparison between more distant age groups, the HR presented must be multiplied to the power of the number of years difference. For example: if HR=1.01, the HR comparing an individual 40 years old to one 20 years old would be 1.0120, or HR=1.23

4.3.8 **Multivariate analysis**

Only adult recipients over 18 years of age and recipients of first grafts were used for multivariate analysis (n=3,475 kidneys).

Primary non-function

The risk of PNF was not associated with the choice of preservation fluid used for aortic flush (p=0.883), kidney storage (p=0.770) or the mixing of fluids (p=0.154). Factors associated with higher risk of PNF were: male recipients (OR=2.18, p=0.006) and older recipients (OR=1.02, p=0.047). Several factors showed a trend towards influencing PNF: CIT over 18 hours (OR=1.57, p=0.057), older donors (OR=1.02, p=0.054), non-white recipient ethnicity (OR=1.65, p=0.051) and donation after circulatory death (OR=1.73, p=0.051).

Delayed graft function

The risk of DGF was not associated with the choice of preservation fluid used for aortic flush (p=0.707), kidney storage (p=0.420) or mixing of fluids (p=0.112). Factors associated with higher risk of DGF were: older donors (OR=1.02, p<0.001), higher HLA mismatch level (OR=1.17, p=0.006), longer CIT (OR=2.06, p=0.010), higher donor terminal creatinine (p<0.001), male donors (OR=1.34, p=-0.005), donor hypertension (OR=1.35, p=0.009) and donation after circulatory death (OR=4.27, p<0.001).

Serum creatinine at 12 months

Serum creatinine at 12 months after transplantation was not associated with the choice of preservation fluid used for aortic flush (p=0.892), kidney storage (p=0.204) or mixing of fluids (p=0.606). Factors associated with higher serum creatinine at 12 months after transplantation were: younger recipients (p<0.001), older donors (p<0.001), female donors (p<0.001), male recipients (p<0.001),

white recipients (p<0.001), non-white donors (p<0.001), DGF (p<0.001), cyclosporine as initial immune suppression (p<0.001), acute rejection (p<0.001), donation after circulatory death (p=0.006). Prednisolone as initial immune suppression was associated with lower serum creatinine at 12 months (p=0.009). Higher donor terminal creatinine showed a trend in association with serum creatinine at 12 months (p=0.087).

Acute rejection

Acute rejection was not associated with the choice of preservation fluid used for aortic flush (p=0.794), kidney storage (p=0.300) or mixing of fluids (p=0.943). Factors associated with acute rejection were older donors (OR=1.02, p<0.001), younger recipients (OR=1.02, p<0.001), and CRF>80% versus CRF<20% (OR=1.87, p=0.005). Non-white donor ethnicity showed a trend towards a higher risk of acute rejection (OR=1.55, p=0.080). Reduced risk of rejection was associated with tacrolimus prescription as initial immune suppression (OR=0.55, p<0.001). The prescription of prednisolone as initial immune suppression showed a trend towards reducing acute rejection (OR=0.79, p=0.066). Increasing HLA mismatch was associated with increasing risk of acute rejection. Compared to a baseline risk of rejection with HLA mismatch Level 1: Level 2 OR=1.51, p=0.018; Level 3 OR=1.77, p=0.001; Level 4 OR=1.97, p=0.001.

Patient survival

Patient survival was not associated with the choice of preservation fluid used for aortic flush (p=0.506), kidney storage (p=0.740) or mixing of fluids (p=0.268). The only predictors of worse patient survival were older recipient age (HR=1.07, 95%CI=1.05-1.08, p<0.001) and donor hypertension (HR=1.35, 95%CI=1.00-1.80, p=0.045). Older donor age showed a trend towards worse patient survival (HR=1.01, 95%CI=1.00-1.02, p=0.076), Table 4.13. When data was split and assessed by donor type the two predictors of worse patient

survival following DCD transplantation were older recipient (HR=1.08 95%CI=1.05-1.12, P<0.001) and mixing of preservation fluids (HR=4.10, 95%CI=1.91-8.79, p<0.001). It should be noted that only 148 DCD kidneys had mixing of preservation fluids. Following DBD transplantation the predictors of worse patient survival were donor hypertension (HR=1.44, 95%CI=1.08-1.93, p=0.013) and older recipient (HR=1.06, 95%CI=1.05-1.08, p<0.001). Two factors showed a trend towards worse patient survival following DBD transplantation: Azathioprine as initial immune suppression (HR=1.44, 95%CI=0.97-2.13, p=0.065) and DGF (HR=1.31, 95%CI=0.97-1.78, p=0.075).

Graft survival

Graft survival was assessed separately for DBD and DCD kidneys given the apparent difference in the association with DGF. For DBD kidneys graft survival was not influenced by the choice of preservation fluid for aortic flush (p=0.402), kidney storage (p=0.823) or mixing of fluids (p=0.255). Factors associated with worse graft survival of DBD kidneys were: DGF (HR=1.81, 95%CI=1.39-2.40, p<0.001), acute rejection (HR=1.66, 95%CI=1.25-2.21, p=0.001), HLA mismatch level 4 (HR=1.66, 95%CI=1.15-2.40, p=0.007) and older donors (HR=1.01, 95%CI=1.00-1.02, p=0.005). Two factors showed a trend towards worse DBD graft survival: donor hypertension (HR=1.30, 95%CI=0.97-1.75, p=0.078), female recipients (HR=1.26, 95%CI=0.97-1.64, p=0.073)

For DCD kidneys graft survival was not associated with the choice of preservation fluid for aortic flush (p=0.292) or for kidney storage (p=0.229). The mixing of preservation fluids showed a trend towards worse graft survival of DCD kidneys (HR=1.98, 95%CI=0.95-4.14, p=0.070). Other predictors of worse graft survival were acute rejection (HR=2.10, 95%CI=1.07-4.10, p=0.030) and female donors (HR=2.01, 95%=1.10-3.69, p=0.023, Table 4.13).

Transplant survival

Transplant survival was also assessed separately for DBD and DCD kidneys given the apparent difference in the association with DGF. For DBD kidneys transplant survival was not associated with the choice of preservation fluid for aortic flush (p=0.811), kidney storage (p=0.595) or mixing of preservation fluids (p=0.685). Factors associated with worse transplant survival of DBD kidneys were: DGF (HR=1.57, 95%CI=1.25-1.97, p<0.001), acute rejection (HR=1.49, 95%CI=1.17-1.91, p=0.001), donor hypertension (HR=1.28, 95%CI=1.01-1.64, p=0.042), older recipients (HR=1.02, 95%CI=1.01-1.03, p<0.001) and older donors (HR=1.01, 95%CI=1.00-1.02, p=0.012).

For DCD kidneys transplant survival was not associated with the choice of preservation fluid for aortic flush (p=0.393) or kidney storage (p=0.10). The mixing of preservation fluids was associated with worse graft survival (HR=2.39, 95%CI=1.47-3.90, p<0.001). Other factors associated with worse transplant survival of DCD kidneys were: acute rejection (HR=1.90, 95%CI=1.20-3.02, p=0.007) and older donors (HR=1.02, 95%CI=1.01-1.04, p=0.002, Table 4.13).

Table 4.13. Results of multivariate cox regression. Variables presented are those with p<0.1. 95%CI= 95% confidence interval. P-value from logrank test.

Survival outcome	Variable	Hazard Ratio	95% CI	P-Value
Patient survival (both donor types)	Donor hypertension	1.39	1.00-1.80	0.045
	Older recipient[12]	1.06	1.05-1.08	<0.001
	Older donor[12]	1.01	1.00-1.02	0.076
Patient survival, DBD kidneys	Donor hypertension	1.44	1.08-1.93	0.013
	Azathioprine	1.44	0.977-2.13	0.065
	Delayed graft function	1.31	0.97-1.78	0.075
	Older recipient[12]	1.06	1.05-1.08	<0.001
Patient survival, DCD kidneys	Mixing of fluids	4.10	1.91	<0.001
	Older recipient[12]	1.08	1.05-1.12	<0.001
DBD Graft survival	Delayed graft function	1.81	1.39-2.27	<0.001
	Acute rejection	1.66	1.25-2.21	0.001
	HLA mismatch Level 4	1.66	1.15-2.40	0.007
	Donor hypertension	1.30	0.97-1.75	0.078
	Female recipient	1.26	0.97-1.64	0.073
	Older donor[12]	1.01	1.00-1.02	0.005
DCD Graft survival	Acute rejection	2.10	1.07-4.10	0.030
	Female donors	2.01	1.10-3.69	0.023
	Mixing of fluids	1.98	0.95-4.14	0.070
DBD Transplant survival	Delayed graft function	1.57	1.25-1.97	<0.001
	Acute rejection	1.49	1.17-1.91	0.001
	Donor hypertension	1.28	1.01-1.64	0.042
	Older recipient[12]	1.02	1.01-1.03	<0.001
	Older donor[12]	1.01	1.00-1.02	<0.001
DCD Transplant survival	Mixing of fluids	2.39	1.47-3.9	<0.001
	Acute rejection	1.90	1.20-3.02	0.007
	Older donor[12]	1.02	1.01-1.04	0.002

[12] Hazard Ratios for recipient age and donor age are presented as the Hazard Ratio between two individuals one year apart in age. For a comparison between more distant age groups, the HR presented must be multiplied to the power of the number of years difference. For example: if HR=1.01, the HR comparing an individual 40 years old to one 20 years old would be 1.0120, or HR=1.23.

4.4 Discussion

This study has analysed the outcomes of kidneys preserved by static cold storage in the United Kingdom over a four year period covering 2005-2008 inclusive. The analysis was based upon a robust data set with excellent levels of follow up information. Hyper-osmolar citrate has not been compared to other currently used preservation fluids in randomised controlled trials, so this data presents a unique opportunity (230). It is difficult to identify the results of kidneys preserved solely with HOC in published reports, so there is little good quality data regarding its efficacy. In 2000 Nicholson et al published a report comparing outcomes of live donor and deceased donor kidneys preserved with HOC in the UK; 301 deceased donor kidneys were included. The DGF rate was 21% for DBD and 84% for uncontrolled DCD, one year graft survival was 84% and 86% respectively (76). In a multicentre, multinational analysis, Opelz and Dohler found HOC had been used to preserve approximately 5% of kidneys in this voluntary database between 1990-2005 (56). The increasing risk of graft loss at longer CIT was worse with HOC than with UW in their analysis (56). Experimental studies have indicated that the more viscous UW Solution cools kidneys more slowly than HOC, but causes less tissue oedema and cellular injury seen on histological analysis than HOC (82).

In this study the use of HOC was associated with certain factors that may be related to worse renal transplant outcomes, including longer CIT, older donors, older recipients and donor hypertension. Some of these factors may be related to the apparent preference by some centres for UW when a liver and/or pancreas were retrieved and a preference for HOC for kidney only donors. A multivariate analysis accounting for these factors allowed an assessment of the influence of the preservation fluid alone. Hyper-osmolar citrate compared to UW Solution, as either aortic flush or kidney storage fluid, was not associated with an increased risk of PNF, DGF, acute rejection or higher serum creatinine

at 12 months. Hyper-osmolar citrate was associated with equivalent patient, graft and transplant survival.

Some centres mixed preservation fluids, either by mixing the aortic flush in pancreas donors (a relatively small number) or by packing kidneys with HOC after an aortic flush in the donor with UW. The biochemical effect of mixing these two fluids is unknown; however there was a trend towards worse DCD graft survival with mixing of fluids. Why the mixing of fluids should be associated with worse graft survival in DCD but not DBD is unclear. Potentially the association may not be causal, and may be related to an unknown confounder in DCD retrievals. The association between mixing fluids and survival was statistically significant with a higher Hazard Ratio for patient and transplant survival than graft survival, which suggests that there is an unknown confounding variable.

Graft survival in this analysis was found to be equivalent between DCD and DBD kidneys, in keeping with other recent analyses of renal transplant outcomes (34). The equal graft survival was apparent despite the much greater risk of DGF in DCD kidneys. This may be explained by the fact that in this study, DGF was associated with worse graft survival of DBD, but not DCD kidneys. Delayed graft function has been established as a risk factor for poor graft survival in several previous studies (206, 232-235), and related to poor renal function in others, if not directly to survival (236). However, these studies were based largely (if not entirely) on the transplantation of kidneys from DBD. The aetiology of DGF may be different in these two donor types, and therefore be of a different predictive value in each. Cytoprotective genes are activated in response to renal ischaemia (237, 238) and these may potentially also play a role in DCD kidneys that experience a longer period of warm ischaemia prior to retrieval. There are now several studies of DCD cohorts which have not found DGF to be a risk factor for graft loss (34, 239-242). Suggestions that the duration

of DGF may be related to DCD graft survival have so far been based on inconclusive studies (243).

The only preservation factor associated with higher risk of DGF and PNF was CIT. In this analysis CIT was not associated directly with worse graft survival and this may be due to covariance with DGF rate in DBD kidneys. It may also be due to the relatively short length of CIT in this study (mean 17 hours), although this is consistent with another recent analysis of UK data (244). Summers et al have recently shown that DCD kidneys are more sensitive to increasing CIT than DBD kidneys (244). Previous analyses of DBD kidney transplant outcomes in the UK showed no increase in risk of transplant failure up to 21 hours CIT (231). A threshold of 18 hours was used for this study as this length of CIT has been shown in a multicentre analysis to be a key threshold above which graft loss increases (56). A maximum of 18 hours CIT has recently been introduced as an audit standard for the transplantation of DBD kidneys in the UK.

There are several criticisms that may be directed at this study. The key limitation is the use of registry data in retrospect. The use of such data is subject to confounding variables influencing outcome that cannot be taken into account; however, many variables that may be related to transplant outcomes were adjusted for. Causation is therefore difficult to prove, and interpretation of the results requires a careful appraisal of the underlying science and the apparent associations shown in the data. The accuracy of the database also depends on the quality of the information fed back to the central co-ordinator from each transplant centre. The preservation protocols that have been reported are also descriptions of procedure in general and there may have been slight variations between surgeons from each centre.

It is particularly difficult to interpret the role of the prescribed drugs as the use of one regimen is associated with the non-prescription of an alternative and therefore a covariance is introduced. For example, the apparent benefit of

tacrolimus in reducing acute rejection is partly contributed to by its comparison with cyclosporine, which would typically have been the alternative drug during the inclusion period. The effect of immune suppressing drugs as a combination (for example triple therapy) was not assessed; data was not available on the use of the induction antibodies basiliximab and alemtuzumab, which are now commonly used in the UK. The role of immune suppression at the time of transplantation was assessed, and this will miss any associations with later changes in the regimen, such as steroid withdrawal.

One criticism may be that the transplant outcomes were not adjusted for the transplant/retrieval centres. However, this would have shown co-linearity with the preservation protocols and immune suppression regimens that were used, as these obviously varied by centre. The large number of centres included, 23, would have meant a division of numbers that would have drastically reduced the power of any statistical analysis.

The relationship between the level of HLA-mismatch and survival outcomes was not completely clear, potentially due to the inclusion of acute rejection as a variable. It may be that HLA-mismatch is associated with survival in all donor types, but via the increased rejection rate at increasing levels, and hence was removed from multivariate models as a covariate of acute rejection.

This work is based upon data from the UK, where the population, patient assessment and drug regimens are relatively standardised. This fact increases the applicability of these results to transplant programs in the UK. However, it affects the relevance of this work to national or supra-national renal transplant programs in other countries, where, for example, average CIT may be longer. The introduction of the NORS retrieval service since 2010 may also impact upon the standard of retrieval and preservation protocols in the UK.

Conclusion

In this large analysis of national data, the use of HOC for the static cold storage of deceased donor kidneys across the UK remains at a high level. It is

associated with acceptable transplant outcomes that are equivalent to those with UW Solution. In view of the comparative costs of HOC and UW Solutions there would seem to be valid arguments in favour of the continued use of HOC for renal preservation.

5. Machine perfusion versus static storage of kidneys

5.1 Introduction

The quality of preservation of renal allografts for transplantation is important for maintaining and improving transplant outcomes. The two prevailing methods for the hypothermic storage of deceased-donor renal allografts are static cold storage on ice and hypothermic machine perfusion. Static cold storage requires the flush-out of blood via the renal artery *in situ* and/or on the theatre back-table with chilled preservation fluid. The kidney is then stored in a bag of preservation fluid on ice. Hypothermic machine perfusion involves cannulation of the renal artery after flush-out, so that cold preservation fluid can be administered in a pulsatile fashion or continuously, within a pump. Hypothermic machine perfusion was the preferred method as renal transplant programs were first developed, particularly for longer periods of preservation and the preservation of (what were considered at the time) marginal kidneys (245). Subsequently SCS became more popular as new preservation fluids were developed, and equivalent outcomes to HMP were demonstrated, at greatly reduced immediate costs and easier management of the stored kidneys (66).

The preferred method of storage remains controversial in scientific terms owing to differing results of recent RCTs and the need to establish cost-effectiveness. Two previous systematic reviews have attempted to address this issue. One review included four studies and was unable to provide a clear recommendation for either modality (83). An earlier systematic review was conducted before the publication of two recent good quality RCTs, concluding

that HMP can reduce DGF by 20%, but does not improve graft survival (246). The increasing use of kidneys from DCD and ECD, has further increased interest in improving the preservation and resuscitation of organs for transplantation (56). The aim of this review was to appraise systematically the available evidence comparing hypothermic machine perfusion with static cold storage, and to compare outcomes for these two methods of kidney preservation. Three other systematic reviews and one large database analysis addressing this issue have been published after work conducted for this thesis, their results are compared and contrasted in the discussion section (62, 129, 132, 247).

5.2 Methods

This study was conducted in accordance with the PRISMA statement (182). The review protocol was prospectively registered with the National Institute for Health Research PROSPERO system on 18th April 2011 and can be found online: Registration Number- CRD42011001080 (183).

5.2.1 Research question

Does hypothermic machine perfusion of deceased donor kidneys result in better transplant outcomes than static cold storage?

5.2.2 Inclusion and exclusion criteria

Inclusion criteria specified any prospective, comparative study of SCS versus HMP of deceased-donor kidneys, from both adult and paediatric donors. All deceased donor types were included. First and subsequent transplants were included. Retrospective, multiple organ, animal, non-comparative and/or live donor studies were excluded. I reviewed abstracts for inclusion independently from, but in duplicate with, Robert Morgan, and differences were agreed by

discussion with Simon Knight and/or Peter Morris. References of included studies, citing articles of included studies and reviews were studied for further potentially relevant references. A subgroup analysis was prospectively specified by donor type (DCD versus DBD) and study quality.

5.2.3 Literature search

A systematic literature search was performed using Ovid MEDLINE (from 1948), Embase (from 1980), the Cochrane Library, the Transplant Library of RCTs from the Centre for Evidence in Transplantation and the International Clinical Trials Registry Platform. The final date for literature searches was 30th November 2012. Searches were conducted using MeSH and EMTREE keywords 'kidney transplantation, organ preservation, perfusion' combined with free-text terms for renal transplantation and machine perfusion. No language limits were applied.

5.2.4 Data extraction

Demographic, quality and outcome data were independently extracted into Microsoft Excel. Data was taken from all papers describing the studies; in the case of discrepancies, the most comprehensive paper was used. Any differences in data extraction were settled by discussion.

5.2.5 Outcomes

The primary outcome was the rate of DGF. Where studies reported DGF in more than one way (for example as a requirement for dialysis or no reduction in serum creatinine level), the method relating to a requirement for dialysis was used to provide data for meta-analysis. There is evidence that functional definitions of delayed graft function may be more reliable and objective (248, 249). However, the definition used was that most commonly employed in RCTs

and observational studies. Secondary outcomes were graft survival, PNF, renal graft function (study defined), acute graft rejection and patient survival.

5.2.6 Quality Assessment

All studies were assessed for general quality measures including sample size calculation, description of statistical tests used and similarity between study group demographics. In addition, RCTs were assessed using the Jadad score (189), the description of intention-to-treat analysis, allocation concealment, and risk of bias using the Cochrane Collaboration risk of bias tool (188). Our assessment of ITT analysis follows the approach outlined in the CONSORT statement (250) and is based on the four analysis strategies proposed by the Centre for Evidence in Transplantation (190).

All first authors were contacted with a standardised letter detailing our assessment and requesting a response. Letters were returned for four RCTs and three Non-RCTs. Eleven groups did not reply, three of these were for RCTs. Responses affected our assessment in three cases where the method of randomisation (251), donor type (252), and blinding methods were clarified (126). No changes were made to our assessment of the other four studies with returned information (131, 253-255).

5.2.7 Data Synthesis

The statistical package "R" (195) was used for statistical analysis in combination with the "metafor" package available for "R" (196). Meta-analysis was conducted using data from RCTs alone. Heterogeneity was analysed using Cochran's Q test, with $P < 0.10$ indicating significant heterogeneity, and the I^2 test, which describes the proportion of variation that is due to heterogeneity beyond chance. In the absence of heterogeneity, studies were combined using a fixed-effect meta-analysis (209). If there was heterogeneity, a cause was sought; if none could be established, the results were combined using a random-effects

meta-analysis (256). Mixed-effects meta-analysis was used to assess for interaction between the study effect and the moderator variables: year of publication and cold ischaemia time. Binary outcomes were analysed using the Pearson Chi^2 test, or Fisher's exact test for samples of less than ten. The test of interaction methods of Altman and Bland were used to compare estimates from different subgroups (203). P-value <0.05 was considered statistically significant.

5.3 Results

Initial literature searches identified 2,619 non-duplicated references across all databases. Eighteen studies (7 RCTs and 11 non-RCTs) described in 35 publications met the full inclusion criteria, Figure 5.1. One study was described in just one abstract, the outcomes were not presented in a useable fashion and the authors declined to provide more information, so results from the study are not discussed further here (257). Two on-going RCTs run by the University of Cambridge, UK, were identified in trial registries (258, 259). The principal investigators were contacted and confirmed that these trials are still recruiting in the UK (Neville Jamieson and Chris Watson, personal communication).

A total of 2,152 kidneys were included, 1,475 of which were from RCTs and were used in the meta-analysis, see Table 5.1. Recent European studies have been reported in papers with overlapping populations of patients (126, 130, 133, 260). Results from these studies were requested from the Scientific Steering Committee so that meta-analysis could be performed without the duplication of patients. The results from these studies in combination are referred to in this thesis as Moers et al 2008.

Five of the included RCTs and six of the included non-RCTs used Waters HMP machines that have the capability for additional oxygen administration, Table 5.1. However, none of these studies specified that additional oxygen was applied, which would have required the connection to an external oxygen supply.

Figure 5.1. PRISMA flow chart of search strategy with inclusions and exclusions. HMP= Hypothermic machine perfusion, ICTRP= International Clinical Trials Registry Platform, SCS= Static cold storage, Transplant Library= Library of RCTs from the Centre for Evidence in Transplantation.

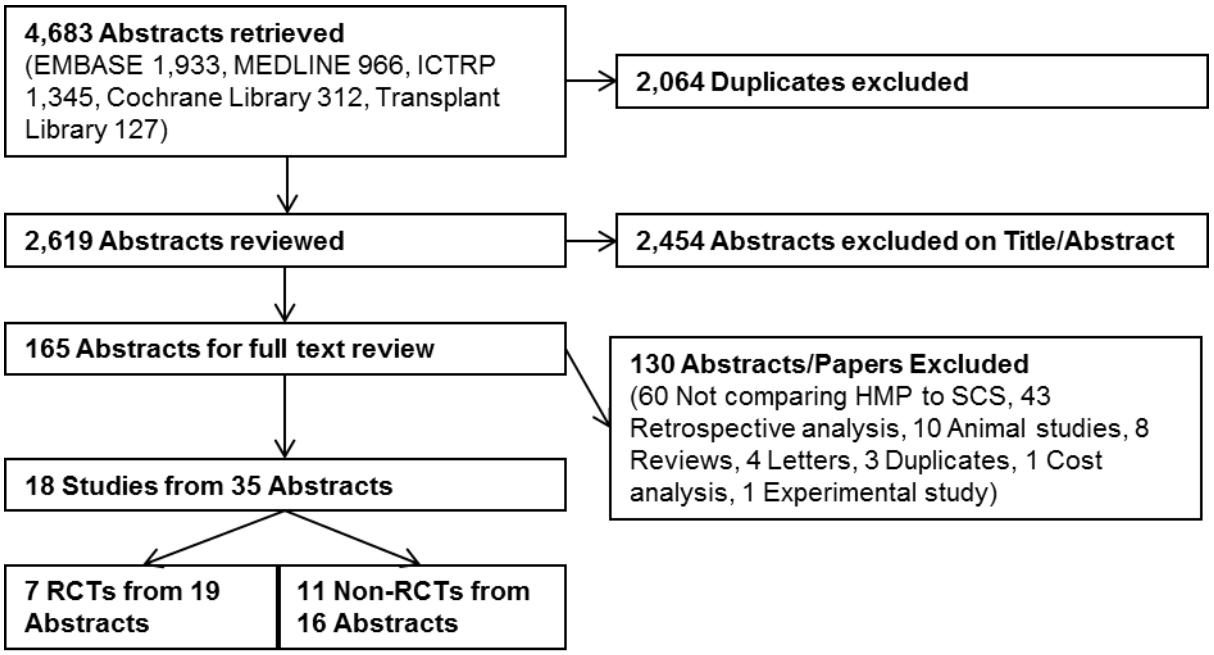


Table 5.1. Details of included studies. CPP=Cryoprecipitated Plasma, DCD= Donor after circulatory death, DBD= Donor after brain-death, EC=Eurocollins Solution, ECD= Expanded criteria donors, HOC=Hyperosmolar Citrate, HTK= Histidine-Tryptophan-Ketoglutarate, MPS= Belzer Machine Perfusion Solution, ORS= Organ Recovery Systems, PPF= Plasma Protein Fraction, RCT= Randomised controlled trial, SCS= Static Cold Storage, TPII= Hyperosmolar Colloid, UW=University of Wisconsin Solution, "Both"= Both DBD and DCD used.

	Study	Kidneys	Machine Type (Perfusate)	SCS Fluids	Donor Types	Country	Refs
RCT	**Halloran 1985**	181	Waters/Gambro (CPP)	Collins	Both	Canada	**(253, 261)**
	Mozes 1985	187	Waters MOX100 (Silica gel fraction)	EC	DBD	USA	**(262)**
	Heil 1987	54	Waters MOX100 (Silica gel fraction)	EC	DBD	USA	**(252)**
	Danielewicz 1997	74	Waters MOX100 (MPS)	UW	Both	Poland	**(263-266)**
	Van der Vliet 2001	76	Gambro (MPS)	UW	DCD	Eurotransplant	**(267)**
	Moers 2008	752	ORS Lifeport (MPS)	UW or HTK	Both	Eurotransplant	**(126, 127, 130, 133, 260, 268-271)**
	Watson 2010	90	ORS Lifeport (MPS)	UW	DCD	UK	**(131)**
Non-RCT	**Sterling 1971**	10	Lifemed Belzer LI400 (CPP)	Unclear	DCD	USA	**(272)**
	Sheil 1975	171	Waters MOX100 (Human albumin)	Collins or Perfudex	DCD	Australia	**(254)**
	Marshall 1977	181	Gambro (CPP)	HOC	DCD	Australia	**(75, 79, 273)**
	Toledo-Pereyra 1983	20	Waters MOX100	TPII	Both	USA	**(274)**
	Alijani 1985	58	Waters MOX100 (PPF)	EC	Both	USA	**(275)**
	Mendez 1987	52	Waters MOX100 (Belzer plasminate)	Collins	Both	USA	**(276)**
	Tamaki 1989	8	Nikiso LPS-II (CPP)	EC	DCD	Japan	**(277)**
	Merion 1990	102	Waters MOX100 (Silica gel)	EC	Both	USA	**(278)**
	Matsuno 1993	46	Nikiso LPS-II (CPP)	UW or EC	DCD	Japan	**(279-281)**
	Veller 1994	36	Waters 1000 (CPP)	UW	DBD	South Africa	**(251)**
	Abboud 2011	44	ORS Lifeport (MPS)	Unclear	ECD	France	**(255, 282)**

5.3.1 Quality of included studies

Three of the seven included RCTs described an adequate method of allocation concealment, see Table 5.3 (126, 131, 253). One RCT used a strict ITT analysis (131), one used a modified ITT analysis (267), three RCTs used a per-protocol analysis (126, 262, 263) and in two RCTs the analysis strategy was unclear (252, 253). One RCT reported a prospective sample size calculation (126). One RCT reported using sequential analysis to establish the study end-point (131). Twelve studies used, and described, appropriate statistical tests (75, 126, 131, 251-253, 255, 262, 263, 276, 278, 279). Three studies used, but did not describe, statistical tests (254, 267, 275) and three studies did not appear to use any statistical tests (272, 274, 277). Thirteen studies provided demographic information that showed the treatment groups to be similar (75, 126, 131, 251, 253, 255, 262, 263, 267, 274-276, 278), three studies had demographic differences between the study arms (254, 272, 277) and in two studies it was unclear (252, 279).

5.3.2 Delayed graft function

Reported rates of DGF varied between the included RCTs (range 28-57%, Table 5.2). DGF was defined as a requirement for any dialysis in the first week in five trials (126, 131, 253, 262, 263), as a requirement for dialysis in one (252) and was not defined in the remaining RCT (267). Funnel plots did not show any asymmetry and suggested no missing studies, although the small number of RCTs included does limit this method of assessment, Figure 5.2 and Figure 5.3. Egger's test for asymmetry (publication bias) and hence missing studies, was not significant for RCTs (p=0.902) nor across all prospective studies (p=0.425).

Figure 5.2. Funnel Plot for relative risk of delayed graft function in RCTs. Each dot represents an included RCT plotted with the study standard error against the log relative risk of DGF in the study. Egger's Test for asymmetry was not significant (p=0.902).

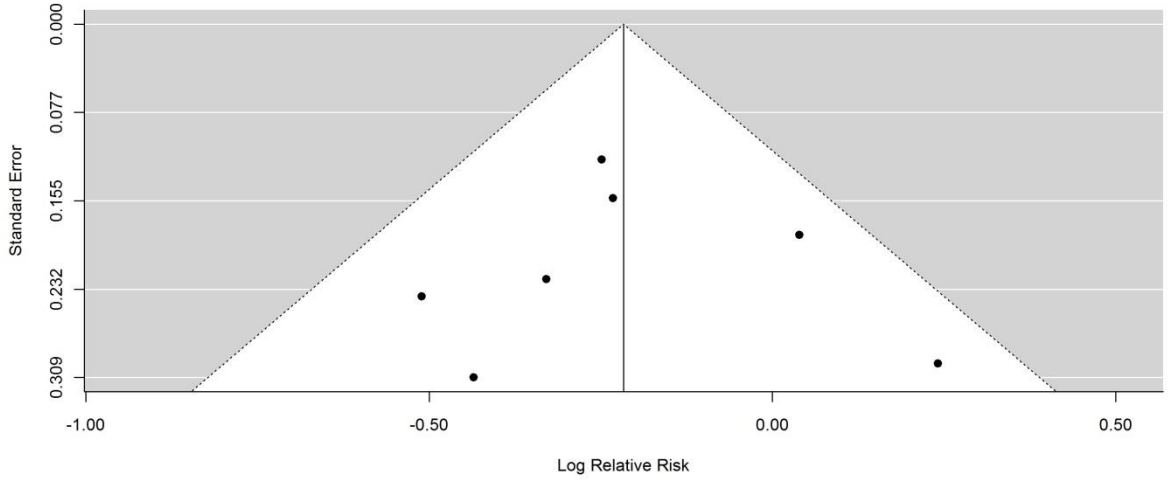
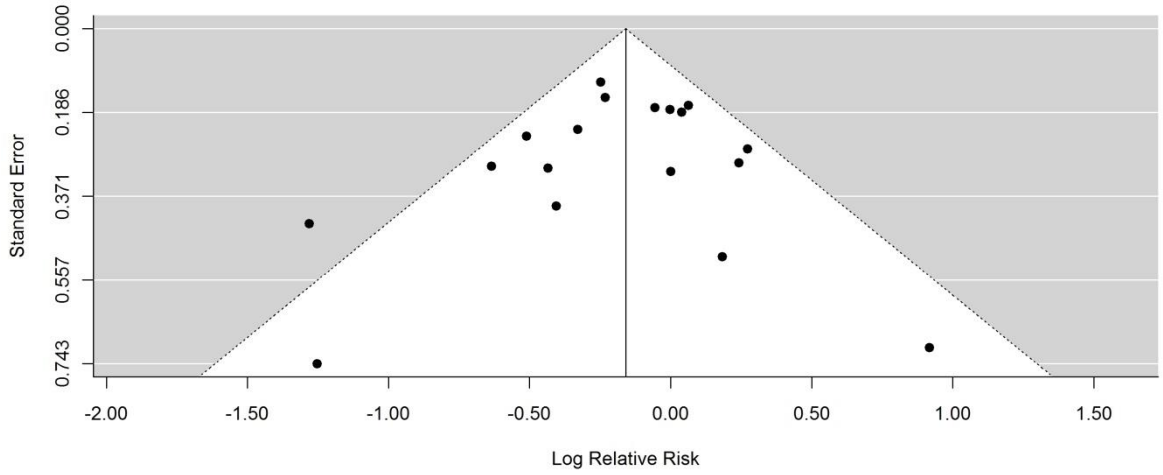


Figure 5.3. Funnel Plot for relative risk of delayed graft function in RCTs and non-RCTs combined. Each dot represents an included study plotted with the study standard error against the log relative risk of DGF in the study. The RCTs cluster in the top of the funnel. Egger's Test for asymmetry was not significant (p=0.425).



Meta-analysis showed that the relative risk of DGF was lower with HMP than with SCS (FEM: RR=0.81, 95%CI=0.70-0.92, p<0.01), Figure 5.4. There was no significant heterogeneity (I^2= 0%, Cochran's Q=6.62, p=0.35).

A sensitivity analysis was performed using good quality RCTs (Jadad score ≥3). Meta-analysis of this subgroup was supportive of the overall analysis (FEM:

RR=0.83, 95%CI=0.70-0.98, p=0.03, Figure 5.5). There was low heterogeneity in this subgroup (I^2= 0%, Cochran's Q=2.12, p=0.35).

Table 5.2. Rate of delayed graft function (DGF) for RCTs. Numbers are kidneys with DGF/kidneys in group (%). Relative Risk (RR) is machine perfusion versus static cold storage, RR <1 favours machine perfusion. 95%CI=95% Confidence Interval, CIT= Cold Ischemic Time, NR= Not Reported.

Study	Mean CIT (hours)	Study total	Static cold storage	Machine perfusion	RR of DGF (95%CI)	P-value
Halloran 1985	29	57/181 (31%)	33/90 (37%)	24/91 (26%)	0.72 (0.46-1.11)	0.14
Mozes 1985	NR	91/187 (49%)	51/94 (54%)	40/93 (43%)	0.79 (0.59-1.07)	0.12
Heil 1987	NR	25/54 (46%)	11/27 (41%)	14/27 (52%)	1.27 (0.71-2.28)	0.41
Danielewicz 1997	31	28/74 (38%)	17/37 (46%)	11/37 (30%)	0.65 (0.35-1.19)	0.13
Van der Vliet 2001	24	38/71 (54%)	24/36 (67%)	14/35 (40%)	0.60 (0.38-0.96)	0.02
Moers 2008	15	236/818 (29%)	132/409 (32%)	104/409 (25%)	0.79 (0.63-0.98)	0.03
Watson 2010	14	51/90 (57%)	25/45 (56%)	26/45 (58%)	1.04 (0.72-1.49)	0.83

RCTs were grouped by included donor types to perform a subgroup analysis for DCD and DBD kidneys. One study provided results for each donor type, the appropriate numbers were included in each subgroup analysis (126, 130). Two small RCTs were excluded from the subgroup analysis as they did not report results split by donor type (253, 263). There was no significant difference in the rate of DGF between HMP and SCS for DCD kidneys (REM: RR=0.80, 95%CI=0.62-1.04, p=0.09, Figure 5.6) or for DBD kidneys (FEM: RR=0.84, 95%CI=0.69-1.03, p=0.09, Figure 5.7). Heterogeneity was low in both cases (I^2=40%, Cochran's Q=3.56, p=0.17 and I^2=0, Cochran's Q=2.20, p=0.33 respectively). When these subgroup summary effects were tested for interaction, there was no evidence to support a different size of treatment effect of HMP in DBD and DCD kidneys (p=0.76).

Table 5.3. Quality assessment of included randomised controlled trials. Quality assessment is a combination of domains specified by the Cochrane Collaboration risk of bias tool and domains specified by the Centre for Evidence in Transplantation. Domains are declared as high, low or unclear risk of bias. The analysis strategy relates to the primary outcome. ITT= Intention-To-Treat, SSC=Sample Size Calculation.

Study	Analysis Strategy (ITT)	Jadad Score	Sequence generation[13]	Allocation concealment	Blinding of patients and staff	Blinding outcomes	Incomplete outcome data	Selective reporting	Other risk of bias	SSC	Statistical Tests Described	Groups Similar
Halloran 1985	Unclear	3	Low	Low	High	High	Low	Low	Unclear[14]	No	Yes	Yes
Mozes 1985	Per protocol	2	Unclear	High	High	High	Low	Low	Low	No	Yes	Yes
Heil 1987	Unclear	2	Low	Low	High	High	Unclear[15]	Low	Low	No	Yes	Unclear[16]
Danielewicz 1997	Per protocol	1	Unclear	High	High	High	High	High	High	No	Yes	Yes
Van der Vliet 2001	Modified ITT	2	Unclear	High	High	High	Low	Unclear[17]	Low	No	No	Yes
Moers 2008	Per protocol	3	Low	Low	Unclear	Unclear	Low	Low	Low	Yes	Yes	Yes
Watson 2010	Strict ITT	3	Low	Low	Unclear	Unclear	Low	Low	Low	Yes	Yes	Yes

[13] Where method of randomisation was not described, the risk of bias is described as unclear.
[14] Large number randomised that could never have received machine perfusion due to lack of equipment.
[15] Very little information provided regarding withdrawals from the study.
[16] Demographic information not provided.
[17] Incomplete follow up

Figure 5.4. Forest plot to show relative risk (RR) of delayed graft function (DGF) comparing hypothermic machine perfusion (HMP) to static cold storage (SCS) in randomised controlled trials. N= total patients in study arm, n= number of patients with DGF. Summary RR calculated by fixed effect meta-analysis, <1 Favours HMP. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =0%, Cochran's Q test for heterogeneity Q=6.62, p=0.36.

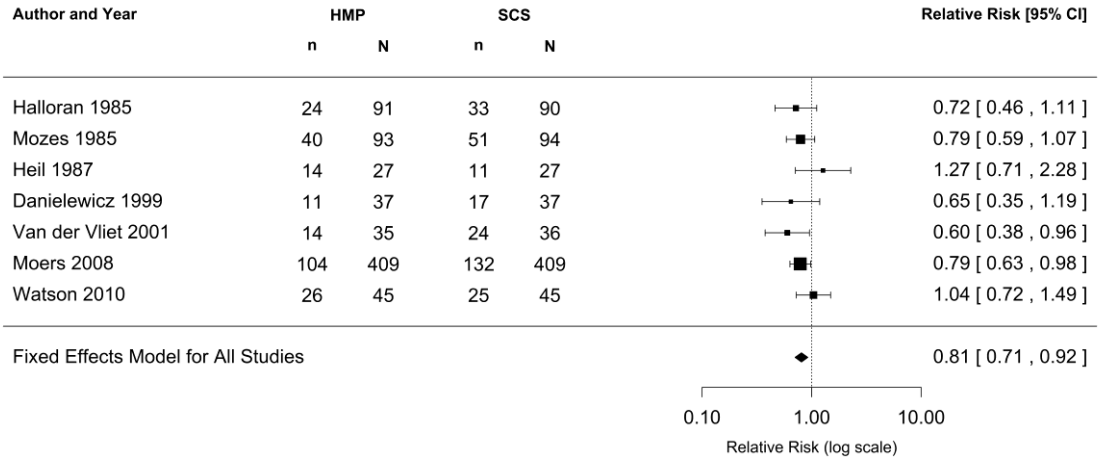


Figure 5.5. Forest plot to show relative risk (RR) of delayed graft function (DGF) comparing hypothermic machine perfusion (HMP) to static cold storage (SCS) in randomised controlled trials with Jadad score ≥3. N= total patients in study arm, n= number of patients with DGF. Summary RR calculated by fixed effect meta-analysis, <1 Favours HMP. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =0%, Cochran's Q test for heterogeneity Q=2.12, p=0.35.

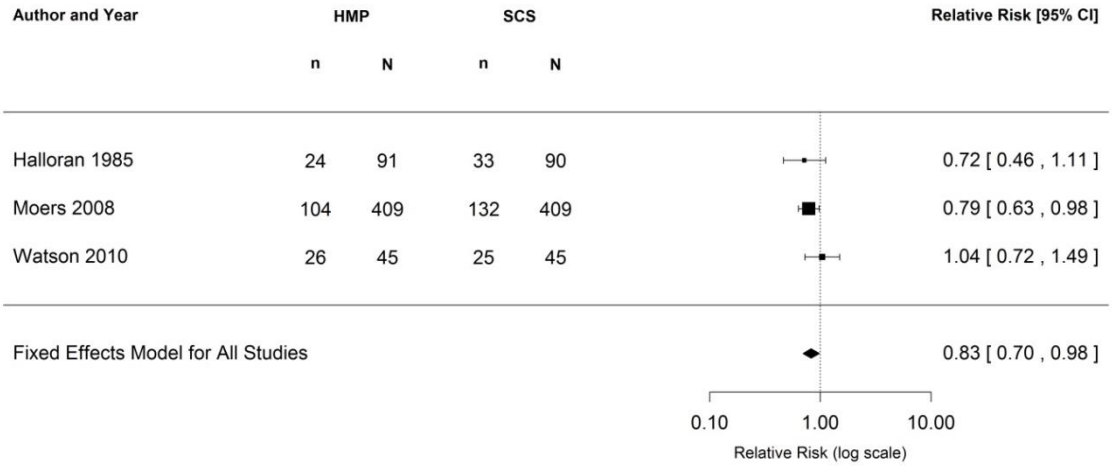


Figure 5.6. Forest plot to show relative risk (RR) of delayed graft function (DGF) comparing hypothermic machine perfusion (HMP) to static cold storage (SCS) in randomised controlled trials of kidneys from donation after circulatory death. N= total patients in study arm, n= number of patients with DGF. Summary RR calculated by random effects meta-analysis, <1 Favours HMP. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =40%, Cochran's Q test for heterogeneity Q=3.56, p=0.17.

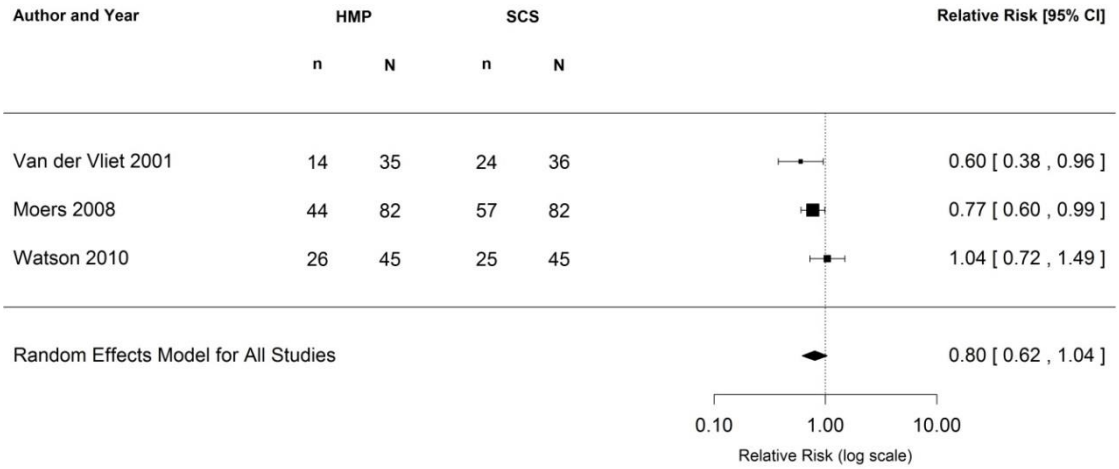
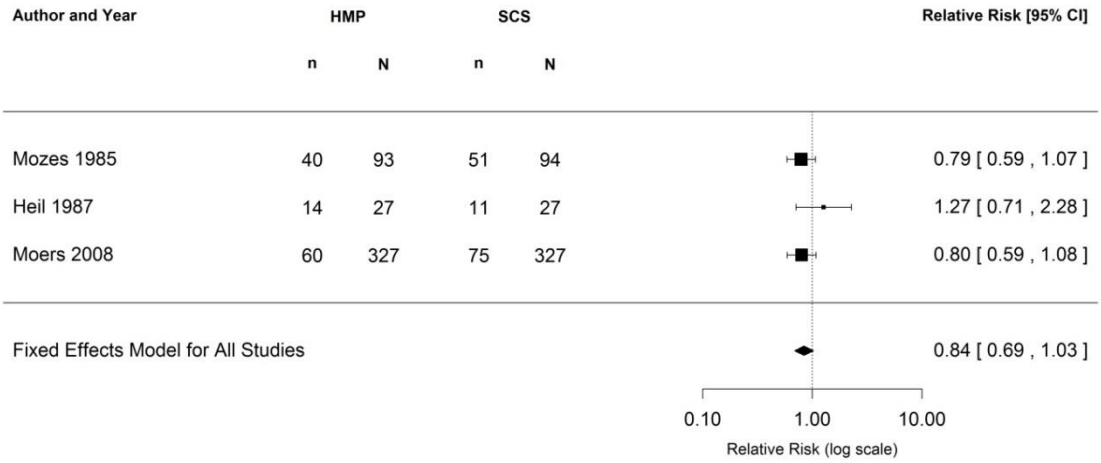


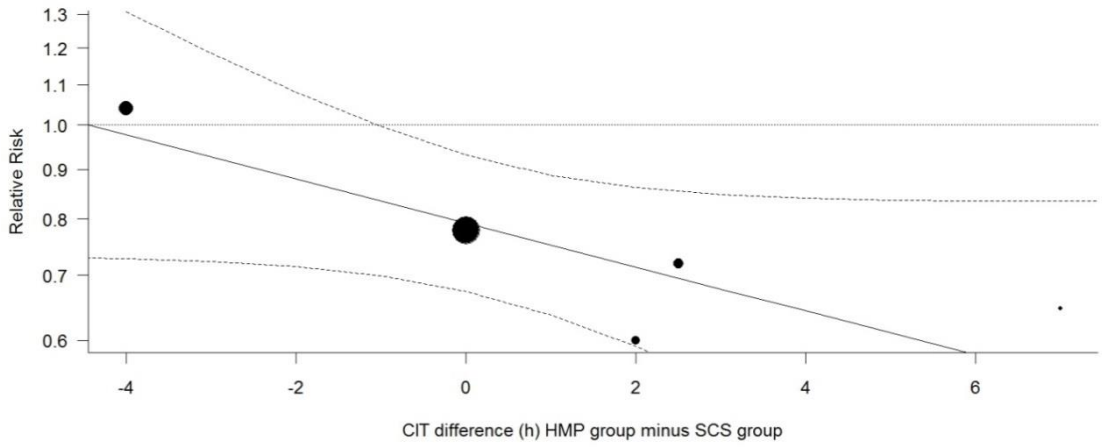
Figure 5.7. Forest plot to show relative risk (RR) of delayed graft function (DGF) comparing hypothermic machine perfusion (HMP) to static cold storage (SCS) in randomised controlled trials of kidneys from donation after brain-death. N= total patients in study arm, n= number of patients with DGF. Summary RR calculated by fixed effect meta-analysis, <1 Favours HMP. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =0%, Cochran's Q test for heterogeneity Q=2.20, p=0.33.



Mixed effects meta-analysis showed no significant interaction between the relative risk of DGF with HMP and the year of publication (p=0.83). This means that studies published in earlier or later years were not more likely to prefer one preservation method.

Mixed effects meta-analysis was also used to assess the relationship between CIT and the relative risk of DGF with HMP. There was no significant association between the relative risk of DGF with HMP and the average CIT in the study (p=0.17), the length of CIT in the machine perfusion arm (p=0.11) or the length of CIT in the static cold storage arm (p=0.17). A trend appeared when looking for interaction between the difference in CIT between the two arms (p=0.07). This may suggest that studies with a longer CIT in the HMP arm were more likely to find a reduced risk of DGF with HMP, Figure 5.8.

Figure 5.8. Mixed effects meta-analysis dot plot for relative risk of delayed graft function with hypothermic machine perfusion (HMP) against difference in mean cold ischaemic time (CIT, hours) between HMP group and static cold storage (SCS) group in RCTs. Each RCT is represented by a dot, the size of which relates to the weighting of the study in the analysis. Straight diagonal line represents fitted correlation, with curved lines representing 95% confidence intervals. As the difference in mean cold ischaemic time between the HMP arm and the SCS arm becomes more positive, the relative risk of DGF with HMP trends down.



Delayed graft function was less consistently defined in the Non-RCTs than the RCTs and also there was a wider range (20-80%), Table 5.4. Delayed graft

function was defined as a requirement for dialysis in the first week by five studies (75, 251, 255, 275, 277), as a requirement for dialysis in the first two weeks by two studies (274, 281) any requirement for dialysis by one study (278), as a lack of urine output more than one litre above normal by one study (276) and in two studies DGF was not defined at all (254, 272). Two studies found a significant reduction in DGF following HMP (275, 276), the remaining nine did not (75, 251, 254, 255, 272, 274, 277-279).

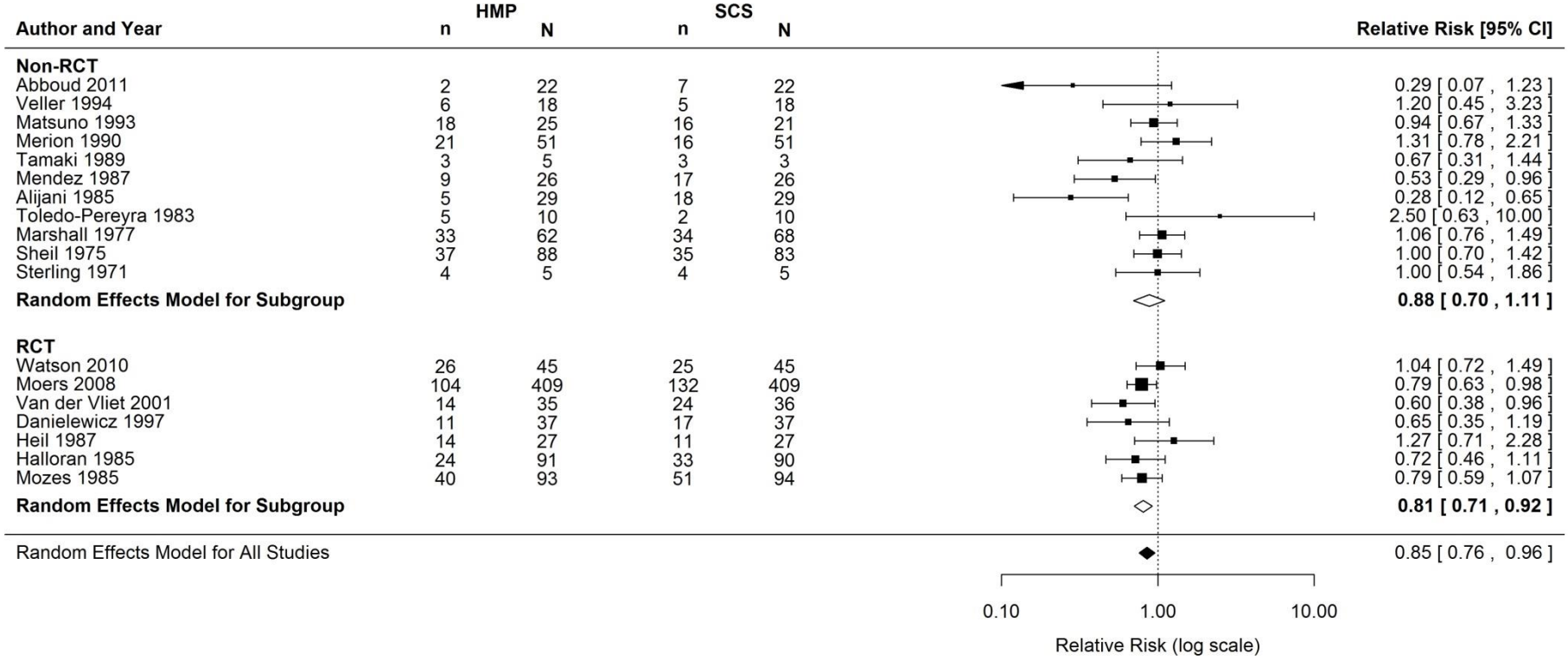
There was a less consistent treatment effect seen in the non-RCTs compared to the RCTs, which is likely due to the smaller size of the non-RCTs, Figure 5.9. Meta-analysis of non-RCTs alone resulted in a wider confidence interval for the treatment effect (REM: RR=0.88, 95%CI 0.70-1.11, p=0.28). When including non-RCTs and RCTs in meta-analysis the overall treatment effect showed a significant benefit of HMP (REM: RR=0.85, 95%CI 0.76-0.96, p=<0.01). Significant heterogeneity was introduced to the overall meta-analysis (I^2= 14.42%, Cochran's Q=27.47, p=0.05) and this was due to the heterogeneity between results of the non-RCTs (I^2= 40.68%, Cochran's Q= 19.20, p=0.04).

Table 5.4. Rate of delayed graft function (DGF) in prospective, non-randomised controlled trials. Numbers are kidneys with DGF/kidneys in group (%). Relative Risk (RR) is machine perfusion versus static cold storage, RR <1 favours machine perfusion. *= DGF defined by lack of urine output more than 1 litre above normal daily output. $= No definition of DGF given. 95%CI=95% Confidence Interval, CIT= Cold Ischemic Time, NR=Not Reported.

Study	Mean CIT (hours)	Study total	Static cold storage	Machine perfusion	RR of DGF (95%CI)	P-value
Sterling 1971[18]	NR	8/10 (80%)	4/5 (80%)	4/5 (80%)	1.00 (0.54-1.86)	1.00
Sheil 1975[18]	11	72/171 (42%)	35/83 (42%)	37/88 (42%)	1.00 (0.70-1.42)	0.99
Marshall 1977	14	67/130 (52%)	34/68 (50%)	33/62 (53%)	1.06 (0.76-1.49)	0.71
Toledo-Pereyra 1983	21	7/20 (35%)	2/10 (20%)	5/10 (50%)	2.50 (0.63-10.00)	0.35
Alijani 1985	31	23/58 (40%)	18/29 (62%)	5/29 (17%)	0.28 (0.12-0.65)	<0.01
Mendez 1987[19]	NR	26/52 (50%)	17/26 (65%)	9/26 (35%)	0.53 (0.29-0.96)	0.05
Tamaki 1989	10	6/8 (75%)	3/3 (100%)	3/5 (60%)	0.67 (0.31-1.44)	0.46
Merion 1990	21	37/102 (36%)	16/51 (31%)	21/51 (41%)	1.31 (0.78-2.21)	0.30
Matsuno 1993	9	34/46 (74%)	16/21 (76%)	18/25 (72%)	0.94 (0.67-1.33)	1.00
Veller 1994	19	11/36 (31%)	5/18 (28%)	6/18 (33%)	1.20 (0.45-3.23)	1.00
Abboud 2011	21	9/44 (20%)	7/22(32%)	2/22 (9%)	0.29 (0.07-1.23)	0.13

[18] No definition of DGF given.
[19] DGF defined by a lack of urine output more than 1 litre above "normal" daily output.

Figure 5.9. Forest plot to show relative risk (RR) of delayed graft function (DGF) comparing hypothermic machine perfusion (HMP) to static cold storage (SCS) in both randomised controlled trials (RCTs) and non-RCTs. The forest plot demonstrates the greater variability between the results of the non-RCTs compared to the RCTs. N= total patients in study arm, n= number of patients with DGF. Summary RR calculated by fixed effects meta-analysis, <1 Favours HMP. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =14.42%, Cochran's Q test for heterogeneity Q=27.47, p=0.05.



5.3.3 **Primary non-function**

In the five RCTs that reported PNF, the rates ranged from 1-15%, Table 5.5 (126, 131, 253, 262, 263). Three RCTs defined PNF as a lack of allograft function from the time of transplantation (126, 253, 263). One trial defined PNF as a "failure to provide 1-month dialysis-free survival, excluding losses attributable to rejection or vascular thrombosis" (131), and it was not defined in the remaining study (262).

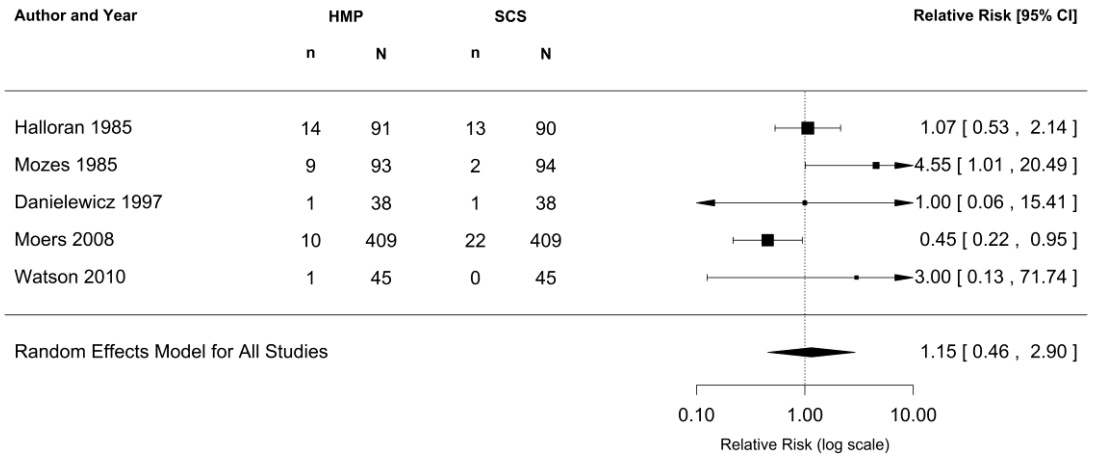
In meta-analysis of RCTs alone, the overall rate of PNF was no different between HMP and SCS groups (REM: RR=1.15, 95%CI=0.46-2.90, p=0.77), Figure 5.10. Heterogeneity was moderate (I^2=60.50%, Cochran's Q=8.55 p=0.07) and may have been due to the low incidence of this outcome and the associated variability in direction of effect. The recent European studies demonstrated a reduction in PNF with HMP (126). This effect comes largely from the inclusion of results from ECD kidneys and the Eurotransplant Senior Programme (kidneys from donors aged at least 65 years are preferentially matched with recipients aged at least 65 years), in which the PNF rate was significantly reduced by HMP (3% versus 12%, p=0.04 and 3.5% versus 12.9%, p=0.02) (133, 260).

Three non-RCTs reported rates of PNF primary non-function (range 2-4%), Table 5.5. No difference between the two preservation modalities was found in the non-RCTs (255, 278, 279).

Table 5.5. Primary non-function (PNF) rates for RCTs and non-RCTs. Numbers are kidneys with PNF/kidneys in group (%). 95%CI= 95% confidence interval, RR= Relative risk of PNF, <1 favours machine perfusion.

Study	Static cold storage	Machine perfusion	RR (95%CI)	P-value
Halloran 1985	13/90 (14%)	14/91 (15%)	1.07 (0.53-2.14)	0.86
Mozes 1985	2/94 (2%)	9/93 (10%)	4.55 (1.01-20.49)	0.03
Danielewicz 1997	1/38 (3%)	1/38 (3%)	1.00 (0.06-15.41)	1.00
Moers 2008	22/409 (5%)	10/409 (2%)	0.45 (0.22-0.95)	0.05
Watson 2010	0/45 (0%)	1/45 (2%)	3.00 (0.13-71.74)	1.00
Merion 1990	3/51 (6%)	1/41 (2%)	0.41 (0.04-3.84)	0.63
Matsuno 1993	1/21 (5%)	0/25 (0%)	0.28 (0.01-6.58)	0.46
Abboud 2011	1/22 (4.5%)	0/22 (0%)	0.33 (0.01-7.76)	1.00

Figure 5.10. Forest plot to show relative risk (RR) of primary non-function (PNF) comparing hypothermic machine perfusion (HMP) to static cold storage (SCS) in randomised controlled trials. N= total patients in study arm, n= number of patients with PNF. Summary RR calculated by random effects meta-analysis, <1 Favours HMP. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I2 test for heterogeneity=60.48%, Cochran's Q test for heterogeneity Q=8.55, p=0.07.



5.3.4 **Renal function**

Renal graft function was reported at several time points after surgery and with different measurements by different studies, so it was not possible to perform a meta-analysis. Two RCTs found a significantly quicker fall in serum creatinine with HMP than SCS (126, 253), but one smaller RCT did not (131). At 14 days there was no significant difference in estimated Glomerular Filtration Rate (eGFR) (131) or serum creatinine clearance (126). One RCT found a significantly lower serum creatinine for HMP kidneys compared to SCS kidneys at both 12 and 24 months (263). Three RCTs found no significant difference in renal function at follow-up time points up to 12 months (126, 131, 267).

One non-RCT provided renal function data on the first 14 days of the post-operative period, finding a significantly quicker fall in serum creatinine with HMP than with SCS (274). One non-RCT provided eGFR results up to 12 months post-operatively, finding no difference between methods of preservation (255). In three non-RCTs trends in renal function after transplantation were described as the same, or similar, for both preservation modalities, but no numbers were provided (272, 278, 281).

5.3.5 **Graft survival**

Graft survival rates varied between included RCTs (126, 131, 252, 262, 267), Table 5.6. Hazard Ratios were reported by one study; Moers et al found that death censored graft survival was improved with HMP compared to SCS, HR=0.52, p=0.03 (126). The effect was particularly evident in the subgroup of ECD kidneys (92.3% versus 80.2%, HR=0.35, p=0.02) (133). Improved one year graft survival was also shown for DBD but not for DCD in the same RCT (130). Three-year graft survival in this study showed a persisting benefit of HMP (91 versus 87%, p=0.04) (268).

Three RCTs found no difference in 12 month graft survival (131, 262, 267). Two of these studies showed the same direction of effect (favouring HMP), and one did not, but each study individually was underpowered. Heil et al reported a difference in 12 month graft survival (89% versus 74%, reported $p<0.05$) but with just 27 kidneys per group we cannot reproduce this statistical analysis.

Graft survival at 12 months was reported by six non-RCTs, and overall it was lower than in the RCTs, at 63%, assuming full follow-up. One non-RCT found a significantly better 12 month survival with HMP (254), the remaining five studies did not (75, 251, 255, 274, 276). One non-RCT declared that graft survival was equal at 12 months but did not provide numbers (275). Graft survival was no different between the two preservation modalities at one month (254, 278, 279) and three months (75, 255) after transplantation in non-RCTs.

Table 5.6. 12 month graft survival rates for randomised controlled trials. P-values are as calculated by the original study.

Study	Static cold storage	Machine perfusion	Definition of graft survival	P-Value
Mozes 1985	48%	52%	Not death censored	Not presented
Heil 1987	74%	89%	Unspecified	0.05
Van der Vliet 2001	72%	84%	Unspecified	Not presented
Moers 2008	90%	94%	Death censored	0.03
Watson 2010	98%	93%	Death censored	0.3

5.3.6 **Acute rejection**

Three RCTs provided rates of Biopsy Proven Acute Rejection (BPAR)(126, 131, 263) and one RCT reported numbers of grafts lost due to BPAR (253). Immunosuppressive regimens are described in Table 5.7. Given the small number of studies reporting this outcome, as well as differences in length of

follow-up and immune suppression regimens, meta-analysis was not appropriate. Danielewicz et al (median follow-up 22 months) reported an increased BPAR with SCS compared to HMP (50% versus 34%, p<0.01) (263). In more recent studies, both Moers et al (14 days follow-up) and Watson et al (12 months follow-up) found no significant difference in BPAR (14% versus 13%, p=0.82 (126) and 22% versus 9%, p=0.10 (131)). A smaller RCT by Halloran et al reported no significant difference in graft loss due to acute rejection (59% versus 71%, p=0.34) (261).

One non-RCT found an increased risk of graft loss due to acute rejection with SCS compared to HMP (30% versus 15%, p=0.03) (254), and one non-RCT found no significant difference (4% versus 10%, p=0.44) (278).

Table 5.7. Immunosuppressive regimens in included randomised controlled trials. ATG= Antithymocyte Globulin.

Study	Antibody Induction	Maintenance Therapy
Halloran 1985	ATG[20]	Prednisolone+/-Azathioprine+/-Cyclosporine
Mozes 1985	ATG[20]	Prednisolone+Azathioprine+/-Cyclosporine
Heil 1987	Not reported	
Danielwicz 1997	None	Cyclosporine+Azathioprine+Prednisolone
Van der Vliet 2001	Not reported	
Moers 2008	Not reported	
Watson 2010	Basiliximab	Tacrolimus +Mycophenolate+Prednisolone

[20] Not given to all patients in this study.

5.3.7 **Patient survival**

Three RCTs reported patient survival at one year post-transplantation (range 89-97%), finding no relationship between the method of preservation and patient survival (126, 131, 262). Two other RCTs reported patient survival at median follow-up of 17 months (253) and median follow-up of 22 months (263) reporting no relationship between preservation method and patient survival. Heterogeneity in the length of follow-up and the number of patients lost to follow-up in some studies meant that meta-analysis was not appropriate.

Three non-RCTs reported patient survival, finding no difference at 9 months (255) and 12 months follow-up (75, 275).

5.4 Discussion

This systematic review has examined the evidence for the use of HMP in comparison with SCS for the preservation of deceased donor kidneys. The RCTs showed a statistically significant reduction in DGF following HMP; however, this reduction may be modest. DGF is an important outcome and may be a surrogate for graft survival owing to its relationship with both early and late graft loss (232).

The present findings suggest that HMP is likely to reduce DGF of kidneys from all donor types: DBD, DCD and ECD. The reduction in relative risk for both DBD and DCD subgroups is the same as the overall meta-analysis, with a trend towards significance in both groups. The fact that these are non-significant possibly can be explained by a lack of statistical power, given the much smaller numbers used for each subgroup analysis.

There does not appear to be an effect on short or longer-term renal function, but this may be confounded by the large number of patients with DGF in the SCS group in these studies. The most recent publications from the European

multicentre trial have shown an improvement in graft survival that is related to the reduction in DGF (268). The other secondary outcomes (PNF, BPAR, and patient survival) were not affected by the preservation method in the present analysis. It is possible that the included studies were not large enough to discern a relationship between the preservation modality and these outcomes. The study from Watson and colleagues stands out by demonstrating no benefit of HMP in DCD kidneys (131). It is possible that this result may arise from the length of SCS prior to HMP in the study group; transplant teams were free to commence HMP when convenient, rather than immediately after retrieval (131). This effect has been demonstrated in animal studies (283).

An important consideration in developing new interventions is cost-effectiveness. The most recent cost-analysis suggests that investment in HMP is cost-effective (284). However, this analysis was based upon the results of the European machine perfusion trial alone, and Dutch costs, which may not be relevant in other countries or healthcare systems.

Registry data from the Collaborative Transplant Study (n=91,674 transplants) shows that HMP is associated with reduced graft survival compared to SCS (56). In this registry analysis, HMP was used more commonly for kidneys from diabetic, older, African-American donors or donors with higher terminal creatinine and longer CIT (128). In contrast, analysis of the Scientific Registry of Transplant Recipients (n=98,736 transplants) showed reduced rates of DGF following HMP, despite the preferential use of HMP for kidneys with risk factors for DGF (128). A more recent analysis of DCD kidneys recorded in the same database (n=4,932 transplants) has also shown a lower risk of DGF with HMP (285).

The present study has several important limitations. The number of studies included is relatively small, and there is unlikely to be adequate power to identify differences in secondary outcomes such as BPAR and PNF. Furthermore, the short follow-up in the majority of studies means that the long-

term risk of the modest increase in DGF identified in the SCS group is unclear. The definition of DGF does vary between studies, although it was the same for five out of seven RCTS. The underlying effect being measured should be the same, and the same definition is used between arms in each individual study. The combination of these studies in meta-analysis is supported by the lack of the heterogeneity and the consistency between the sensitivity analysis using the best quality studies and the overall analysis.

The results described here may be confounded by the fact that the three earliest RCTs used Collins or Eurocollins as the cold-storage solution in the SCS arm. A recent systematic review has suggested that Eurocollins is associated with a higher risk of DGF than UW (230). Therefore, any benefit of HMP seen in these earlier studies may have been mitigated if newer, more effective cold-storage solutions had been used. The preferred machine and machine perfusate has also changed over time. This suggests that the relative benefit of HMP has been maintained over the cold-storage fluids available, and this is reinforced by the lack of heterogeneity.

While there have been small animal experiments using UW as a pump fluid (286, 287), Belzer Machine Perfusion Solution (MPS) has not been tested as a preservation fluid for static storage. If MPS is as effective a preservative in a static system as it is within a pump system, then the apparent effect of the pump may be negated. It should be noted that no included study has used the same fluid for HMP and SCS. The included studies were also conducted over a long interval (25 years for RCTs), but despite this, we found no interaction between the year of publication and the relative risk of DGF in each study.

Three other systematic reviews comparing the outcome of kidneys preserved by HMP compared to SCS have been published in the last year (129, 132, 247). A recently published a systematic review from China, comparing the outcomes in patients receiving kidneys from DCD, conducted a meta-analysis using the results from four RCTs (n=351 kidneys) (132). Machine perfusion reduced the

rate of DGF compared to SCS (OR=0.56, p<0.01). A second group, from Ontario, Canada, conducted a meta-analysis using the results from the same four RCTs, but very slightly different numbers of kidneys, with essentially the same results (OR=0.59, p=0.02) (247). Another group, from the University of Sydney, Australia, included both DCD and DBD kidneys (n=1,353 kidneys), also finding a reduced risk of DGF (RR=0.83, p=0.01) (129).

Whilst in the overall meta-analysis of all donor types HMP was associated with a significant reduction in DGF (RR=0.81, p<0.01) there was not a significant reduction with kidneys from circulatory death alone (RR=0.80, p=0.09). This could be due to two key factors; firstly we did not include data from the study by Matsuno et al, as we felt it was not a randomised controlled trial (280). It is relatively small however, and may not have contributed greatly to the overall meta-analysis by Deng et al (132) or Bathini et al (247). Secondly, I^2 testing showed heterogeneity of 40%, random effects meta-analysis was therefore used for this analysis, which will result in wider confidence intervals. Both Deng et al and Bathini et al used fixed effects meta-analysis given the lower heterogeneity between their included studies.

Both of these factors will result in less power to demonstrate a significant effect in the subgroup analysis of DCD kidneys performed in this review. Overall the results of this review agree with the conclusions of Deng et al and Bathini et al.

In comparison to the study by Lam et al, the results presented here are very similar, including the point estimate of the summary effect and the 95% confidence intervals (129). The slight differences in relative risk can be attributed to two small differences between the studies; firstly we were fortunate to receive extra information from the steering committee of the Eurotransplant study, which allowed us to incorporate results from the study extensions of DCD, ECD, and senior program kidneys without overlap. Secondly, the paper by Jaffers and Banowsky was excluded, as we felt it was a retrospective study and did not meet our inclusion criteria (288). We instead

included a study by Van der Vliet et al (267). These slight differences in methods lead to the small differences in summary effects and confidence intervals. Overall the results of this study agree with the conclusions of Lam et al.

Interestingly, none of the recent reviews found conclusive evidence for a difference in graft survival in RCTs, either by using meta-analysis, or in narrative review. Further weight is added to this discussion by the recent review of UNOS data by Cannon et al at the University of Louisville, USA (62). Across multivariate analysis, paired kidney analysis, and propensity-matched comparisons, Cannon et al found a consistent reduction in delayed function associated with machine perfusion, but no difference in graft survival (62).

Conclusion

This analysis of the available studies suggests that HMP reduces DGF compared to SCS. This reduction in DGF may result in improved graft survival but robust evidence is not available to support that claim. Furthermore, there is no evidence to show a difference in PNF, renal function, BPAR or patient survival. If HMP is to become a standard feature of renal preservation, more evidence is required and this will only come from further large, well planned RCTs directed at ECD and DCD donors in particular.

6. Preservation solutions for static storage of livers

6.1 Introduction

In 2011 over 5,600 adult liver transplants from deceased donors were performed in the USA and 88% of these patients are expected to survive longer than 1 year after transplantation (3). Whilst patient and graft survival following liver transplantation has improved over time, in the USA 3 and 5 year graft survival are still only 78% and 72% respectively (based on Organ Procurement and Transplantation Network data, as of February 8, 2013). The implications for the recipient of a poorly functioning graft, or early graft loss are catastrophic. Adequate preservation of liver grafts during retrieval and storage, and the prevention of ischaemia reperfusion injury, are therefore of critical importance. The most commonly used method of preservation of livers for transplantation is still static cold storage. Livers are flushed *in situ* in the donor with a chilled preservation fluid through the aorta and/or portal vein. The liver may also be further flushed on the back-table before packing into an ice box. The constituents of commercially available preservation fluids have been designed to attenuate any deterioration in organ quality during the preservation period. These preservation fluids differ in cost and also in their composition, such that they can have considerable differences in electrolyte combinations, buffering capacity, osmolality and viscosity.

The use of UW for liver preservation increased after clinical comparisons with previously developed fluids, such as Eurocollins solution, demonstrated superior results with UW, even with longer cold ischaemic times (289, 290). University of Wisconsin solution has since become the most widely used fluid for the preservation

of liver allografts from deceased donors, followed by HTK (291). Multivariate analysis of large databases, corrected for confounding variables, has suggested an association between HTK preservation and increased graft loss compared to UW (291). Registry data however, is subject to selection bias, even after correcting for known confounders, while clinical trials of preservation methods have been, on the whole, individually underpowered for important outcomes (230).

Two previous systematic reviews have explored this topic, each with its limitations. Feng et al included prospective and retrospective comparisons of UW with HTK, in both live-donor and deceased-donor studies (292). The group excluded one large study (293), concluding that there were no differences in outcome between UW and HTK. Zuluagu et al made an indirect comparison of HTK with Celsior solution (an extracellular-type low viscosity solution) in the preservation of deceased-donor livers, by including studies that did not directly compare these two fluids (294). The group did not include results from one unpublished study (295) and concluded that HTK and Celsior performed similarly. The aim of this study was to systematically appraise the evidence in RCTs comparing available preservation solutions for static cold storage of deceased-donor liver allografts, and to conduct meta-analysis if appropriate.

6.2 Methods

This study was conducted in accordance with the PRISMA statement (182). The review protocol was prospectively registered with the National Institute for Health Research PROSPERO system on 4TH April 2012 and can be found online (registration number CRD42012001720) (183).

6.2.1 Research question

Which preservation fluid is best for the static and hypothermic preservation of deceased donor liver allografts?

6.2.2 Inclusion and exclusion criteria

Inclusion criteria specified any RCT comparing two or more preservation solutions for the static cold storage of deceased-donor livers, from both adult and paediatric donors. Multi-organ transplants and re-transplants were included. Retrospective, animal, non-comparative and/or live donor studies were excluded. I reviewed abstracts for inclusion independently from, but in duplicate with, Robert Morgan. Differences were agreed by discussion with Simon Knight and/or Peter Morris.

6.2.3 Literature search

A systematic literature search was performed using Ovid MEDLINE (from 1948), EMBASE (from 1980), the Cochrane Library, the Transplant Library of RCTs from the Centre for Evidence in Transplantation and the International Clinical Trials Registry Platform. Searches were conducted using MeSH and EMTREE keywords, combined with free-text searching for various terms for liver transplantation and different perfusion fluids. No language limits were applied. The final date for literature searches was 31st March 2013. References of included studies, citing articles of included studies and reviews were studied for further potentially relevant references.

6.2.4 Data extraction

Demographic, quality and outcome data were extracted independently into Microsoft Excel. Data was taken from all papers describing the studies; in the case of discrepancies, the paper with the largest number of patients was used. Any differences in data extraction were settled by discussion.

6.2.5 Outcomes

The primary outcomes were early dysfunction (as defined by the authors of each individual study), primary non-function, re-transplantation rate, patient survival and graft survival. Secondary outcomes were peak biochemical parameters in the

first week after transplantation including serum transaminases, bilirubin and clotting profiles and biliary complications. Early dysfunction of the liver graft, defined by abnormal biochemical parameters in the first week, has been validated as a predictor of worse graft and patient survival in analyses of large multicentre databases (296, 297). Early dysfunction, sometimes referred to as initial poor function, has been defined in other ways, with slightly different thresholds for biochemical parameters or additional parameters (59, 298-302). The definition provided in the original manuscripts was used.

6.2.6 **Quality assessment**

Included studies were assessed using the Jadad score (189), the description of intention-to-treat analysis, allocation concealment, and the risk of bias using the Cochrane Collaboration risk of bias tool (188). A score of three or more out of five on the Jadad scale indicates good quality. The assessment of intention-to-treat analysis follows the approach outlined in the CONSORT statement (250) and uses the four analysis strategies proposed by the Centre for Evidence in Transplantation (190). All studies were assessed for general quality measures including sample size calculation, description of statistical tests and similarity between the study group and control group. All first authors were contacted with a standardised letter detailing the assessment and requesting a response. Letters were returned for six studies (295, 303-307).

6.2.7 **Data synthesis**

The statistical package "R", was used for statistical analysis (195) in combination with the "metafor" package available for "R" (196). Relative risk (RR) was used as a summary statistic for binary outcomes. Binary outcomes have been analysed using the Pearson Chi^2 test, unless $n<10$, where Fisher's exact test has been used. Where hazard ratios for survival outcomes were not reported, binary data were converted to Relative Log Survival (RLS) in order to estimate the hazard ratio in a validated

method, allowing comparisons to be made between outcomes reported at different lengths of follow-up (201). P-value <0.05 has been used to indicate statistical significance. Heterogeneity was analysed using the Cochran's Q test, with p<0.1 indicating significant heterogeneity, and the I^2 test, which describes the proportion of variation that is due to heterogeneity beyond chance. In the absence of heterogeneity, studies were combined using a fixed effect meta-analysis (209). In the presence of heterogeneity a cause was sought, and if none could be established then results were combined using a random effects meta-analysis (256). Publication bias using funnel plot could not be assessed accurately given the small number of studies for each comparison. Subgroup analysis was planned by donor type but there was insufficient information regarding liver transplants from donation after circulatory death.

6.3 Results

Initial literature searches identified 3,546 unique references across all databases and 16 RCTs described in 24 publications met the inclusion criteria (1,619 livers, Figure 6.1, Table 6.1). One study was unpublished as a full paper and described in an abstract only, it was presented at the International Liver Transplantation Society Annual Congress, Paris 2008 (295).

Four studies achieved at least three points on the Jadad scale (295, 303, 306, 307), ten studies scored 2 points (95, 105, 293, 304, 308-313), one study scored one point (305), and one study scored zero points (314), Table 6.2. Six studies described an adequate method of randomisation (105, 293, 295, 303, 306, 307), four studies described an adequate method of allocation concealment (293, 303, 306, 309) and only one study described blinding of participants (306).

Studies by Nardo et al (2001) (93, 104, 303, 315, 316), Nardo et al (2004) (304, 317), Garcia-gil et al (309, 318), Lopez-Andujar et al (305, 319) and Dondero et al (314, 320),

were reported in two or more publications. These studies are referred to by the author and date of the earliest related reference.

Perfusion fluids were used at three stages of the liver retrieval process; initial donor aortic perfusion, back-table flush and storage, and flush prior to implantation in the recipient. Eleven of the included studies investigated the same fluid for donor aortic perfusion (and portal flush, if done) as well as the back-table and packing fluid, Table 6.1. Results from these studies will be discussed first. Two studies examined the effect of varying the initial aortic perfusion in the donor (311, 312). Three studies examined the effect of the fluid used to flush the liver before blood reperfusion in the recipient (306, 307, 313). Results from these five studies will be discussed later.

No study presented Donor Risk Index in the published report. Two studies presented recipient MELD score, reporting no significant difference between study and control groups; Garcia-gil et al reported MELD score of 15.3+/- 5.7 versus 15.7+/- 9.9 (318) and Dondero et al reported 17+/9 versus 15+/-8 (314). Five studies presented Child-Pugh score; each one reported an equal distribution of classes between study and control arms (293, 295, 303, 305, 317).

Figure 6.1. PRISMA flow chart of search strategy with inclusions and exclusions. ICTRP= International Clinical Trials Registry Platform, SCS= Static cold storage, Transplant Library= Library of RCTs from the Centre for Evidence in Transplantation.

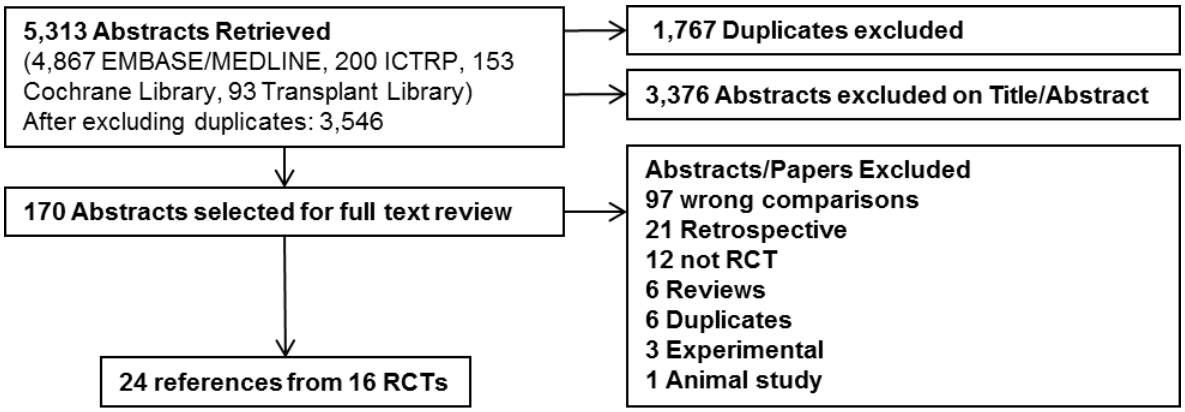


Table 6.1. Details of included studies. CIT= mean cold ischaemia time, HTK= Histidine-Tryptophan-Ketoglutarate, IGL-1= Institut-Georges-Lopez Solution, UW= University of Wisconsin Solution.

Study	Control group					Study group					Refs
	Fluid	Aortic flush volume	Portal flush volume	N	Mean CIT (h)	Fluid	Aortic flush volume	Portal flush volume	N	CIT (h)	
In the eleven studies below, livers received the same fluid for initial aortic perfusion and storage.											
Erhard 1994	UW	2L in situ	2L in situ 200-300ml ex situ	30	9.39	HTK	150ml/kg in situ	150ml/kg in situ 500ml ex situ	30	9.66	(95)
Meine 2006	UW	2L in situ 500ml ex situ (Hepatic artery)	1L in situ 500ml ex situ	65	9.66	HTK	4L in situ 500ml ex situ (Hepatic artery)	1L in situ 500ml ex situ	37	9.8	(293)
Brolese 2008 (three arm study)	UW	Unclear	Unclear	74	Unclear	HTK	Unclear	Unclear	148	Unclear	(295)
						Celsior	Unclear	Unclear	82	Unclear	
Nardo 2001	UW	30ml/kg in situ 300ml ex situ (Hepatic artery)	30ml/kg in situ 700ml in situ	90	7.3	Celsior	60ml/kg in situ 300ml ex situ (Hepatic artery)	30ml/kg in situ 700ml ex situ	83	7.4	(93, 104, 303, 315, 316)
Lama 2002	UW	Unclear	Unclear	10	Unclear	Celsior	Unclear	Unclear	10	Unclear	(308)
Pedotti 2004	UW	4.7L on average, centres could decide to use aortic or aortic and portal flush		96	8.2	Celsior	5.8L on average, centres could decide to use aortic or aortic and portal flush		79	8.7	(105)
Garcia-Gil 2006	UW	3L in situ	2L in situ 1L ex situ	51	6.6	Celsior	4L in situ	2L in situ 1L ex situ	51	6.41	(309, 318)
Lopez-Andujar 2009	UW	2L in situ	2L in situ	103	6.01	Celsior	2L in situ	2L in situ	92	5.38	(305, 319)
Nardo 2004	Celsior	60ml/kg in situ	30ml/kg in situ	20	7.63	HTK	120ml/kg in situ	30ml/kg in situ	20	7.5	(304, 317)
Dondero 2010	UW	3L in situ	1L in situ 750ml ex situ	92	7.95	IGL-1	3L in situ	1L in situ 750ml ex situ	48	7.87	(314, 320)
Kurzawinski 1994	UW	2L Baxter's solution aortic and 2L Baxter's solution portal in situ. 1L portal and 500ml hepatic artery UW ex situ.		20	11.26	High Sodium UW	2L Baxter's solution aortic and 2L Baxter's solution portal in situ. 1L portal and 500ml hepatic artery study fluid ex situ.		20	11.7	(310)
Two studies below randomised the initial aortic flush in the donor. Livers in all groups were then back-table flushed and stored in UW.											
Schwartz 1991	UW	4L in situ	1L in situ	32	Unclear	Ringer's Lactate	1L in situ 3L of UW ex situ	1L in situ	34	Unclear	(311)
Cofer 1992	UW	2-3L mixed aortic and portal in situ. 500ml aortic and portal ex situ.		32	10.2	Eurocollins	2-3L mixed aortic and portal in situ. 500ml UW aortic and portal ex situ.		24	10.6	(312)
Three studies below stored all livers in UW. Livers were then randomised to one of two fluids for flush-out before reperfusion.											
Adam 1991	Ringer's Lactate	Unclear	50% weight of liver pre- reperfusion	41	9.28	Albumin	Unclear	50% weight of liver pre-reperfusion	42	9.7	(313)
Sanchez-Urdazpal 1993	Plasmalyte	150ml hepatic artery pre-reperfusion	350ml pre-reperfusion	20	8.7	Carolina Rinse	150ml hepatic artery pre-reperfusion	350ml pre-reperfusion	23	8.33	(306)
Bachmann 1997	Albumin	250ml hepatic artery pre-reperfusion	750ml pre-reperfusion	10	Unclear	Carolina Rinse	250ml hepatic artery pre-reperfusion	750ml pre-reperfusion	10	Unclear	(307)

Table 6.2. Quality assessment of included studies. Risk of bias is assessed in each domain of the Cochrane Collaboration Risk of Bias Tool. ITT= Intention to treat analysis, SSC= Sample size calculation, Strict= Strict intention to treat analysis.

Study	Analysis strategy (ITT)	Jadad score (/5)	Risk of bias							SSC	Statistical tests described	Groups similar
			Sequence generation	Allocation concealment	Blinding of participants	Blinding outcomes	Incomplete outcome data	Selective reporting	Other risk			
Erhard 1994	Per-protocol	2	Unclear	High	High	High	High	Low	High	Yes	Yes	No
Meine 2006	Strict ITT	2	Low	Low	High	High	High	Low	Low	No	Yes	No
Nardo 2001	Modified ITT	3	Low	Low	High	High	Low	Low	Low	No	Yes	Yes
Lama 2002	Unclear	2	Unclear	High	High	High	Low	Low	Low	No	Yes	Yes
Pedotti 2004	Per-protocol	2	Low	High	High	High	High	Low	Low	No	Yes	Yes
Garcia-Gil 2006	Strict ITT	2	Unclear	Low	High	High	Low	Low	Low	Yes	Yes	Yes
Lopez-Andujar 2009	Modified ITT	1	High	High	High	High	Low	Low	Low	Yes	Yes	Yes
Nardo 2004	Strict ITT	2	Unclear	High	High	High	Low	Low	Low	No	Yes	Yes
Dondero 2010	Per-protocol	0	High	High	High	High	Low	Low	Low	No	Yes	Yes
Kurzawinski 1994	Strict ITT	2	Unclear	High	High	High	Low	Low	Low	No	Yes	Yes
Brolese 2008	Modified ITT	3	Low	High	High	High	Low	Low	Unclear[21]	Yes	Yes	Yes
Schwartz 1991	Strict ITT	2	Unclear	High	High	High	Low	High	Low	No	Yes	Yes
Cofer 1992	Modified ITT	2	Unclear	High	High	High	Unclear[22]	Low	Low	No	Yes	Yes
Adam 1991	Per-protocol	2	Unclear	High	High	High	Low	Low	Low	No	Yes	Yes
Sanchez-Urdazpal 1993	Strict ITT	3	Low	Low	Low	High	Low	Low	Low	No	Yes	Yes
Bachmann 1997	Strict ITT	3	Low	High	High	High	Low	Low	Low	No	None	Unclear

[21] Unpublished study. Information available from lead author and conference abstract only.
[22] Recipients were excluded from the analysis if not all biochemical results were available.

6.3.1 **Early dysfunction**

Eleven studies reported early dysfunction rates in terms of livers that did not function optimally after transplantation but subsequently went on to provide life-sustaining function. Early dysfunction rates ranged from 2-26%, Table 6.3. In six of these studies early dysfunction was defined by an elevation in serum transaminases beyond a pre-defined threshold within the first week (293, 295, 303, 305, 309, 314). The specific threshold varied between studies and other factors were also used to define early dysfunction including: prolonged stay in intensive care, or need for clotting factor support (293), elevated bilirubin (314), reduced prothrombin activity (303, 305, 309), and rise in serum ammonia (303, 305). In all studies presenting early dysfunction as an outcome, the same definition was applied to all arms of the study.

Meta-analysis showed no significant difference in early dysfunction rates between Celsior and UW (5 studies, FEM: RR=1.08, 95%CI=0.63-1.86, p=0.77; Figure 6.2). There was no evidence of a difference between HTK and UW (3 studies, FEM: RR=0.73, 95%CI=0.23-2.35, p=0.59; Figure 6.3). Despite the variations in definition, heterogeneity was low in both analyses (Celsior versus UW: Cochran Q=4.73, p=0.32, I^2=17.11%, HTK versus UW: Cochran Q=1.78, p=0.41, I^2=11.67%). No difference was found between Celsior and HTK in two studies (295, 304), between UW and IGL-1 in one study (314), or between UW and high-sodium UW in one study (310).

Table 6.3. Early dysfunction rates in included studies. Studies are grouped by preservation solutions compared. CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate Solution, IGL-1= Institut Georges-Lopez-1 Solution, UW= University of Wisconsin Solution, UW High Na= Modified UW with high sodium content. N= number in group, n= number with early dysfunction, RR=Relative risk of early dysfunction, Solution 1 versus Solution 2.

Study	Solution 1				Solution 2						Definition of Early Dysfunction
		N	N	%		N	n	%	RR	P-Value	
Erhard 1994	UW	30	1	3.33	HTK	30	0	0	3.00	0.50	Undefined
Meine 2006	UW	65	6	9.23	HTK	37	1	2.7	3.42	0.25	Need for clotting factor support, prolonged intensive care or persistent liver enzyme elevation during week 1
Brolese 2008	UW	74	2	2.7	HTK	148	6	4.05	0.67	0.61	ALT>1500 days 2-7
Nardo 2001	UW	90	6	6.67	Celsior	83	2	2.41	2.77	0.20	AST>2000, prothrombin time >16seconds, Ammonia>50 days 2-7
Lama 2002	UW	10	1	10	Celsior	10	3	30	0.33	0.30	Undefined
Garcia-Gil 2006	UW	51	0	0	Celsior	51	0	0	1.00	1.00	Transaminases >30 times normal and prothrombin activity <50% for 4 days in days 1-7
Brolese 2008	UW	74	2	2.7	Celsior	82	7	8.54	0.32	0.14	ALT>1500 days 2-7
Lopez-Andujar 2009	UW	103	16	15.5	Celsior	92	14	15.2	1.02	0.95	AST>2000, prothrombin time >16seconds, Ammonia>50 days 2-7
Nardo 2004	HTK	20	1	5	Celsior	20	0	0	3.00	0.49	Undefined
Brolese 2008	HTK	148	6	4.05	Celsior	82	7	8.54	0.47	0.17	ALT>1500 days 2-7
Dondero 2010	UW	92	24	26.1	IGL-1	48	13	27.1	0.96	0.90	AST/ALT>2000 days 1-7, Bilirubin>10 on day 7
Kurzawinski 1994	UW	20	0	0	UW High Na	20	0	0	1.00	1.00	Undefined
Bachmann 1997	Albumin	10	4	40	Carolina Rinse	10	0	0	9.00	0.12	Undefined

Figure 6.2. Forest plot to show Relative Risk (RR) of early dysfunction comparing Celsior with University of Wisconsin Solution (UW) in randomised controlled trials. N= total patients in study arm, n= number of patients with early dysfunction. Summary RR calculated by fixed effect meta-analysis, <1 Favours Celsior. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =17.11%, Cochran's Q test for heterogeneity Q=4.73, p=0.32.

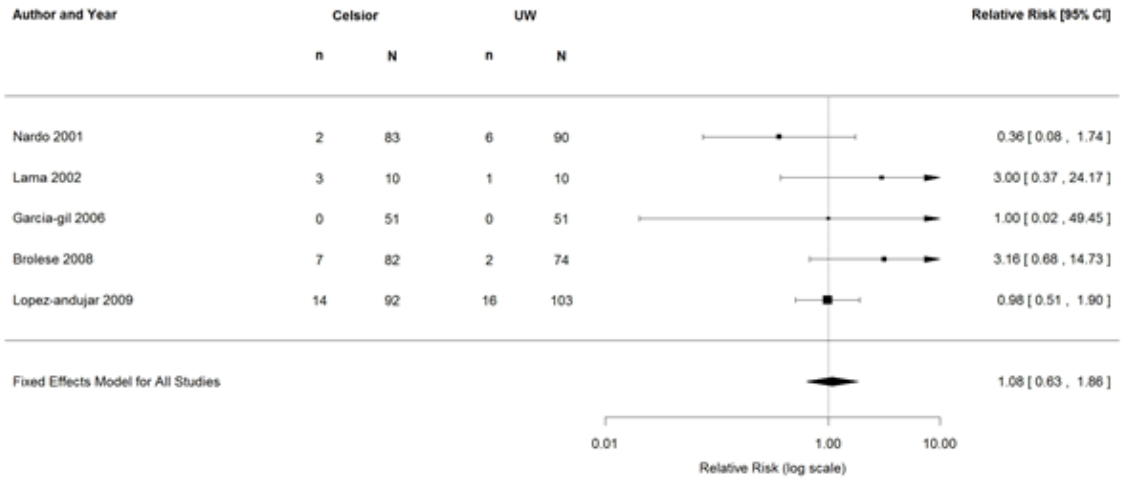
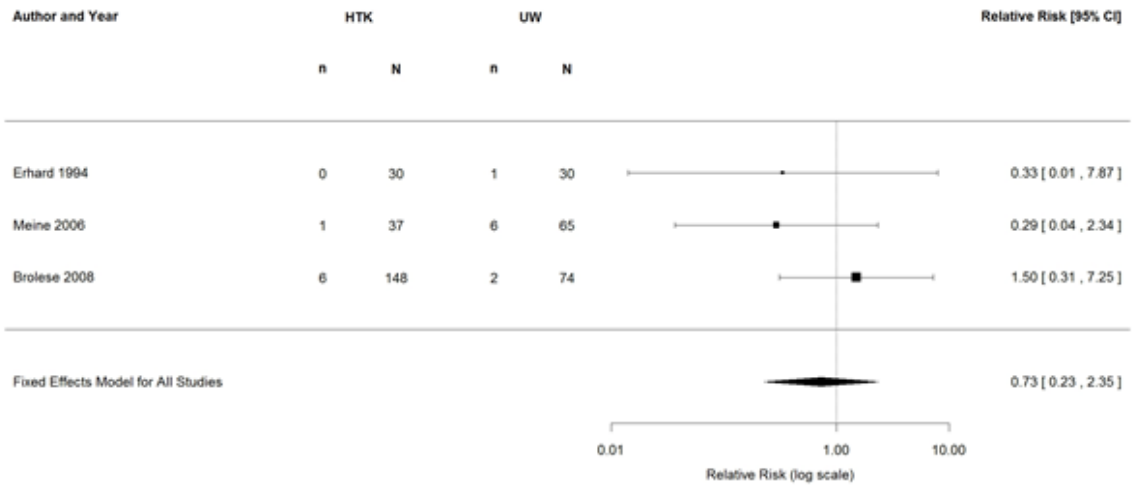


Figure 6.3. Forest plot to show Relative Risk (RR) of early dysfunction comparing HTK with UW Solution in randomised controlled trials. N= total patients in study arm, n= number of patients with early dysfunction. Summary RR calculated by fixed effect meta-analysis, <1 Favours HTK. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =11.67%, Cochran's Q test for heterogeneity Q=1.78, p=0.41.



6.3.2 **Primary non-function**

Primary non-function rates were reported in 13 studies, Table 6.4. In four studies PNF was defined as patient death or re-transplantation in the first week. In nine studies primary non-function was undefined. Overall rates of PNF were very low (range 0-7.3%). In only three studies was the overall primary non-function rate greater than 3% (95, 311, 314). No study was individually powered to detect differences for such a low event rate. Meta-analysis showed no significant difference in risk of primary non-function between Celsior and UW (6 studies FEM: RR=0.83, 95%CI=0.25-2.73, p=0.76, Figure 6.4) with low heterogeneity (Cochran Q=2.18, p=0.82, I^2=0%).

Figure 6.4. Forest plot to show Relative Risk (RR) of primary non-function comparing Celsior Solution with University of Wisconsin Solution (UW) in randomised controlled trials. N= total patients in study arm, n= number of patients with early dysfunction. Summary RR calculated by fixed effect meta-analysis, <1 Favours Celsior. 95%CI=95% confidence interval. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =0%, Cochran's Q test for heterogeneity Q=2.18, p=0.82.

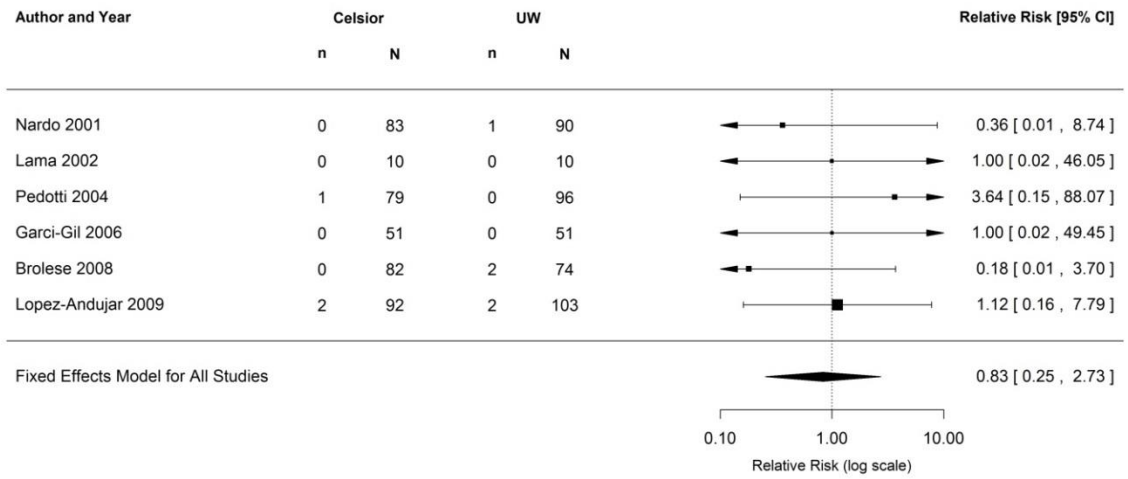


Table 6.4. Primary non-function rates in included studies. Studies are grouped by preservation solutions compared. CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate Solution, IGL-1= Institut Georges-Lopez-1 Solution, UW= University of Wisconsin Solution, UW High Na= Modified UW with high sodium content. N= number in group, n= number with primary non-function, RR=Relative risk of primary non-function, Solution 1 versus Solution 2.

Study	Solution 1				Solution 2				RR	P-Value	Definition of Primary Non-Function
		N	n	%		N	n	%			
Erhard 1994	UW	30	1	3.33	HTK	30	1	3.33	1.00	1.00	Undefined
Brolese 2008	UW	74	2	2.70	HTK	148	7	4.73	0.57	0.47	Undefined
Nardo 2001	UW	90	1	1.11	Celsior	83	0	0.00	2.77	0.53	Death or re-transplantation days 1-7
Lama 2002	UW	10	0	0.00	Celsior	10	0	0.00	1.00	1.00	Undefined
Pedotti 2004	UW	96	0	0.00	Celsior	79	1	1.27	0.27	0.43	Undefined
Garcia-Gil 2006	UW	51	0	0.00	Celsior	51	0	0.00	1.00	1.00	Death or re-transplantation days 1-7
Brolese 2008	UW	74	2	2.70	Celsior	82	0	0.00	5.53	0.46	Undefined
Lopez-Andujar 2009	UW	103	2	1.94	Celsior	92	2	2.17	0.89	0.91	Death or re-transplantation days 1-7
Nardo 2004	HTK	20	1	5.00	Celsior	20	0	0.00	3.00	0.49	Undefined
Brolese 2008	HTK	148	7	4.73	Celsior	82	0	0.00	8.36	0.14	Undefined
Dondero 2010	UW	92	4	4.35	IGL-1	48	1	2.08	2.09	0.51	Death or re-transplantation days 1-7
Schwartz 1991	UW	32	1	3.13	Ringers	34	2	5.88	0.53	0.60	Undefined
Adam 1991	Albumin	42	1	2.38	Ringers	41	3	7.32	0.34	0.34	Undefined
Sanchez-Urdazpal 1993	Plasmalyte	20	0	0.00	Carolina Rinse	23	0	0.00	1.14	0.95	Undefined
Bachmann 1997	Albumin	10	0	0.00	Carolina Rinse	10	0	0.00	1.00	1.00	Undefined

6.3.3 **Re-transplantation**

Re-transplantation rates varied from 0-12%, Table 6.5. Re-transplantation was treated as a negative survival outcome and hazard ratio was estimated by converting events to RLS for meta-analysis. Meta-analysis showed no significant difference in risk of re-transplantation between Celsior and UW (3 studies, FEM: RLS=0.95, 95%CI=0.47-1.92, p=0.89, Figure 6.5). Heterogeneity was low in this analysis (Cochran Q=0.81, p=0.64, I^2=0%). No difference in re-transplantation rates was found between HTK and UW in two studies (95, 293), between Celsior and HTK in two studies (295, 317), between UW and IGL-1 in one study (314), or between UW and high-sodium UW in one study (310).

Figure 6.5. Forest plot to show risk of re-transplantation, presented as Relative Log Survival (RLS), comparing Celsior with UW Solution in randomised controlled trials. Summary RLS calculated by fixed effects meta-analysis, <1 Favours Celsior. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. I^2 test for heterogeneity =%, Cochran Q=0.81, p=0.64).

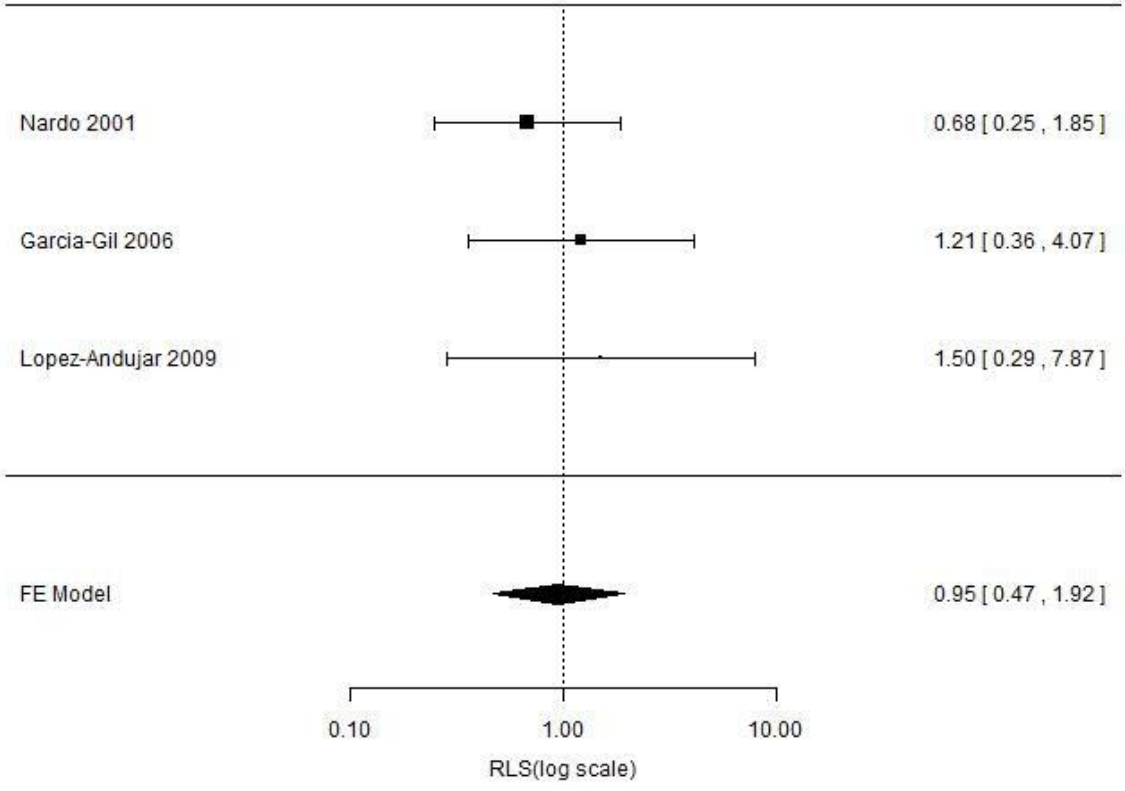


Table 6.5. Re-transplantation rates in included studies. Studies are grouped by preservation solutions compared. CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate Solution, IGL-1= Institut Georges-Lopez-1 Solution, NA= Relative risk not calculated against zero events, UW= University of Wisconsin Solution, UW High Na= Modified UW with high sodium content. N= number in group, n= number re-transplanted, RR=Relative risk of re-transplantation, Solution 1 versus Solution 2.

Study	Solution 1				Solution 2				RR	P-Value	Follow up
		N	N	%		N	N	%			
Erhard 1994	UW	30	1	3.33	HTK	30	1	3.33	1.00	1.00	Unclear
Brolese 2008	UW	74	0	0	HTK	148	5	3.38	0.18	0.24	3 months
Nardo 2001	UW	90	11	12.22	Celsior	83	7	8.43	1.45	0.42	12 months
Garcia-Gil 2006	UW	51	5	9.80	Celsior	51	6	11.76	0.83	0.75	36 months
Brolese 2008	UW	74	0	0	Celsior	82	3	3.65	0.16	0.22	3 months
Lopez-Andujar 2009	UW	103	3	2.91	Celsior	92	4	4.35	0.67	0.59	26 months mean
Nardo 2004	HTK	20	4	20	Celsior	20	4	20	1.00	1.00	1 month
Brolese 2008	HTK	148	5	3.38	Celsior	82	3	3.65	0.92	0.91	3 months
Dondero 2010	6% versus 7% but unclear which is which (UW or IGL-1).										1 month
Kurzawinski 1994	UW	20	0	0	UW high Na	20	1	5	0.33	0.49	9 months
Adam 1991	Albumin	42	3	7.14	Ringers	41	3	7.32	0.98	0.98	1 month
Bachmann 1997	Albumin	10	0	0	Carolina Rinse	10	0	0	1.00	1.00	12 months

6.3.4 **Patient survival**

Patient survival was reported by 13 studies (Table 6.6). No study was individually powered for small differences in patient survival and no study reported a difference related to the preservation fluid used. There was no difference between Celsior and UW (6 studies, FEM: RLS= 0.90, 95%CI=0.60-1.36, p=0.63). Heterogeneity was low in this analysis (Cochran Q=1.35, p=0.93, I^2=0%). There was no difference in patient survival between HTK and UW in two studies (95, 293), between Celsior and HTK in two studies (295, 317), or between UW and IGL-1 in one study (314).

6.3.5 **Graft survival**

Graft survival was reported by ten studies and closely followed patient survival in these studies. No study was individually powered for small differences in graft survival and no study reported a difference related to the preservation fluid used. There was no difference between Celsior and UW in terms of graft loss (6 studies, FEM: RLS=0.85, 95%CI=0.59-1.23, p=0.39). Heterogeneity was low in this analysis (Cochran Q=1.40, p=0.39, I^2=0%). There was no evidence of a difference between HTK and UW in two studies (95, 293), between Celsior and HTK in two studies (295, 317), or between UW and IGL-1 in one study (314)

Figure 6.6. Forest plot to show patient survival, presented as Relative Log Survival (RLS), comparing Celsior with UW Solution in randomised controlled trials. Summary RLS calculated by fixed effect meta-analysis, <1 Favours Celsior. Squares represent individual study effects, with the size of the box relating to the weight of the study in the meta-analysis. Diamond represents summary effect from meta-analysis. Horizontal bars represent 95% confidence intervals. Heterogeneity is low: I^2 test for heterogeneity =0%, Cochran Q=1.35 p=0.93.

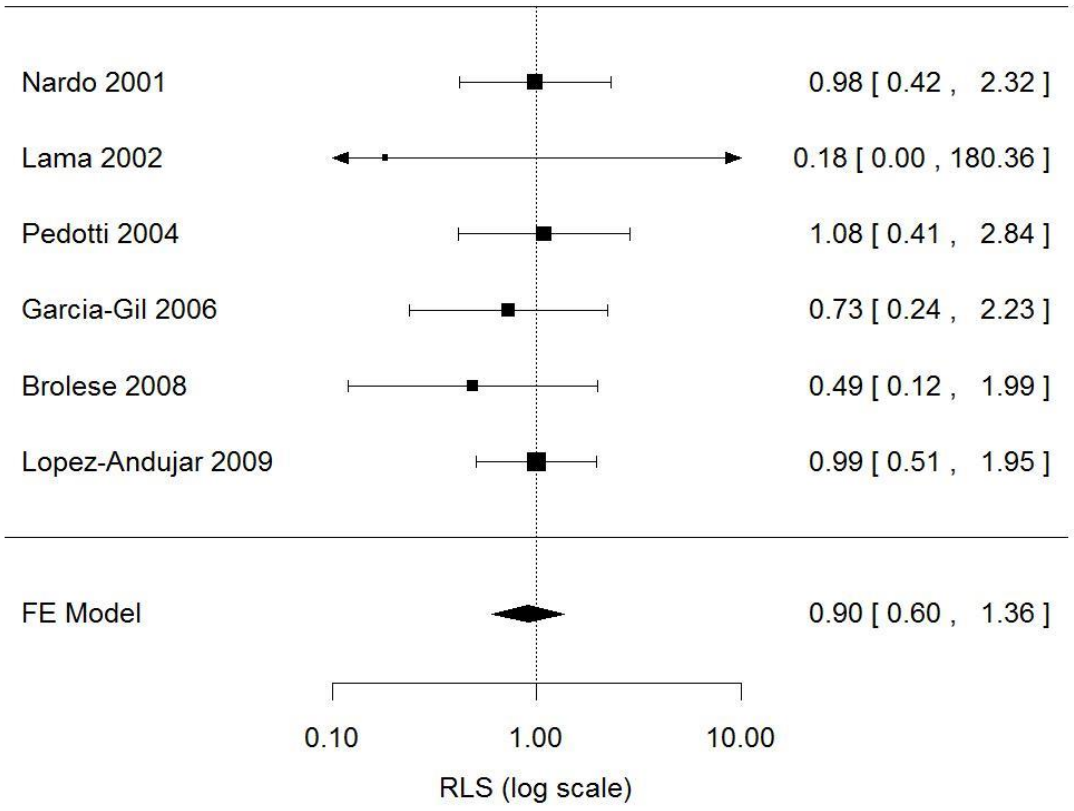


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Table 6.6. Patient survival in included studies. Percentage survival is that presented in the original papers or calculated from numbers followed up. Where more than one paper reports the same study, numbers from the paper including more patients are displayed. Blank spaces indicate no data reported at that time-point. CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate, IGL-1= Institut Georges Lopez-1 Solution, UW= University of Wisconsin Solution.

Study	Solutions compared	Follow up (Months)									
		1	3	4	6	12	18	24	30	36	60
Erhard 1994	UW v HTK		86 v 87%						73 v 77%		
Meine 2006	UW v HTK			92 v 89%				94 v 86%			
Brolese 2008	UW v HTK v Celsior	92 v 95 v 94%	88 v 93 v 94%								
Nardo 2001	UW v Celsior	96 v 95%				88 v 88%					
Lama 2002	UW v Celsior				80 v 100%						
Pedotti 2004	UW v Celsior	92 v 96%				91 v 90%					
Garcia-gil 2006	UW v Celsior	98 v 96%	94 v 94%		90 v 90%	80 v 85%		80 v 85%			67 v 82%
Lopez-Andujar 2009	UW v Celsior					83 v 83%		80 v 77%		76 v 70%	
Nardo 2004	HTK v Celsior	90 v 95%	90 v 95%			85 v 90%					
Dondero 2010	UW v IGL-1					86 v 83%				83 v 81%	
Cofer 1992	UW v Eurocollins					97 v 91%	97 v 84%				
Adam 1991	Albumin v Ringers	93 v 87%									
Bachmann 1997	Albumin v Carolina Rinse				100 v 100%	100 v100%					

6.3.6 **Biochemical parameters**

The majority of studies presented serum transaminases, bilirubin and clotting profiles with graphs alone. One study found a significantly higher peak alanine aminotransferase on day three post-operatively with HTK than with Celsior solution (304), Table 6.7, and one study found a significantly higher peak bilirubin on day five post-operatively with UW than with Celsior (105), Table 6.8. One study found a lower prothrombin activity on day five with Celsior solution than with UW (105), one study found a lower prothrombin activity on day one with HTK than with Celsior solution (304), Table 6.9.

6.3.7 **Biliary complications**

Overall biliary complication rates were 11% in the studies that reported this outcome (nine studies, range 0-18%, including leaks, stenosis and other, Table 6.10. Overall rates of biliary stenosis (including anastomotic and non-anastomotic) were less than 5%, in keeping with large series (321). No study found a difference in risk of biliary stenosis or leak between preservation fluids. However, one study did find a greater total number of biliary complications with HTK than UW (Meine et al, p=0.03) (293).

Table 6.7. Peak alanine aminotransferase within first seven days after transplantation. Presented as either mean +/-standard deviation or as median and range. CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate, NS= Not Significant as reported by original paper, UW= University of Wisconsin Solution. Statistical tests and P-values are those presented in the original papers.

Study	Solution 1, Number of livers, Mean+/- SD (numbers in brackets are median and range).			Solution 2, Number of livers, Mean+/- SD (numbers in brackets are median and range).			Units	P-Value
Erhard 1994	UW	30	599.5 +/- 585.1	HTK	30	786.5 +/-118.3	Units/L	0.16[23]
Brolese 2008	UW	74	Graph presented	HTK	148	Graph presented	Units/L	NS
Nardo 2001	UW	90	Graph presented	Celsior	83	Graph presented	Units/L	NS
Lama 2002	UW	10	22.7 +/- 21	Celsior	10	30.1 +/- 28	µkat/L	0.50
Pedotti 2004	UW	96	(8.46, 0.8 to 55.65)	Celsior	79	(9.05, 0.28 to 77.02)	µkat/L	NS[24]
Garcia-gil 2006	UW	51	Graph presented	Celsior	51	Graph presented	Units/L	NS[24]
Brolese 2008	UW	74	Graph presented	Celsior	82	Graph presented	Units/L	NS
Lopez-Andujar 2009	UW	103	Graph presented	Celsior	92	Graph presented	Units/L	NS[25]
Nardo 2004	HTK	20	1145 +/- 937	Celsior	20	702 +/- 533	Units/L	0.03[24]
Brolese 2008	HTK	148	Graph presented	Celsior	82	Graph presented	Units/L	NS
Dondero 2010	UW	92	1188 +/- 668	IGL-1	48	1208 +/- 589	Units/L	NS[24]
Kurzawinski 1994	UW	20	(429, 50 to 2214)	High Na UW	20	(518, 121 to 2768)	Units/L	NS[25]
Schwartz 1991	UW	32	1431 +/- 256	Ringers	34	1642 +/- 405	Units/L	NS[24]
Cofer 1992	UW	32	Graph presented	Eurocollins	24	Graph presented	Not given	0.60[23]
Adam 1991	Albumin	42	454 +/- 434	Ringers	41	779 +/- 929	Units/L	0.05[24]
Sanchez-Urdazpal 1993	Plasmalyte	20	Graph presented	Carolina Rinse	23	Graph presented	Units/L	NS[26]
Bachmann 1997	Albumin	10	434 +/- 297	Carolina Rinse	10	428 +/- 421	Units/L	Not tested

[23] Analysis of variance.
[24] Student's T-test.
[25] Mann Whitney U-test.
[26] Wilcoxon test.

Table 6.8. Peak serum bilirubin after within first seven days transplantation. Presented as either mean and standard deviation (SD) or as median and range.CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate, NS= Not Significant as reported by original paper, UW= University of Wisconsin Solution. Statistical tests and P-values are those presented in the original papers.

Study	Solution 1, Number of livers, Mean Bilirubin +/- SD (numbers in brackets are median and range).			Solution 2, Number of livers, Mean Bilirubin +/- SD (numbers in brackets are median and range).			Units	P-Value
Brolese 2008	UW	74	Graph presented	HTK	148	Graph presented	Mg/dl	NS
Nardo 2001	UW	90	Graph presented	Celsior	83	Graph presented	Mg/dl	NS
Lama 2002	UW	10	80.3 +/- 82	Celsior	10	85.1 +/- 62	Mmol	0.88
Pedotti 2004	UW	96	(90.6, 8.2 to 641.3)	Celsior	79	(51.3, 17.1 to 331.7)	Micromol	0.006[27]
Garcia-gil 2006	UW	51	Graph presented	Celsior	51	Graph presented	Mg/dl	NS[27]
Brolese 2008	UW	74	Graph presented	Celsior	82	Graph presented	Mg/dl	NS
Lopez-Andujar 2009	UW	103	Graph presented	Celsior	92	Graph presented	Mg/dl	NS[28]
Nardo 2004	HTK	20	9.3 +/- 6.6	Celsior	20	10.4 +/- 6.4	Mg/ml	NS[27]
Brolese 2008	HTK	148	Graph presented	Celsior	82	Graph presented	Mg/dl	NS
Dondero 2010	UW	92	Graph presented	IGL-1	48	Graph presented	Micromol	NS[27]
Kurzawinski 1994	UW	20	(112, 30 to 318)	High Na UW	20	(124, 45 to 256)	Micromol	NS[28]
Cofer 1992	UW	32	Graph presented	Eurocollins	24	Graph presented	Not given	0.77[29]
Sanchez-Urdazpal 1993	Plasmalyte	20	Graph presented	Carolina Rinse	23	Graph presented	Mg/dl	Not given
Bachmann 1997	Albumin	10	Graph presented	Carolina Rinse	10	Graph presented	Mg/dl	NS

[27] Student's T-test.
[28] Mann Whitney U-test.
[29] Analysis of variance.

Table 6.9. Clotting profile within first seven days after transplantation. APTT= Activate Partial Thromboplastin Time, CR= Carolina Rinse, HTK= Histidine-Tryptophan-Ketoglutarate, INR= International Normalised Ratio, NS= Not Significant, PT= Prothrombin Time (% activity), UW= University of Wisconsin Solution. Statistical tests and P-values are those presented in the original papers.

Study	Solutions compared	Mean and standard deviation, median and range in brackets.	Units	P-Value
Brolese 2008	UW vs HTK	Graph presented	INR	NS
Lama 2002	UW vs Celsior	Peak: 1.65+/-0.6 versus 1.8+/-0.6	INR	0.56[30]
Pedotti 2004	UW vs Celsior	Nadir: (82, 13.7 to 108 versus 80, 12.7 to 106	PT (%)	0.04
Brolese 2008	UW vs Celsior	Graph presented	INR	NS
Garcia-Gil 2006	UW vs Celsior	Graph presented	PT (%)	NS[31]
Lopez-Andujar 2009	UW vs Celsior	Graph presented	APTT (%)	0.76[30]
Nardo 2004	HTK vs Celsior	Nadir: 42+/-9 versus 53+/-18	PT (%)	0.05[31]
Brolese 2008	HTK vs Celsior	Graph presented	INR	NS
Dondero 2010	UW vs IGL-1	Graph presented	PT (%)	NS[31]
Kurzawinski 1994	Ringers vs UW	Peak: 15.1+/-0.8 versus 15.5+/- 0.6	PT (min)	NS
Cofer 1992	Eurocollins vs UW	Graph presented	PT (min)	0.24[31]
Sanchez-Urdazpal 1993	Plasmalyte vs Carolina Rinse	Data not presented	PT (min)	NS[32]

Table 6.10. Biliary complications in included studies. CR=Carolina Rinse, HTK=Histidine Tryptophan Ketoglutarate, IGL-1=Institut Georges Lopez-1 Solution, NAS= Non-anastomotic stenosis, UW=University of Wisconsin solution.

Study	Complication rates, Number (%)		Follow up	P-Value
Erhard 1994	HTK (n=30) Stenosis 1 (3.3)	UW (n=30) Stenosis 1 (3.3)	30 months	 1.00
Meine 2006	HTK (n=31) Stenosis 4 (12.9) Leak 2 (6.5) Ischaemic 2 (6.5)	UW (n=58) Stenosis 4 (6.9) Leak 0 (0) Ischaemic 1 (1.7)	4 months	 0.44 0.12 0.55
Nardo 2001	"No difference was found"		12 months	NA
Lama 2002	Celsior (n= 10) Leak 0 (0)	UW (n=10) Leak 0 (0)	6 months	 1.00
Garcia-Gil 2006	Celsior (n=51) Leak 2 (3.9) Stenosis 7 (13.7) NAS 1 (1.9)	UW (n=51) Leak 2 (3.9) Stenosis 5 (9.8) NAS 1 (1.9)	5 years	 1.00 0.76 1.00
Lopez-Andujar 2009	Celsior (n=92) Leak 9 (9.7) Stenosis 2 (2.2) Other 5 (5.4)	UW (n=103) Leak 9 (9.8) Stenosis 2 (2.2) Other 9 (9.8)	26 months (mean)	 0.81 1.00 0.42
Nardo 2004	HTK (n=20) Stenosis 0 (0)	Celsior (n=20) Stenosis 1 (5)	12 months	 1.00
Dondero 2010	UW (n=92) NAS 3 (3.3)	IGL-1 (n=48) NAS 1 (2.1)	3 years	 1.00
Bachmann 1997	Albumin (n=10) Total 0 (0)	CarolinaRinse (n=10) Total 0 (0)	12 months	 1.00

[30] Mann Whitney U-test.
[31] Student's T-test.
[32] Wilcoxon test.

6.3.8 **Studies of initial aortic perfusion in the donor**

Two small studies examined the effect of varying the fluid used for the initial aortic perfusion before ex situ flush and storage with UW. One study compared Ringer's lactate to UW for this purpose, finding no difference in primary non-function rate, peak alanine aminotransferase or prothrombin time (311). One study compared Eurocollins solution to UW for this purpose, finding no difference in patient survival or trend in serum bilirubin, alanine aminotransferase or prothrombin time (312).

6.3.9 **Studies of pre-reperfusion flush in the recipient**

Three studies examined the effect of varying the fluid used to wash UW out of the liver before blood reperfusion in the recipient. One study found a lower bilirubin for 96 hours and lower alanine aminotransferase for 12 hours with Carolina rinse compared to plasmalyte, but no difference in primary non-function or peak prothrombin time (306). One study found a lower alanine aminotransferase on day five using albumin for this purpose rather than Ringer's lactate, but no difference in primary non-function, patient or graft survival (313). In a small study of only 20 livers, there was no difference in primary non-function, re-transplantation, patient survival, graft survival, peak alanine aminotransferase, or biliary complications comparing albumin to Carolina rinse for this purpose (307).

6.4 Discussion

This study reviews the evidence comparing preservation fluids for the static, hypothermic preservation of deceased donor livers for transplantation. Overall the quality of individual studies was poor and individually most studies were under-powered, even for their stated primary outcome. However, in meta-analysis there were results from 821 livers comparing UW with Celsior solution. The best available evidence suggests there is no difference between UW and Celsior solution in terms of early dysfunction, primary non-function, re-transplantation, patient survival or graft survival. There is little good evidence to support a difference in other outcomes between these two fluids. There is also little good evidence to support a difference in outcomes between the other commonly used preservation fluids.

Serum enzyme levels and clotting factor profiles in the first week post-transplant are inconsistently reported. However, they may represent the status of the recipient as much as they represent the status of the donor liver.

Biliary stenotic lesions are a major source of morbidity following liver transplantation (321). We did not find evidence of an increased risk of biliary stenotic lesions with any preservation fluid, although all included studies were underpowered for this relatively rare outcome. One study found a higher risk of biliary complications with HTK than with UW, but this was inclusive of leaks and anastomotic strictures (293). This study had a large number of dropouts and also reported older donors in the HTK group (293). A large registry analysis (n=1,771 livers) found that UW preservation was associated with more ischaemic biliary lesions than HTK in univariate analysis, although in multivariate analysis UW and HTK preservation were not independently associated with the development of ischaemic biliary lesions (321). This multivariate analysis included data from liver transplants where different preservation fluids were used during different time periods, so era-effect may

have played a part. Biliary stenotic lesions may also develop months or years after transplantation, and so the follow up in many of the included studies might not be long enough to fully evaluate this outcome (322). The relatively high viscosity of UW has been considered a barrier to adequate preservation of peri-biliary vasculature, and hence a risk factor for the development of ischaemic biliary lesions. Studies that support this hypothesis have been retrospective analyses of data collected over many years, or between-centre comparisons (84, 322).

Other arguments against the use of UW have included its relatively high potassium content, although there is no evidence that this is reflected in a higher serum potassium in the recipient after reperfusion (323). The high potassium content and potential for cardiac arrhythmia after reperfusion is the reason for flushing UW from the liver before blood reperfusion in the recipient. The three studies included here that compared fluids for this purpose were too small to draw firm conclusions. A previous systematic review of the reperfusion technique for orthotopic liver transplantation found no evidence to support any specific technique (324).

In registry analyses of large numbers of liver transplants (n=17,428 transplants), HTK has been associated with worse graft survival than UW, particularly for livers from donation after circulatory death (HR=1.44) (291). This review did not find such an association in the included studies, but there was inadequate power. A previous systematic review has also concluded that there is no significant difference between HTK and UW from live-donor and deceased-donor studies, not all of which were RCTs (292). A more recent systematic review and meta-analysis by Zuluagu et al, using an indirect meta-analysis to compare Celsior with HTK, found no significant difference in primary dysfunction, initial poor function, or primary non-function (294). Zuluagu et al also found no evidence of a difference in primary dysfunction, primary non-function and graft survival when comparing Celsior or HTK with UW (294).

Studies of live-donor livers for transplantation have found similar results with the use of HTK and UW for back-table flush (325).

The three most commonly used preservation solutions do not vary greatly in price; Celsior costs US$250 per litre, HTK costs US$195 per litre and UW costs US$190 per litre. It was not within the study protocol to conduct a cost-effectiveness analysis and there are currently no published assessments of cost-effectiveness in liver preservation which address this issue.

The present study has several important limitations. Many of the included studies are individually underpowered and even in meta-analysis of multiple studies there may not be adequate power for some outcomes. However, this is the largest comparison of UW with Celsior solution using data from RCTs. For this particular comparison we have good power to detect clinically relevant differences in early dysfunction, graft and patient survival. The follow up period of the included studies does vary, but a validated method to estimate hazard ratios from binary survival data was used. The definition of early dysfunction varies between studies but the underlying effect should be the same. Furthermore, the same definition is used between arms in each individual study. The combination of these results in meta-analysis is supported by the lack of heterogeneity. The included studies were mostly of poor to moderate quality with only four achieving 3 or more points on the Jadad scale. Despite this, all included studies were RCTs and therefore represent the best level of evidence currently available. All studies included livers from donation after brain-death, which remains the most common source of livers for transplantation. Livers from donation after brain-death are associated with improved graft and patient survival and less biliary complications compared to livers from donation after circulatory death (45, 326). There may therefore be more potential to improve upon outcomes for livers from donation after circulatory death. New technologies such as

hypothermic machine perfusion (123) and normothermic machine perfusion (327) of the liver remain in the early stages of clinical evaluation.

Conclusion

This review appraises the best available evidence comparing currently available preservation fluids in deceased-donor liver preservation. There is good evidence that University of Wisconsin and Celsior Solutions are associated with equivalent outcomes. There is not good evidence from RCTs that there is any difference in efficacy between UW and HTK or between Celsior and HTK. There is so far only one small RCT comparing UW to IGL-1 Solution.

7. Discussion

The adequate hypothermic preservation of livers and kidneys for transplantation minimises the risk of cellular damage to the organ both during and after the preservation period. Clinical protocols for solid organ preservation should be based upon the best level of evidence available. The aim of this thesis was to provide a thorough assessment of hypothermic preservation fluids for deceased donor livers and kidneys and the potential of hypothermic machine perfusion of kidneys. The research undertaken was primarily in the form of systematic review and meta-analysis, with an analysis of registry data that was prompted by the conclusions of the first systematic review. The results of the original work included in this thesis have been discussed in detail at the end of each chapter. Therefore, this discussion will summarise these findings, make recommendations for future practice and research, and appraise the methodology used.

7.1 Recommendations for clinical practice

7.1.1 Kidney preservation

There is evidence from RCTs that the use of Celsior, HTK and UW Solutions for kidney preservation, are all associated with similar rates of DGF and graft survival rates. There is also clear, randomised, evidence that HTK and UW Solution are superior to EC Solution. The majority of these studies were conducted using organs from DBD, however, the graft survival of controlled DCD kidneys is similar to DBD kidneys, and they are therefore unlikely to have different requirements for static cold storage fluids.

This work also identified that HOC had not been compared to any of the commonly used preservation fluids in a prospective study; the analysis of national data demonstrated that the use of HOC, in the particular population and transplant program of the UK, was associated with similar outcomes to UW Solution. There was a potential association between the mixing of preservation fluids for DCD kidneys and worse graft survival. There is not good clinical evidence comparing IGL-1 Solution to UW Solution.

The results of RCTs examining HMP of kidneys with Belzer MPS show consistent evidence of reduced rates of DGF compared to SCS in other preservation fluids. An effect is present in all donor types. It should be noted that no included study has used the same preservation fluid for both HMP and SCS. The absolute reduction in risk does depend upon the baseline risk, and the number-needed-to-treat for each donor type therefore varies. The relationship between this reduction in DGF and graft survival is somewhat complicated by mixing populations of different donor types in some studies. For DCD kidneys, HMP considerably reduces the absolute risk of DGF; however this does not translate to improved graft survival in this donor type. In DBD kidneys, HMP may be linked to an improvement in graft survival, although this has only been strongly evident in ECD kidneys, which have the worst baseline risk for graft loss.

7.1.2 **Liver preservation**

The situation is slightly different in liver preservation where, generally speaking, trials of preservation fluids have been smaller than for kidneys. There is good evidence that Celsior and UW Solutions are associated with equivalent functional and survival outcomes. This is subtly different from the situation regarding HTK, where we do not have good evidence that it is any worse, or better, than the other two commonly used fluids. The continued use of HTK for liver preservation is therefore based upon experiential evidence rather than that

from clinical trials. There is also not good evidence comparing IGL-1 Solution to UW Solution. Only one RCT reports the use of HOC in liver preservation, although it was used only as an *in situ* flush before UW was used *ex situ*, to further flush the liver. This regimen has not been compared to others in any RCT. The majority of RCTs used both aortic and portal perfusion *in situ*, with some also using additional flushing *ex situ*.

7.2 Recommendations for future work

Before proposing clinical trials that may answer questions that have been raised by this work, it may be wise to identify the pitfalls in clinical trials of preservation methods. These can be divided into generic and specific methods that will improve the quality of clinical trials in this field, and produce results with internal and external validity.

7.2.1 General recommendations

The overall quality of the preservation studies identified in the systematic reviews and included in this work, was poor; the quality of RCTs in transplantation has previously been criticised (179). The publication and development of the SPIRIT and CONSORT guidance will improve the quality of protocols and trial reports in coming years as long as they are followed (250, 328). Improving internal validity of trials in organ preservation for transplantation depends upon improving the standards that are now expected of clinical trials in all fields of medicine: adequate randomisation method, allocation concealment, blinding, adequate power, completeness of follow up and intention-to-treat analysis.

7.2.2 **Recommendations for trials in organ preservation**

Achieving the quality measures above can be obstructed by problems specific to the nature of a specialty such as transplantation. However, there are ways that these problems can be solved. For example, the limited time frames involved in emergency transplantation mean that there is a time pressure on consenting recipients and donor families depending on the legal framework in each country. This has implications for the quality of randomisation method and allocation concealment if complex algorithms are required out of hours, but centralised or web-based systems can achieve this. Patients can also be consented to the study whilst on the waiting list.

The blinding of surgeons to the preservation method can prove difficult, but every attempt to do this should be made; one preservation fluid looks much like another in standardised packaging. Digital displays on perfusion pumps can also be obscured. Emergency un-blinding procedures can be put in place if needed. Also, the outcome assessors and data processors can potentially be blinded to the preservation method more easily than staff on the ground.

Completeness of follow up becomes increasingly important the less common an outcome is (181). With longer follow up, more patients tend to be lost and in transplantation this means that data on long term graft survival in trials is at risk. In this specialty we are however lucky to have national registries in many countries, which can increase the potential for full follow up of trial patients. Clinical trials co-ordinators should engage with these services.

Intention-to-treat analysis proves to be a particular sticking point in studies of transplantation. For example, if a recipient is randomised to receive a kidney preserved by a particular method, but is not fit for the transplant on the day, how do we deal with this outcome? Without a transplanted organ to follow up, there can be no transplant-related outcomes to monitor. What if a donor is randomised for their liver to be preserved by a particular method but the

retrieval does not proceed? The answers to these concerns lie in getting to the bottom of all withdrawals from the trial at any stage and comparing between study arms. Organs that are randomised but not transplanted must be assessed as a graft loss, as the reason for non-transplantation could be intimately linked to problems with the preservation method. If the reasons for non-transplantation are unrelated to the preservation method, then these will even out between the study arms with adequate randomisation.

The external validity of preservation trials depends more upon the specifics of transplantation programs. Adequate follow up to discern the impact of interventions on graft survival is imperative, relating to full follow up of included patients as well as adequate time for differences in outcome to become apparent. Survival outcomes should also be explicitly defined to avoid confusion, for example: graft loss (including or excluding death with a functioning graft and non-transplantation of a randomised organ).

Beyond graft survival the choice of surrogate outcomes, which permit earlier assessment of intervention effects, should be based upon those which have been shown empirically to be related to worse graft survival. The two most well established are DGF of DBD kidneys (235) and EAD of livers (296).

Delayed graft function has been defined in different ways, but the most recent assessment seems to show that almost 9 out of 10 definitions of DGF were equally sensitive for graft survival of DBD kidneys (242). No definition of DGF was associated with worse DCD graft survival and only one was associated with worse eGFR at one year. The authors concluded that given these results, the most common and most simple definition should be used, that of the requirement for dialysis in the first week after transplantation (242). Given the costs associated with DGF however, it may still be wise to consider it as an important outcome in trials of DCD kidney preservation.

The baseline risks for graft loss and DGF in DBD kidneys are low, meaning that large studies are required to demonstrate effects in a specialty that may not

have large numbers of patients to include. Multicentre and multinational studies could provide this power.

The interpretation of study outcomes could be helped by avoiding mixing different donor types, which may have different baseline risks for different outcomes. If different donor types are to be included, then it may be wise to structure or size the study, and subdivide the report, in a manner that allows interpretation for each donor type.

7.2.3 Clinical trials

Indications for specific clinical trials have been highlighted by the work conducted for this thesis. A few examples are described below in the style of research questions, with suggested primary outcomes.

Kidney

The potential to improve graft survival of deceased donor kidneys through improved preservation methods lies mostly with kidneys from older donors and donors with comorbidities. These donors make up a large latent donor pool in the UK. The use of paired data analysis could potentially reduce the number of required inclusions (sample size), if each kidney from the same donor is allocated to a different preservation method. The power would depend upon the correlation in outcomes between two kidneys from the same donor, which may differ by donor type. All sample size calculations are alpha 0.05 with power of 80%.

1. *Does the static cold storage of DCD and DBD kidneys from donors >60 years old with UW Solution improve graft survival compared to HOC?*

2. *Does short-term machine perfusion (once the kidney has arrived at the transplant centre) improve the graft survival of DCD and DBD kidneys from donors >60 years old compared to static cold storage?*

For the two studies above, the primary outcome would be three year graft survival, which excludes death with a functioning graft. A non-transplanted kidney that has been randomised counts as a graft loss, it would therefore be important to blind surgeons to the preservation method. The sample size for either study, for unpaired analysis would be calculated thus: Graft survival for donors >60 years old is ~80% (244). To demonstrate an improvement to the level of donors aged 40-59 years (~90% three year graft survival) (244) would require approximately 400 kidneys (200 in each arm). The second study could potentially be paired in analysis, so that kidneys from the same donor are randomised to machine perfusion or not and thereby reduce the required sample size. The degree of reduction would depend on the correlation between the graft survival of two kidneys from the same donor, which is not known. Some work has been conducted to assess the correlation in renal function at one year and DGF rates in kidneys from the same donor (329).

3. *Does oxygenated, hypothermic machine perfusion improve the graft survival of DCD and DBD kidneys from donors >60 years old compared to standard hypothermic machine perfusion?*

The primary outcome for the study above would be one year graft survival. The sample size for this analysis, for unpaired analysis would be calculated thus: Graft survival for older donor kidneys treated with non-oxygenated HMP is ~90% (133). To demonstrate improvement to the level of one year graft survival across all donors (~96%) would require 540 kidneys (270 in each arm). The study could be paired to reduce numbers required.

Liver

As for kidneys, it may be wise to focus on donors that are currently considered to have worse outcome and may have the greatest potential for improvement.

4. *Does hypothermic machine perfusion of DCD livers improve graft survival compared to static cold storage?*

The primary outcome for the study above would be three year graft survival, which excludes death with a functioning graft. A non-transplanted liver that has been randomised counts as a graft loss. The sample size for this analysis would be calculated thus: To demonstrate an improvement in the graft survival of DCD livers (~64%) to that of DBD livers (~73%) (45) would require approximately 840 livers (420 in each arm).

There is a temptation in developing medical technology to bypass intermediary stages for clinical studies. This leads to clinical comparisons between basic preservation techniques and complex ones, where "middle-level" techniques may have shown a significant benefit if only given the chance, but do not make it beyond experimental studies. This may prove to be the case for liver preservation, where the accelerating pace of development of normothermic machines has overtaken that of hypothermic machines. The cost implications however, remain important.

7.3 Novelty of the work

For each of the research questions that were identified at the start of this work, and during its course, a step forwards has now been taken in terms of the level of evidence available. A number of RCTs have been appraised and synthesised where available to provide a more powerful analysis than had previously been published. The systematic reviews of liver and kidney preservation were novel in their scope by including all potential comparisons between fluids, which other reviews had not (83, 207, 292, 294). The reviews conducted for this thesis provide robust meta-analytical results that increased the power of available study results.

The review of HMP in kidney preservation was published around the same time as three other similar reviews of the topic. However, the review conducted for this thesis encompassed all donor types, whereas others restricted themselves to DCD or DBD (129, 132, 247). The review conducted for this thesis was also augmented by additional details regarding the overlap of patients in the European Machine Perfusion Trial, which had been published in a number of reports (126, 130, 133, 260). Other reviewers did not attempt to access this information.

The registry analysis of outcomes of kidneys preserved by HOC provides a unique analysis of kidney allografts preserved by this method.

7.4 Critique of the methodology used

The key limitation of the methodology used is the underlying weakness of the included data, and this is an inherent problem in both systematic review and also registry analysis. The majority of the studies included in the review of kidney preservation solutions were either not adequately randomised or of poor overall quality if RCTs. However, they are the best level of evidence available, and the conclusions drawn from this review take into account the relative strengths of each study.

Only RCTs were used for the meta-analysis in the kidney HMP review and also for the liver preservations review. Having said this, no RCT included in either review achieved a Jadad score of 4 or 5. In most cases this was due to a lack of blinding; the impact this would have on the objective results of individual studies is debatable. Another problem is the size of included trials; it is possible even with the meta-analysis of several trials that statistical power is still not above generally accepted levels. Despite this, it may still be possible to identify a true effect of an intervention. There was good power for the comparisons of Celsior and UW Solution in liver preservation.

It was particularly difficult to extract data regarding survival outcomes from the included studies, as the numbers followed up were not always well described. Follow up times were also different between studies. The calculation of relative log survival did address this issue to some extent, and allows further use of data that otherwise could not be used to compare studies. As well as graft survival there are also other outcomes which are dependent on the length of follow up of a study. The length of follow up in some included studies was too short to demonstrate differences in risk for some important outcomes. It is also possible that reports of trials do not include all data or outcomes that may have been available. This type of information was not requested on a routine basis for the reviews, as it is not often accessible. All authors were however asked to respond to the quality assessment of their trial, to help with assessing its validity.

It is possible that studies are missed when conducting a systematic review. For the reviews included in this thesis several major databases were included, which covered conference abstracts as well as published and unpublished trials, so it is likely that all relevant trials were identified. No language limits were applied either, so studies originally published in languages other than English were identified. I searched through references in duplicate with Robert Morgan to increase sensitivity.

The use of data from unpublished studies can face criticism, however it is possible that studies remain unpublished because of negative results rather than study quality. The inclusion of data from an unpublished study in the review of liver preservation added an important body of work to the overall assessment of the relative effectiveness of the three most commonly used preservation fluids.

The submission of transplant activity data to the NHS Blood & Transplant registry is mandatory. This registry therefore includes very good information on the follow-up of patients. It does however have some missing data in some

fields, which is inevitable. There was also information unrecorded regarding preservation protocols, which has now been addressed with adaptations to the mandatory recording forms completed at the time of retrieval. This information had to be sought from individual transplant centres for this work and while the general procedure was reported for all centres, there may have been slight differences between retrieval surgeons that could confound the data.

Registry databases are not designed for comparative analyses to prove causal associations, so caution should be used when drawing conclusions based upon this type of data. Whilst we can be quite confident that associations between two variables are present, establishing the causal relationship can be much more difficult. Part of the problem is confounding, for which it is possible to correct, however there are likely to be unknown factors that are also involved. Lastly, it is important when using large databases with many thousands of individuals that statistical significance is not confused with clinical significance.

The studies included in this thesis do not include a cost-effectiveness analysis of the organ preservation methods compared, so the recommendations made are on a scientific basis only. With technological advances the direct costs of preservation methods are likely to become more expensive initially. There are also costs associated with negative outcomes of transplantation. The final decision whether or not to invest in medical technologies to improve transplant outcomes, will require an assessment of the relative costs, and be balanced against the cost of not investing.

7.5 Conclusion

The method used for preservation of livers and kidneys is associated with the outcome of the transplant and by improving preservation methods it is possible to improve results. It is one of the many factors that contribute to the life-span of a transplanted organ and retrieval protocols should therefore take into account evidence-based recommendations. We should be working over coming

years to improve early graft function and survival times by developing preservation methods that protect organs from damage and cause no deterioration in potential function. We should be working to reverse any damage that is unpreventable. In doing this we will not only improve the outcome of standard transplants, but also open up a latent pool of donors that would not be considered today.

8. References

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9. Appendices

9.1 Associated publications and presentations

9.1.1 Publications

The effect of preservation solutions for storage of liver allografts on transplant outcomes. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. *Annals of Surgery* 2014; 260(1):46-55.

Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. *British Journal of Surgery* 2013; 100(8):991-1001.

Preservation Solutions for Static Cold Storage of Kidney Allografts: A Systematic Review and Meta-Analysis. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. *American Journal of Transplantation* 2012; 12(4): 896-906.

Kidney Preservation, Chapter 9 in: *Kidney Transplantation, Principles and Practice* 7th Edition. Saunders Ltd. Editor Peter J Morris and Stuart J Knechtle. Authors: O'Callaghan J, Leuvenink H, Friend P, Ploeg R.

Preservation and perfusion of abdominal organs for transplantation, Chapter 6 in: *Transplantation: A Companion to Specialist Surgical Practice* 5th Edition. Saunders Ltd. Editor John LR Forsythe. Authors: O'Callaghan J, Friend P, Ploeg R.

9.1.2 **Online**

http://www.transplantevidence.com/blog/2013/05/multiple-reviews-of-machine-perfusion-find-reduced-delayed-function-of-kidneys-but-no-effect-on-graft-survival/

9.1.3 **Presentations**

A national registry analysis of kidney allograft preservation with Marshall's Solution in the United Kingdom. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. Presented at the Annual Congress of the British Transplantation Society, Glasgow, UK, and at the World Transplant Congress, San Francisco, USA 2014.

Liver Allograft Preservation Fluids: A Systematic Review and Meta-Analysis. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Presented at the 15th Congress of the European Society for Organ Transplantation, Vienna, Austria 2013.

Preservation solutions for static cold storage of liver allografts. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Presented at the Joint Congress of the British Transplantation Society and Renal Association, Bournemouth, UK 2013.

Systematic review and meta-analysis of hypothermic machine perfusion versus static cold storage of kidney allografts on transplant outcomes. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Presented at the 24th International Congress of The Transplantation Society, Berlin, Germany 2012.
Abstract in: *Transplantation*. 2012; 94 (10S) 277.

Hypothermic machine perfusion of kidneys. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Presented at the 16th National Organ Retrieval Workshop, NHSBT, Oxford, UK 2012.

Evidence based use of organ preservation fluids. O'Callaghan JM. Presented at the Royal College of Surgeons, NHSBT Organ Perfusion Protocol Meeting, London, UK 2012.

Preservation solutions for static cold storage of kidney allografts: a systematic review and meta-analysis. O'Callaghan JM, Knight SR, Morgan RD, Morris PJ. Presented at the 14th Congress of the European Society for Organ Transplantation, Glasgow, UK 2011.
Abstract in: *Transplant International.* 2011; 24 (S2) 204

9.2 Sample search strategies

9.2.1 Kidney preservation solutions

Example search strategy for Ovid EMBASE
1. kidney transplantation/
2. ((kidney or renal) adj5 (graft$ or allograft$ or transplant$)).ti,ab.
3. organ transplantation/
4. *organ preservation/ or exp kidney preservation/
5. exp preservation solution/
6. celsior.ti,ab.
7. (htk or custodiol or bretschneider or (histidine and tryptophan)).ti,ab.
8. (eurocollins or collins).ti,ab.
9. (polysol or vasosol).ti,ab.
10. igl 1.ti,ab.
11. (marshall or soltran).ti,ab.
12. (viaspan or cardiosol or belzer or uw solution or (university adj3 wisconsin)).ti,ab.
13. 1 or 2 or 3
14. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
15. animal/
16. human/
17. 15 not 16
18. 13 and 14
19. 18 not 17

9.2.2 Kidney machine perfusion

Example search strategy for Ovid EMBASE
1. kidney transplantation/
2. ((kidney or renal) adj5 (graft$ or allograft$ or transplant$)).ti,ab.
3. organ transplantation/
4. *organ preservation/
5. exp kidney perfusion/ or "perfusion and superperfusion"/ or perfusion/
6. (lifeport or RM3).ti,ab.
7. (machine adj3 perfus$).ti,ab.
8. (pulsat$ adj3 perfus$).ti,ab.
9. (machine adj3 preserv$).ti,ab.
10. (pulsat$ adj3 preserv$).ti,ab.
11. 1 or 2 or 3 or 4
12. 5 or 6 or 7 or 8 or 9 or 10
13. 11 and 12
14. animal/
15. human/
16. 14 not 15
17. 13 not 16

9.2.3 **Liver preservation solutions**

Example search strategy for Ovid EMBASE
1. exp ORGAN PRESERVATION SOLUTIONS/
2. celsior.ti,ab.
3. (histidine-tryptophan-ketoglutarate or HTk or custodiol or bretschneider).ti,ab.
4. (eurocollins or euro-collins or collins or collin's).ti,ab.
5. polysol.ti,ab.
6. vasosol.ti,ab.
7. IGL-1.ti,ab.
8. (marshall's or marshalls).ti,ab.
9. ((preservation or storage) adj5 solution).ti,ab.
10. ("University of wisconsin" or "UW solution" or viaspan or cardiosol or "belzer solution").ti,ab.
11. LIVER TRANSPLANTATION/
12. ORGAN PRESERVATION/
13. exp LIVER TRANSPLANTATION/
14. ((liver$ or hepatic$ or viscera$) adj5 transplant$).ti,ab.
15. ((liver$ or hepatic$ or viscera$) adj5 graft$).ti,ab.
16. ((liver$ or hepatic$ or viscera$) adj5 allograft$).ti,ab.
17. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
18. 11 or 12 or 13 or 14 or 15 or 16
19. 17 and 18
20. human.sh.
21. animal.sh.
22. 21 not 20
23. 19 not 22

9.3 Review protocols registered with PROSPERO

9.3.1 Hypothermic machine perfusion versus static cold storage of kidney allografts for transplantation- Protocol

Review title and timescale

1 Review title

Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.

Hypothermic machine perfusion versus static cold storage of kidney allografts for transplantation

2 Original language title

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 Anticipated or actual start date

Give the date when the systematic review commenced, or is expected to commence.

25/02/2011

4 Anticipated completion date

Give the date by which the review is expected to be completed.

23/03/2012

5 Stage of review at time of this submission

Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ✗

Review stage	Started	Completed
Preliminary searches	No	Yes
Piloting of the study selection process	No	Yes
Formal screening of search results against eligibility criteria	No	Yes
Data extraction	No	Yes
Risk of bias (quality) assessment	No	Yes
Data analysis	No	Yes

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

John O'Callaghan

7 Named contact email

Enter the electronic mail address of the named contact.

jocallaghan@rcseng.ac.uk

8 Named contact address

Enter the full postal address for the named contact.

Centre for Evidence in Transplantation Royal College of Surgeons of England 35-43 Lincoln's Inn Fields London WC2A 3PE

9 Named contact phone number

Enter the telephone number for the named contact, including international dialing code.

+44 (0)7825 363 337

10 Organisational affiliation of the review

Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Centre for Evidence in Transplantation, Royal College of Surgeons of England

Website address:

www.transplantevidence.com

11 Review team members and their organisational affiliations

Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mr	John	O'Callaghan	CET
Mr	Simon	Knight	CET
Professor	Peter	Morris	CET
Dr	Robert	Morgan	CET

12 Funding sources/sponsors

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

Centre for Evidence in Transplantation Royal College of Surgeons of England 35-43 Lincoln's Inn Fields London WC2A 3PE

13 Conflicts of interest

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

Are there any actual or potential conflicts of interest?

None known

14 Collaborators

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.

Is hypothermic machine perfusion better than static cold storage for preservation of kidneys for transplantation?

16 Searches

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

Systematic literature search of Ovid MEDLINE, EMBASE, The Cochrane Library, The Integrated Clinical Trials Registy Platform, The Transplant Library. Mesh/EMTREE keywords combined with free text aliases. No language or date limits.

17 URL to search strategy

If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

18 Condition or domain being studied

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.

Kidney transplantation for end-stage renal disease removes the need for dialysis and has proven quality of life, morbidity and mortality outcomes for patients. Kidneys removed from deceased donors suffer injury during the procedure of retrieval, preservation and reperfusion. Improving preservation methods may improve the short and long term outcomes of the transplanted organ.

19 Participants/population

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

Any adult or paediatric patients receiving their first or subsequent kidney transplant. Kidneys from DCD and DBD.

20 Intervention(s), exposure(s)

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed

Intervention: Hypothermic machine perfusion of the kidney for at least part of the preservation period.

21 Comparator(s)/control

Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).

Comparator: Static cold storage without any machine perfusion during the preservation period.

22 Types of study to be included initially

Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.

Any prospective randomized controlled trial or quasi-randomized control trial. Prospective controlled trials.

23 Context

Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)

Give the most important outcomes.

Delayed graft function whereby dialysis is required within the first week post-transplantation.

Give information on timing and effect measures, as appropriate.

25 Secondary outcomes

List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.

Patient and graft survival, primary graft dysfunction, incidence of acute and chronic rejection, vascular complications (e.g. thrombosis), post-transplant creatinine or GFR.

Give information on timing and effect measures, as appropriate.

26 Data extraction, (selection and coding)

Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

After the initial search duplicates will be removed. 2 authors will then review the abstracts and select articles for full text review. These will be reviewed and excluded if not achieving the inclusion criteria. Discrepancies will be decided by discussion, if no conclusion can be met a third author will be asked to comment. The remainder of articles will be grouped by study and data extracted by 2 authors to a predesigned excel spreadsheet. Data will be exported to the statistical package "R" for analysis.

27 Risk of bias (quality) assessment

State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.

All studies will be assessed in keeping with the Cochrane Handbook risk of bias tool as well as intention-to-treat analysis and allocation concealement. RCTs will also be assessed by Jadad score.

28 Strategy for data synthesis

Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.

Publication bias will be assessed by funnel plot. Data will be assessed for heterogeneity using I squared and Chi Sqaured testing, reasons for heterogeneity will be explored. A meta-analysis will be performed if appropriate, using fixed effects or random effects analysis as appropriate.

29 Analysis of subgroups or subsets

Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.

Data will be analysed in subgroups DBD and DCD.

Review general information

30 Type of review

Select the type of review from the drop down list.

Prevention

31 Language

Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.

English

Will a summary/abstract be made available in English?

Yes

32 Country

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.

England

33 Other registration details

List places where the systematic review title or protocol is registered (such as with he Campbell Collaboration, or The Joanna Briggs Institute). The name of the organisation and any unique identification number assigned to the review by that organization should be included.

34 Reference and/or URL for published protocol

Give the citation for the published protocol, if there is one.

Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

35 Dissemination plans

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

Do you intend to publish the review on completion?

Yes

36 Keywords

Give words or phrases that best describe the review. (One word per box, create a new box for each term)

Transplantation
Kidney
Preservation
Perfusion

37 Details of any existing review of the same topic by the same authors

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38 Current review status

Review status should be updated when the review is completed and when it is published.

Completed and published
01/07/2013

39 Any additional information

Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)

This field should be left empty until details of the completed review are available. Give the full citation for the final report or publication of the systematic review.

British Journal of Surgery, July 2013; 100(8): 991-1001

Give the URL where available.

9.3.2 Preservation solutions for static storage of livers- Protocol

Review title and timescale

1 Review title

Give the working title of the review. This must be in English. Ideally it should state succinctly the interventions or exposures being reviewed and the associated health or social problem being addressed in the review.

Preservation solutions for static cold storage of liver allografts

2 Original language title

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3 Anticipated or actual start date

Give the date when the systematic review commenced, or is expected to commence.

08/11/2011

4 Anticipated completion date

Give the date by which the review is expected to be completed.

31/07/2012

5 Stage of review at time of this submission

Indicate the stage of progress of the review by ticking the relevant boxes. Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. This field should be updated when any amendments are made to a published record.

The review has not yet started ×

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	No	Yes
Formal screening of search results against eligibility criteria	No	Yes
Data extraction	No	Yes
Risk of bias (quality) assessment	No	Yes
Data analysis	No	Yes

Provide any other relevant information about the stage of the review here.

Review team details

6 Named contact

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

John O'Callaghan

7 Named contact email

Enter the electronic mail address of the named contact.

jocallaghan@rcseng.ac.uk

8 Named contact address

Enter the full postal address for the named contact.

Centre for Evidence in Transplantation Royal College of Surgeons of England 35-43 Lincoln's Inn Fields London WC2A 3PE

9 Named contact phone number

Enter the telephone number for the named contact, including international dialing code.

07825 363 337

10 Organisational affiliation of the review

Full title of the organisational affiliations for this review, and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Centre for Evidence in Transplantation
Website address:
www.transplantevidence.com

11 Review team members and their organisational affiliations

Give the title, first name and last name of all members of the team working directly on the review. Give the organisational affiliations of each member of the review team.

Title	First name	Last name	Affiliation
Mr	John	O'Callaghan	CET
Professor	Peter	Morris	CET
Mr	Simon	Knight	CET
Dr	Robert	Morgan	CET

12 Funding sources/sponsors

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Any unique identification numbers assigned to the review by the individuals or bodies listed should be included.

No funding was received for the conduct of the research.

13 Conflicts of interest

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

Are there any actual or potential conflicts of interest?

None known

PJM chairs a DSMB for Bristol M

14 Collaborators

Give the name, affiliation and role of any individuals or organisations who are working on the review but who are not listed as review team members.

Title	First name	Last name	Organisation details

Review methods

15 Review question(s)

State the question(s) to be addressed / review objectives. Please complete a separate box for each question.

Which is the best preservation solution for the static cold storage of liver allografts for transplantation?

16 Searches

Give details of the sources to be searched, and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

Systematic literature searches of MEDLINE, EMBASE, The Cochrane Library, The Integrated Clinical Trials Registy Platform and The Transplant Library from the Centre for Evidence in Transplantation. Mesh/EMTREE keywords combined with free text aliases. No language or date limits.

17 URL to search strategy

If you have one, give the link to your search strategy here. Alternatively you can e-mail this to PROSPERO and we will store and link to it.

18 Condition or domain being studied

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.

Liver transplantation is the only option for the treatment for end-stage liver failure. Livers removed from donors suffer injury during the procedure of retrieval, preservation and reperfusion. Improving preservation methods may improve the short and long term outcomes of the transplanted organ.

19 Participants/population

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

Any adult or paediatric patients receiving their first or subsequent liver transplant.

20 Intervention(s), exposure(s)

Give full and clear descriptions of the nature of the interventions or the exposures to be reviewed
The intervention and comparator would be one preservation solution compared to another for the storage of liver grafts between the point of retrieval and transplantation.

21 Comparator(s)/control
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group).
The intervention and comparator would be one preservation solution compared to another for the storage of liver grafts between the point of retrieval and transplantation.

22 Types of study to be included initially
Give details of the study designs to be included in the review. If there are no restrictions on the types of study design eligible for inclusion, this should be stated.
Types of study to be included: Randomised controlled trials. Types of study to be excluded: Registry data, retrospective single centre study, prospective non-randomised study, non-randomised study, case-controlled study, cohort study.

23 Context
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24 Primary outcome(s)
Give the most important outcomes.
Early dysfunction Primary non function Retransplantation Patient survival Graft survival
Give information on timing and effect measures, as appropriate.

25 Secondary outcomes
List any additional outcomes that will be addressed. If there are no secondary outcomes enter None.
Highest mean blood tests in the first week post-operatively. To include transaminases, bilirubin, INR.
Give information on timing and effect measures, as appropriate.
Within the first week post-operatively.

26 Data extraction, (selection and coding)
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.
After the initial searches duplicates will be removed. Two authors will then review the abstracts and select articles for full text review. These will be reviewed and excluded if not achieving the inclusion criteria. Discrepancies will be decided by discussion, if no conclusion can be met a third author will be asked to comment. The remainder of the articles will have data extracted by 2 authors to a predesigned excel spreadsheet. Data will be exported to the statistical package "R" for analysis.

27 Risk of bias (quality) assessment
State whether and how risk of bias will be assessed, how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
All studies will be assessed in keeping with the Cochrane Handbook risk of bias tool as well as intention-to-treat analysis, allocation concealement and the Jadad score will be used.

28 Strategy for data synthesis
Give the planned general approach to be used, for example whether the data to be used will be aggregate or at the level of individual participants, and whether a quantitative or narrative (descriptive) synthesis is planned. Where appropriate a brief outline of analytic approach should be given.
Publication bias will be assessed by funnel plot. Data will be assessed for heterogeneity using I-squared and Cochrane Q testing, reasons for heterogeneity will be explored. A meta-analysis will be performed if appropriate, using fixed effects or random effects analysis as appropriate.

29 Analysis of subgroups or subsets
Give any planned exploration of subgroups or subsets within the review. 'None planned' is a valid response if no subgroup analyses are planned.
Studies will be grouped by the preservation solutions used. Subgroup analysis by donor type may be done if available data permits.

Review general information

30 Type of review
Select the type of review from the drop down list.
Prevention

31 Language
Select the language(s) in which the review is being written and will be made available, from the drop down list. Use the control key to select more than one language.
English
Will a summary/abstract be made available in English?
Yes

32 Country
Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved. Use the control key to select more than one country.
England

33 Other registration details
List places where the systematic review title or protocol is registered (such as with he Campbell Collaboration, or The Joanna Briggs Institute). The name of the organisation and any unique identification number assigned to the review by that organization should be included.

34 Reference and/or URL for published protocol
Give the citation for the published protocol, if there is one.
Give the link to the published protocol, if there is one. This may be to an external site or to a protocol deposited with CRD in pdf format.

35 Dissemination plans
Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
Do you intend to publish the review on completion?
Yes

36 Keywords
Give words or phrases that best describe the review. (One word per box, create a new box for each term)
Organ preservation
Liver transplantation
Organ preservation solutions

37 Details of any existing review of the same topic by the same authors
Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38 Current review status
Review status should be updated when the review is completed and when it is published.
Completed but not published

39 Any additional information
Provide any further information the review team consider relevant to the registration of the review.

40 Details of final report/publication(s)
This field should be left empty until details of the completed review are available.
Give the full citation for the final report or publication of the systematic review.
Give the URL where available.

9.4 NHS Blood & Transplant application for data

NATIONAL TRANSPLANT DATABASE

Application for Data REF No -

NHS

UK Transplant

APPLICANT DETAILS	Section 1
Title	Mr
Name	John Matthew O'Callaghan
Institution address	Centre for Evidence in Transplantation Royal College of Surgeons of England 35-43 Lincoln's Inn Fields London
Postcode	W C 2 A 3 P E
Co-authors and affiliation	Centre for Evidence in Transplantation, Royal College of Surgeons of England, AND Oxford Transplant Centre, Oxford Radcliffe NHS Trust
Study title	How does the preservation solution for static cold storage of renal allografts impact upon delayed graft function and long-term transplant outcomes?
Deadline *(Date the data are required)*	0 1 0 7 2 0 1 1

STUDY DESCRIPTION	Section 2
Study summary *(Provide a brief summary of the proposed study in abstract form)*	We aim to assess the relationship between the available preservation solutions for static cold storage of renal allografts and transplant outcomes. So far we have done a systematic review and meta-analysis of the limited number of RCTs and prospective comparative studies. The evidence for the efficacy of the available preservation solutions is of mixed quality and relies upon very few prospective studies. There is registry data relating preservation solution to graft survival (Collaborative Transplant Study), however this database does not collect information on delayed graft function, which is currently a primary outcome measure for trials of preservation techniques. We aim to provide an analysis of transplant outcomes in relation to the preservation solution used. There is some evidence that donor characteristics and cold ischemic time impact upon the effectiveness of the preservation solution, we would therefore like to include this analysis.
Study aims *(State the specific aims of the study)*	What is the relationship between the preservation solution used for static cold storage of renal allografts and transplant outcomes? What is the relative risk of delayed graft function with each preservation solution? What is the relative risk of acute rejection with each preservation solution? What is the relative risk of primary non-function with each preservation solution? What is the hazard ratio for graft failure with each preservation solution? What is the hazard ratio for patient survival with each preservation solution? Are these effects more pronounced at longer cold ischaemia times? Are these effects more pronounced with increasing donor age? Are these effects more pronounced in DCD versus DBD donors?
Background information *(Explain how this study will benefit transplantation)*	Static cold storage is the most prevalent method for renal allograft preservation worldwide. Numerous preservation solutions have been designed to counteract the detrimental effects of the retrieval process, graft cooling and reperfusion injury. Currently the data available to support the use of the various solutions depends largely on retrospective data analysis from individual centres and registries (UNOS, CTS) and a limited number of randomized controlled trials and prospective trials. With changes in practice in the 4 decades of renal transplantation, historic analysis can provide skewed results, particularly in data combined from numerous centres in a diverse array of socio-economic settings. We believe that this may bias registry data against some solutions. An analysis of UKT data would provide an insight into the relationship between preservation solution and transplant outcome in our population and healthcare system.

DATA AND ANALYSIS **Section 3**

Study cohort	All cadaveric renal transplants from DBD and DCD donors. Donors and recipients of all age groups. Exclude kidneys stored by machine perfusion.
Data requirements *(List all data required)*	Preservation solution, cold ischaemic time (hours), donor age (years), donor sex (m/f), donor type (DBD versus DCD), recipient age (years), recipient sex (m/f), acute rejection episode (y/n), graft survival at all available time points (time to event), creatinine at all available time points (mg/dl), primary non-function (y/n), patient survival at all available time points (time to event), year of transplant, cause of death, immune suppression regime.
Statistical analysis *(Provide a brief outline of the proposed statistical analysis)*	We intend to use the following statistical methods for analysis: Chi squared test for analysis of binary outcomes (i.e. rate of delayed graft function by preservation solution, rate of primary non-function by preservation solution and acute rejection episodes by preservation solution). Kaplan-Meier computation of graft survival, (plus death censored graft survival) and patient survival. Logistic regression and multivariate logistic regression will be used for covariables: length of cold ischaemic time. P values <0.05 will be taken to indicate statistical significance.
Publication *(State intentions for publishing study results eg name meetings, conferences and journals)*	We intend to publish the results in a peer reviewed journal and to present the results at one or more society conferences (eg. British Transplantation Society, European Society of Organ Transplantation, The Transplantation Society).

PATIENT CONSENT AND DATA PROTECTION **Section 4**

Is any patient identifiable information required? Yes / No? No

If Yes,

a) Do you have patient consent? Yes / No? _____

b) If you do not have patient consent, have you got approval to use patient identifiable information for the study from the Patient Information Advisory Group and your local Ethics Committee? Yes / No? _____

If Yes, please provide documentary evidence of this approval

Data protection *(Indicate any safeguards set in place to limit use of, and access to, the data)*	Data will be stored on CEU's analysis server which resides in a physically secure building and on a firewalled network. Access will be limited to authorised, named users who are required to provide a username and password to access the data. Data will be securely destroyed when it is no longer required.

For UKT use only	UKT's Caldicott Guardian	UKT's Data Protection Officer
Application approved:	Yes / No? _____ Date: _____	Yes / No? _____ Date: _____
Name:	_____	_____
If No, state why:		

	AG Chair	AASG	Unit Directors
Application approved:	Yes / No / Not sought? _____ Date: _____	Yes / No / Not sought? _____ Date: _____	Yes / No / Not sought? _____ Date: _____
If No or Not sought, state why:			