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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-093001
Article Type:	Protocol
Date Submitted by the Author:	28-Aug-2024
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Keywords:	TRANSPLANT MEDICINE, Renal transplantation < NEPHROLOGY, Transplant surgery < SURGERY

Protocol for a randomised pilot study to assess the safety and feasibility of adding a CytoSorb® filter during kidney normothermic machine perfusion to remove inflammatory and immune mediators

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Word count: 2993
Keywords: normothermic machine perfusion, kidney transplantation, cytokine filter
Protocol version 1.2, 4th July 2023. Rewritten for publication.

ABSTRACT

Introduction

The introduction of perfusion technologies in kidney transplantation has the potential to improve graft function and survival and increase utilisation. Our previous work demonstrated that kidneys with an enhanced inflammatory and immune response during normothermic machine perfusion (NMP) had significant graft dysfunction after transplantation. The addition of a cytokine filter (CytoSorb®) to the NMP circuit dramatically reduces both circulating inflammatory mediators and inflammatory gene expression but this has not been trialled in clinical practice.

Methods and analysis

This is a randomised phase I pilot study to evaluate the safety and feasibility of using a CytoSorb® filter in clinical NMP to remove inflammatory and immune mediators. Eligible kidney transplant recipients on the waiting list in the East of England will be approached for consent. A total of 20 patients will be recruited and randomised in a 1:1 ratio for the donor kidney to receive either NMP or NMP with a CytoSorb® filter pre-transplantation. The kidney will be transplanted according to standard practice after NMP. The primary endpoint is inflammatory and immune gene expression measured in a cortical biopsy from the kidney 60 minutes post-transplant. Secondary endpoints include rates and duration of delayed graft function, and graft function as assessed by change in creatinine clearance and estimated glomerular filtration rate 2 days, 5 days, 1 month and 3 months post-transplant. Additionally, inflammatory mediators and injury markers will be measured in peripheral blood and urine samples taken pre-operatively and on days 2 and 5 after transplant.

Ethics and dissemination

This study has been approved by the Health Research Authority (HRA) Health and Care Research Wales (HCRW) Committee (REC 23/WM/0141) and by NHS Blood and Transplant (NHSBT) (Ref: Study 148). Findings will be published in a peer-reviewed journal and disseminated at scientific conferences. The dataset will be made available on request.

Trial registration

The study is prospectively registered on the ISCRTN registry (ID: 13698207).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first prospective randomised pilot study evaluating the safety and feasibility of cytokine filtration during kidney NMP in the clinical setting.
- Whilst not its primary aim, the study will contribute to the evidence base for the safety and feasibility of intermediate durations of kidney NMP (2–6 hours).
- The global transcriptomic analysis of post-reperfusion kidney biopsies will provide novel and high-resolution mechanistic insights into the impact of cytokine removal on the inflammatory status of the donor kidney immediately post-transplant.
- The study is not sufficiently powered to support the inference of causal relationships between NMP and clinical outcomes.
- The study is not double-blinded.

INTRODUCTION

An increasing number of kidneys from extended criteria donors (ECD) and donation after circulatory death donors (DCD) are being used in transplantation due to the shortage of organs. These marginal kidneys offer a survival benefit to transplant patients compared to remaining on dialysis but portend a higher risk of delayed graft function (DGF) and graft loss compared to alternative kidney allografts[1,2]. Prolonged DGF is independently associated with longer hospital stays, increased cost, higher incidence of acute rejection, poorer 12-month graft function and higher rates of graft loss and death[3–5].

Traditional hypothermic kidney preservation techniques suppress cellular metabolism and oxygen requirements during transport; however, these conditions cause gradual depletion of energy substrates culminating in cellular damage. After transplantation, restoration of blood flow to the kidney causes further insult via ischaemia reperfusion injury (IRI), which involves a cascade of inflammatory and immune mediators that can lead to graft dysfunction.

Normothermic machine perfusion (NMP) offers an alternative organ preservation technique that recirculates an oxygenated red blood cell-based solution through the donor kidney at near physiological pressure and temperature[6]. It restores oxidative phosphorylation and cellular function following hypothermic preservation and prior to implantation in the recipient. NMP can potentially reduce injury caused by hypothermic conditions and provides a platform for organ assessment and the delivery of targeted treatments. The safety and feasibility of NMP in clinical practice has been established. The results of a randomised controlled trial of NMP in DCD kidneys showed no benefit of 1 hour of NMP in reducing rates of DGF post-transplant[7]. Longer durations of NMP have since been trialled and shown to be safe in clinical practice[8,9].

Evidence from non-transplanted human kidneys has shown that during NMP inflammatory and immune mediators are also upregulated similarly to the reperfusion response following graft implantation and restoration of blood flow[10]. Correlations with early outcome after transplantation show that kidneys with an enhanced inflammatory profile after NMP are more likely to have prolonged DGF[7,10]. Moreover, systemic inflammation early after kidney transplantation is associated with long-term graft loss[11].

Our group has shown in transplant-declined human kidneys that adding a CytoSorb® filter to the kidney NMP circuit removes the inflammatory and immune mediators from the circulating perfusate

and attenuates a DGF-associated inflammatory gene signature in renal tissue[10,12]. CytoSorb® is a Class IIb medical device made of a bio- and haemo-compatible porous bead-based polymer, which adsorb hydrophobic molecules up to 55kDa (including cytokines and chemokines) in a concentration-dependent manner.

Clinically, the CytoSorb® filter has been used successfully to treat patients with sepsis, COVID-19 and severe inflammatory response syndrome[13–16]. It has also been used in several clinical trials during cardiac bypass[17]. Experimental studies in lung NMP have shown a beneficial effect after transplantation[18].

The primary aim of this study is to establish the safety and feasibility of adding a CytoSorb® filter in clinical kidney NMP to reduce the inflammatory and immune response within the kidney after transplantation. Secondary outcome measures include rates and duration of delayed graft function, and graft function as assessed by change in creatinine clearance and estimated glomerular filtration rate 2 days, 5 days, 1 month and 3 months post-transplant. Additionally, inflammatory mediators and injury markers will be measured in peripheral blood and urine samples taken pre-operatively and on days 2 and 5 after transplant.

METHODS

Study design

This is a randomised, single-blinded, single centre phase 1 pilot study. The protocol is written in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) guidelines[19]. Figure 1 summarises the study design including the temporal course of events for the patient and the kidney after arrival at the centre and the key time points for sample collection.

Recruitment

A total of 20 patients will be recruited from a single centre, Cambridge University Hospitals NHS Foundation Trust (Addenbrooke's site). The deceased donor kidney transplant waiting list at Cambridge will be used to identify eligible participants. Participant information sheets will be sent to eligible participants beforehand. The transplant co-ordinators will contact the research team when the patient is called into the transplant centre after the identification of a suitably matched donation after brain death (DBD) or DCD donor kidney. Once assessed and the inclusion criteria met, the patient will be offered the opportunity to ask any further questions.

Inclusion criteria

Kidney transplant recipients who meet all of the criteria below are eligible to participate.

1. Aged ≥18 years
2. On dialysis (any modality) prior to transplantation
3. Undergoing a first or second kidney transplant
4. Receiving a kidney from a DBD or DCD donor aged ≥50 years
5. Able to provide written informed consent

Exclusion criteria

Kidney transplant recipients who meet any of the criteria below are ineligible to participate.

1. Pre-dialysis (i.e. undergoing a pre-emptive transplant)
2. Undergoing a third or subsequent kidney transplant
3. Receiving a multi-organ transplant (e.g. simultaneous pancreas–kidney transplant)
4. Receiving a dual kidney transplant
5. Receiving a paediatric en-bloc kidney transplant
6. Receiving a kidney from a DBD or DCD donor aged <50 years
7. Receiving a kidney with complex vascular anatomy

Consent

Informed consent will be obtained by a qualified member of the research team on the day the patient is called into hospital and prior to the kidney transplant taking place.

Randomisation

Patients receiving a deceased donor kidney transplant who meet the eligibility criteria and provide informed consent will be randomised in a 1:1 ratio for the donor kidney to receive NMP alone or NMP with the CytoSorb® filter prior to transplantation. Randomisation will be performed after the transplant recipient and kidney have both arrived in the transplant centre and a final decision to proceed with transplantation has been made. The randomisation will be performed by a member of the research team using the sealed envelope™ simple randomisation service. The patient and medical staff caring for the patient after surgery will be blinded to the groups.

Clinical care

Following organ retrieval, the kidney will be transported to the transplant centre on ice as per standard protocol. Bench preparation of the kidney will take place and any anatomical abnormalities that preclude inclusion in the trial will be noted.

NMP with or without the CytoSorb® filter will then be carried out as described below by members of the research team and clinical staff who are independent to the care of the patient.

The recipient will concurrently be anaesthetised according to local protocols. Anti-microbial prophylaxis, anti-thrombotic prophylaxis and immunosuppressive medication will be given according to local protocols. It is expected that patients will also receive prophylaxis against Pneumocystis jiroveci pneumonia, oral candidiasis and cytomegalovirus.

The kidney will be transplanted using standard techniques into either iliac fossa. The renal artery will be anastomosed to either the common, external or internal iliac arteries, and the renal vein to either the common or the external iliac vein. The ureteric anastomosis will be performed as an extravesical onlay over a double J stent.

A cortical 4mm punch biopsy of the kidney will be taken post-reperfusion (45–60 minutes), and recipient blood and urine samples will be collected at pre-determined time points as described below.

Study groups

Normothermic machine perfusion

Kidneys will be placed on the NMP system (Kidney Assist, XVIVO) and perfused with a red cell-based solution mixed with a priming solution (human serum albumin 5%, dexamethasone, heparin, calcium gluconate 10% and sodium bicarbonate 8.4% to adjust pH within normal range). Infusion pumps will deliver the following:

- 5% glucose 2–4ml/h as required
- Amino acid/electrolyte solution (with 100units of Actrapid insulin, 15ml sodium bicarbonate 8.4% and 5ml Cernevit) 5–10ml/h
- Prostacyclin 0.5mg x1 (i/v infusion 16ml in 100ml 0.9% sodium chloride) 3ml/h as required
- Verapamil 2.5mg as required
- Ringer's solution to replace urine output

Kidneys will be perfused for a minimum of 2 hours at a mean arterial pressure of 80–85mmHg and temperature of 35–37.4°C. Renal blood flow (RBF) will be monitored continuously during NMP. Intra-renal resistance (IRR) will be calculated (mean arterial pressure/RBF) until the end of perfusion. The total urine output will be recorded. Blood gas analysis will be used to measure the acid–base balance during NMP.

After NMP kidneys will be flushed with cold preservation solution and placed on ice until transplantation.

Normothermic machine perfusion with CytoSorb® filter

Kidneys will be perfused as per the standard NMP protocol but with the CytoSorb® filter (CytoSorbents Europe GmbH) added to the circuit for the duration of perfusion (Figure 2).

Follow up

Patients will be followed up at the transplant clinic or local centre at 1 and 3 months as normal practice after discharge from hospital.

Withdrawal from the study

Withdrawal from the study is likely to be uncommon but may occur on account of withdrawal of consent by the patient or the kidney being deemed untransplantable following final bench surgery.

Patients who are randomised but withdraw before intervention will receive standard clinical care according to the local protocol. If patients undergo the intervention but subsequently withdraw, they will also receive standard clinical care. In the unexpected situation where consent to use data and samples that have already been collected is withdrawn, these will be discarded.

Protocol deviations

One-off protocol deviations will be documented and reported to the Chief Investigator and Sponsor. Frequent deviations will be reported to the Sponsor and REC.

OUTCOMES

Primary outcome

The primary outcome measure is inflammatory and immune gene expression measured in post-reperfusion renal cortical biopsies.

Secondary outcomes

The secondary outcome measures are:

1. Rates of DGF
2. Duration of DGF
3. Incidence of primary non-function (PNF)

4. Graft function at 3 months post-transplant
5. Graft survival at 3 months post-transplant
6. Patient survival at 3 months post-transplant
7. Incidence of biopsy-proven acute rejection
8. Complications within 3 months of transplant (infection, re-operation due to bleeding)
9. Length of hospital stay
10. Levels of inflammatory/immune and injury markers in perfusate and urine samples taken during NMP
11. Isolation of peripheral blood mononuclear cells from recipient blood samples pre-transplant and on days 2 and 5 post-transplant to examine the inflammatory/immune response
12. Biomarkers of kidney injury on days 2 and 5 post-transplant.

SAMPLES AND ANALYSIS

Sample collection

- A cortical 4mm punch biopsy of the kidney will be taken from the transplant kidney at 45–60 minutes after reperfusion. Tissue samples will be divided. Half will be fixed in 10% formalin and the other half placed in RNA later.
- Samples of the perfusate and urine from the kidneys during NMP will be collected at the start and hourly until the end of NMP.
- Peripheral blood (10ml) and urine (10ml) samples from the transplant recipient will be collected at the start of surgery and then at days 2 and 5 post-transplant.

Sample storage

Samples will be processed as required and then stored at –80°C or in liquid nitrogen. Frozen tissue samples will be stored in the research laboratories at the University of Cambridge, Department of Surgery. Fixed tissue will be processed by the tissue bank at Addenbrooke's Hospital and stored within the Department of Surgery laboratories.

Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation and the requirements set out in the 2004 Human Tissue Act. Samples will be labelled in compliance with the 1998 Data Protection Act. On completion of the trial samples will be disposed of in accordance with the Human Tissue Authority's Code of Practice.

Data collection and storage

Patient related data will be collected from computerised health records. Each participant will be allocated a study code to facilitate data anonymisation for storage on a password protected database.

Analysis

- Donor and recipient characteristics, levels of creatinine clearance, eGFR, graft and patient survival, complications, length of hospital stay, incidences of DGF and PNF, episodes of acute rejection and perfusion parameters during NMP will be compared between the two groups using the appropriate statistical tests.
- Cortical kidney tissue will be analysed as follows: a gene expression profile will be performed using bulk RNA sequencing. Bulk RNA sequencing and sample analysis will be performed at a laboratory with expertise in this area. Fixed tissue will be used to assess the level of injury and inflammation.
- Samples of perfusate and urine from the kidney during NMP, and of urine from the recipient will be analysed as follows: levels of cytokines will be measured using a Cytokine Luminex panel and biomarkers using a Proteome Profiler Human Kidney Biomarker Array Kit. Changes in the level of cytokines and injury markers will be calculated and compared between groups using repeated measures and ANOVA analysis.
- Peripheral blood samples from recipients will be analysed as follows: peripheral blood mononuclear cells will be isolated from whole blood using density-gradient separation, and their relative abundance, phenotype and activation status determined using cell sorting, flow cytometry, transcriptomic analysis and cell culture techniques. Levels of inflammation/immune mediators will be measured using a Luminex panel.

ETHICS AND DISSEMINATION

Ethics

This study has been approved by the Health Research Authority (HRA) Health and Care Research Wales (HCRW) Committee (REC 23/WM/0141) and by NHS Blood and Transplant (Ref: Study 148).

Confidentiality

Patient identifiable information will be accessed and handled by members of the research team in compliance with the 1998 Data Protection Act. Only patient unidentifiable data will be transmitted to the sponsor and co-investigators. The data will be stored for 5 years after the end of study. The custodian of the data will be the Chief Investigator.

Adverse events

The risk to participants entering the study is low. Any adverse events directly linked to the intervention will be recorded and reported to the Chief Investigator and Sponsor immediately.

Indemnity

Cambridge University Hospitals NHS Foundation Trust will accept full financial liability for harm caused to participants in the study through the negligence of its employees and honorary contract holders. The University of Cambridge will arrange insurance for negligent harm on account of protocol design and for non-negligent harm arising through participation in the study.

Dissemination

Findings will be published in a peer-reviewed journal and disseminated at scientific conferences. The dataset will be made available on request.

Patient and Public Involvement

This research proposal was presented to the NHIR Blood and Transplant Research Unit in Organ Donation and Transplantation (BTRU in ODT) Patient and Public Research Panel who provided guidance on the protocol design. On completion of the study, we will present the results to panel members at an annual NIHR BTRU in ODT progress meeting.

DISCUSSION

Anticipated benefits

To our knowledge, this is the first clinical study to test the safety and feasibility of using CytoSorb® therapy during kidney NMP. It is anticipated that the analysis will provide novel mechanistic insights. The use of global transcriptomic techniques on post-reperfusion kidney biopsies will elucidate the role played by cytokine signalling in reperfusion injury to the graft. Further, the isolation and characterisation of peripheral blood mononuclear cells and inflammatory mediators from recipient blood samples taken in the days following transplant will provide valuable information about the host systemic inflammatory response in the early postoperative period, and whether this is altered by pre-transplant CytoSorb® therapy.

Limitations

The study design has some limitations. Firstly, whilst the sample size is large enough to prove feasibility, the study is not sufficiently powered to draw causal inferences about the relationship

between the interventions and clinical endpoints. However, the results will inform future efficacy trials. Secondly, a clinical and ethical decision was made to take a single renal cortical biopsy post-reperfusion to minimise the risk of bleeding. The lack of a pre-transplant (pre-NMP) biopsy makes it difficult to assess the contribution of baseline inflammation in the donor allograft at the time of organ retrieval, as well as the impact of NMP on the inflammatory status of the donor kidney. To mitigate the impact of this on the analysis, access to QUOD renal cortical biopsy samples taken at organ retrieval will be requested, where available.

ACKNOWLEDGEMENTS

Author contributions

MM revised and wrote the protocol for publication, designed the figures and edited the manuscript. SH and MLN designed the study and wrote the protocol, co-wrote the published version and edited the manuscript.

Sponsors

This study is sponsored by the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust.

Funding

This study is funded by the National Institute for Health and Care Research (NIHR) Blood and Transplant Research Unit in Organ Donation and Transplantation (NIHR203332), a partnership between NHS Blood and Transplant, University of Cambridge and Newcastle University. The views expressed are those of the author(s) and not necessarily those of the NIHR, NHS Blood and Transplant or the Department of Health and Social Care.

Competing interests

Sarah Hosgood received an honorarium from CytoSorbents Europe GmbH. The other authors have no competing interests to declare.

Figures used in this publication were created using Biorender.com.

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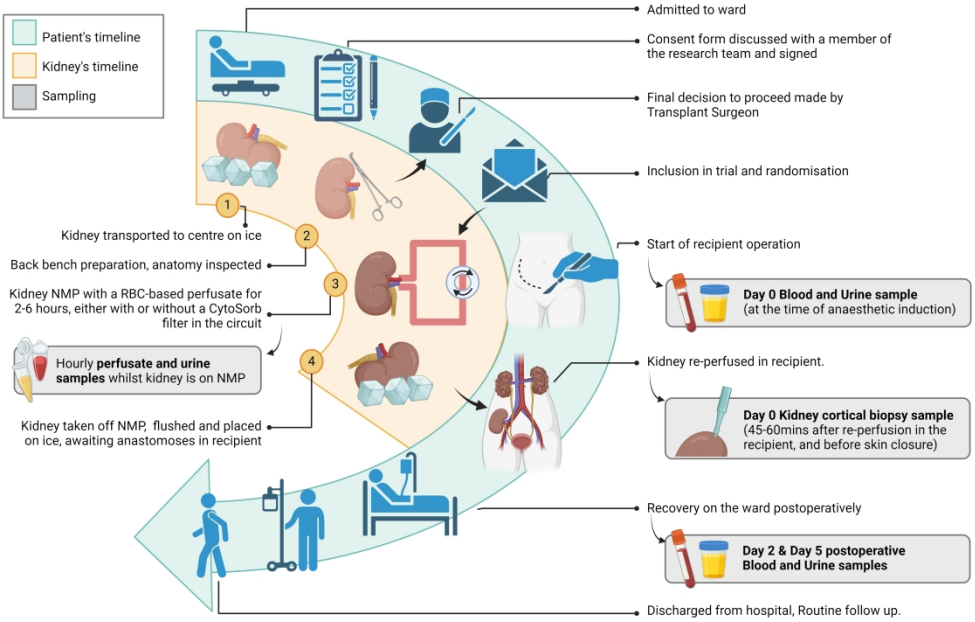
Figure 1: Participant timeline, delineating the patient's clinical care whilst enrolled in the trial (blue panel), the kidney's temporal course (yellow panel) and the key time points for sample collection (grey inserts).

Figure 2: Schematic representation of the XVIVO Kidney Assist perfusion circuit with a CytoSorb® filter incorporated. The kidney is placed in a sterile organ chamber with a lid. A patch clamp is used to connect the arterial end of the tubing to the renal artery. The renal vein and ureter drain directly into the venous reservoir (urine recirculation). Additional component parts (not shown) include a pressure transducer, an arterial flow sensor, temperature sensors and sampling ports.



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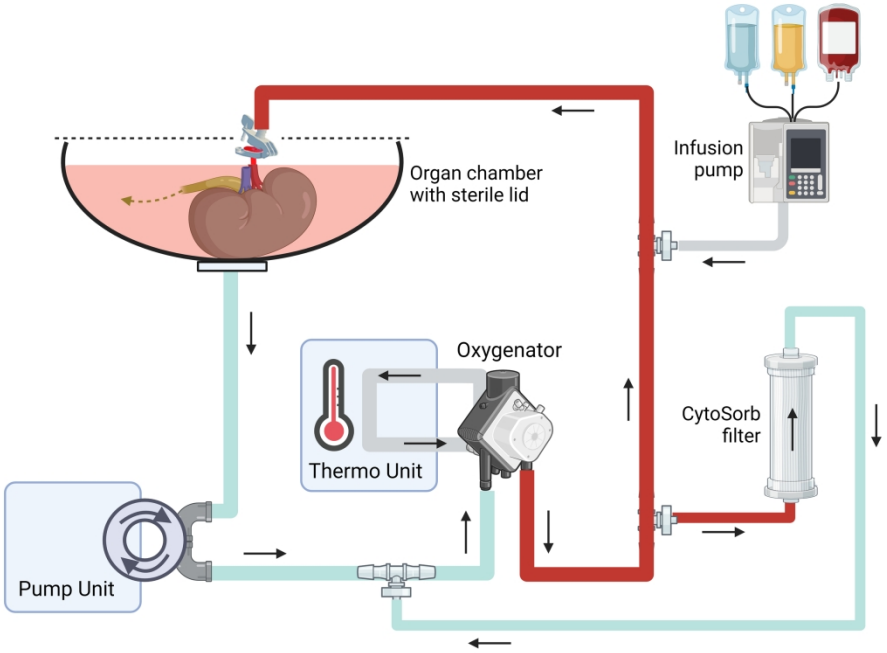

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-093001.R1
Article Type:	Protocol
Date Submitted by the Author:	06-Feb-2025
Complete List of Authors:	Mehta, Maithili; University of Cambridge, Department of Surgery Hosgood, Sarah; University of Cambridge, Department of Surgery Nicholson, Michael; University of Cambridge, Department of Surgery
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Renal medicine, Surgery
Keywords:	Renal transplantation < NEPHROLOGY, Transplant surgery < SURGERY, Transplant medicine < INTERNAL MEDICINE

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Trial registration

The study is prospectively registered on the ISCRTN registry (ID: 13698207).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study uses a highly sensitive and unbiased measure (inflammatory gene expression in post-reperfusion kidney cortical biopsies) as its primary endpoint
- The study is not sufficiently powered to support the inference of causal relationships between NMP and clinical outcomes.
- The study is not double-blinded.

INTRODUCTION

An increasing number of kidneys from extended criteria donors (ECD) and donation after circulatory death donors (DCD) are being used in transplantation due to the shortage of organs. These marginal kidneys offer a survival benefit to transplant patients compared to remaining on dialysis but portend a higher risk of delayed graft function (DGF) and graft loss compared to alternative kidney allografts[1,2]. Prolonged DGF is independently associated with longer hospital stays, increased cost, higher incidence of acute rejection, poorer 12-month graft function and higher rates of graft loss and death[3–5].

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1. Aged ≥18 years
2. On dialysis (any modality) prior to transplantation
3. Undergoing a first or second kidney transplant
4. Receiving a kidney from a DBD or DCD donor aged ≥50 years
5. Able to provide written informed consent

Exclusion criteria

Kidney transplant recipients who meet any of the criteria below are ineligible to participate.

1. Pre-dialysis (i.e. undergoing a pre-emptive transplant)
2. Undergoing a third or subsequent kidney transplant
3. Receiving a multi-organ transplant (e.g. simultaneous pancreas–kidney transplant)
4. Receiving a dual kidney transplant
5. Receiving a paediatric en-bloc kidney transplant
6. Receiving a kidney from a DBD or DCD donor aged <50 years
7. Receiving a kidney with complex vascular anatomy

Consent

Written informed consent (see supplement) will be obtained by a qualified member of the research team on the day the patient is called into hospital and prior to the kidney transplant taking place.

Randomisation

Patients receiving a deceased donor kidney transplant who meet the eligibility criteria and provide informed consent will be randomised in a 1:1 ratio for the donor kidney to receive NMP alone or NMP with the CytoSorb® filter prior to transplantation. Randomisation will be performed after the transplant recipient and kidney have both arrived in the transplant centre and a final decision to proceed with transplantation has been made. The randomisation will be performed by a member of the research team using the sealed envelope™ simple randomisation service. The patient and medical staff caring for the patient after surgery will be blinded to the groups.

Clinical care

Following organ retrieval, the kidney will be transported to the transplant centre on ice as per standard protocol. Bench preparation of the kidney will take place and any anatomical abnormalities that preclude inclusion in the trial will be noted.

NMP with or without the CytoSorb® filter will then be carried out as described below by members of the research team and clinical staff who are independent to the care of the patient.

The recipient will concurrently be anaesthetised according to local protocols. Anti-microbial prophylaxis, anti-thrombotic prophylaxis and immunosuppressive medication will be given according to local protocols. It is expected that patients will also receive prophylaxis against Pneumocystis jiroveci pneumonia, oral candidiasis and cytomegalovirus.

The kidney will be transplanted using standard techniques into either iliac fossa. The renal artery will be anastomosed to either the common, external or internal iliac arteries, and the renal vein to either the common or the external iliac vein. The ureteric anastomosis will be performed as an extravesical onlay over a double J stent.

A cortical 4mm punch biopsy of the kidney will be taken post-reperfusion (45–60 minutes), and recipient blood and urine samples will be collected at pre-determined time points as described below.

Study groups

Normothermic machine perfusion

Kidneys will be placed on the NMP system (Kidney Assist, XVIVO) and perfused with a red cell-based solution mixed with a priming solution (human serum albumin 5%, ringer's solution, dexamethasone, heparin, meropenem, calcium gluconate 10% and sodium bicarbonate 8.4% to adjust pH within normal range). Infusion pumps will deliver the following:

- 5% glucose 2–4ml/h as required
- Amino acid/electrolyte solution (with 100units of Actrapid insulin, 15ml sodium bicarbonate 8.4% and 5ml Cernevit) 5–10ml/h
- Prostacyclin 0.5mg x1 (i/v infusion 16ml in 100ml 0.9% sodium chloride) 3ml/h as required
- Verapamil 2.5mg as required
- Ringer's lactate solution to replace urine output

Kidneys will be perfused for a minimum of 2 hours and a maximum of 6 hours at a mean arterial pressure of 80–85mmHg and temperature of 35–37.4°C. Renal blood flow (RBF) will be monitored continuously during NMP. Intra-renal resistance (IRR) will be calculated (mean arterial pressure/RBF) until the end of perfusion. Blood gas analysis will be used to measure the acid–base balance during NMP.

After NMP kidneys will be flushed with cold preservation solution and placed on ice until transplantation.

Normothermic machine perfusion with CytoSorb® filter

Kidneys will be perfused as per the standard NMP protocol but with the CytoSorb® filter (CytoSorbents Europe GmbH) added to the circuit for the duration of perfusion (Figure 2).

Follow up

Patients will be followed up at the transplant clinic or local centre at 1 and 3 months as normal practice after discharge from hospital.

Withdrawal from the study

Withdrawal from the study is likely to be uncommon but may occur on account of withdrawal of consent by the patient or the kidney being deemed untransplantable following final bench surgery.

Patients who are randomised but withdraw before intervention will receive standard clinical care according to the local protocol. If patients undergo the intervention but subsequently withdraw, they will also receive standard clinical care. In the unexpected situation where consent to use data and samples that have already been collected is withdrawn, these will be discarded.

Protocol deviations

One-off protocol deviations will be documented and reported to the Chief Investigator and Sponsor. Frequent deviations will be reported to the Sponsor and REC.

Patient and Public Involvement

This research proposal was presented to the NHIR Blood and Transplant Research Unit in Organ Donation and Transplantation (BTRU in ODT) Patient and Public Research Panel who provided guidance on the protocol design. On completion of the study, the results will be presented to panel members at an annual NIHR BTRU in ODT progress meeting.

OUTCOMES

Primary outcome

The primary outcome measure is inflammatory and immune gene expression measured in post-reperfusion renal cortical biopsies.

Secondary outcomes

The secondary outcome measures are:

1. Rates of DGF, defined as the need for dialysis within the first seven postoperative days
2. Duration of DGF
3. Incidence of primary non-function (PNF), defined as dialysis dependence or creatinine clearance≤20 ml/min at 3 months post-transplant
4. Graft function at 3 months post-transplant as measured by estimated glomerular filtration rate (eGFR)
5. Graft survival at 3 months post-transplant
6. Patient survival at 3 months post-transplant
7. Incidence of biopsy-proven acute rejection
8. Complications within 3 months of transplant (infection, re-operation due to bleeding)
9. Length of hospital stay
10. Levels of inflammatory/immune and injury markers in perfusate and urine samples taken during NMP
11. Isolation of peripheral blood mononuclear cells from recipient blood samples pre-transplant and on days 2 and 5 post-transplant to examine the inflammatory/immune response
12. Biomarkers of kidney injury on days 2 and 5 post-transplant.

SAMPLES AND ANALYSIS

Sample collection

- A cortical 4mm punch biopsy of the kidney will be taken from the transplant kidney at 45–60 minutes after reperfusion. Tissue samples will be divided. Half will be fixed in 10% formalin and the other half placed in RNA later.
- Samples of the perfusate and urine (if any) from the kidneys during NMP will be collected at the start and hourly until the end of NMP.
- Peripheral blood (10ml) and urine (10ml) samples from the transplant recipient will be collected at the start of surgery and then at days 2 and 5 post-transplant.

Sample storage

Samples will be processed as required and then stored at –80°C or in liquid nitrogen. Frozen tissue samples will be stored in the research laboratories at the University of Cambridge, Department of Surgery. Fixed tissue will be processed by the tissue bank at Addenbrooke's Hospital and stored within the Department of Surgery laboratories.

Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation and the requirements set out in the 2004 Human Tissue Act. Samples will be labelled in compliance with the 1998 Data Protection Act. On completion of the trial samples will be disposed of in accordance with the Human Tissue Authority's Code of Practice.

Data collection and storage

Patient related data will be collected from computerised health records. Each participant will be allocated a study code to facilitate data anonymisation for storage on a password protected database.

Analysis

- Donor and recipient characteristics, levels of creatinine clearance, eGFR, graft and patient survival, complications, length of hospital stay, incidences of DGF and PNF, episodes of acute rejection and perfusion parameters during NMP will be compared between the two groups using the appropriate statistical tests.
- Cortical kidney tissue will be analysed as follows. RNA will be extracted from RNALater biopsies and bulk sequenced. The data will thereafter be demultiplexed, assessed for quality control using FASTQC, the Fastq files aligned to the human genome using Hisat2, and normalisation and differential gene expression analysis performed using DESeq2 . RNA extraction, library preparation, sequencing and sample analysis will be performed at a laboratory with expertise in this area. Additionally fixed tissue will be used to assess the level of injury and inflammation.
- Samples of perfusate and urine from the kidney during NMP, and of urine from the recipient will be analysed as follows. Levels of cytokines will be measured using a Cytokine Luminex panel. Biomarkers will be quantified using a Proteome Profiler Human Kidney Biomarker Array Kit. Changes in the level of cytokines and injury markers will be calculated and compared between groups using repeated measures and ANOVA analysis.
- Peripheral blood samples from recipients will be analysed as follows: peripheral blood mononuclear cells will be isolated from whole blood using density-gradient separation, and their relative abundance, phenotype and activation status determined using cell sorting, flow cytometry, transcriptomic analysis and cell culture techniques. Levels of inflammation/immune mediators will be measured using a Luminex panel.

Cytokines and chemokines of particular interest are G-CSF, GM-CSF, IFN-α, IFN-γ, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, TNF-α, CXCL2, CXCL3, CCL5

and CXCL4. Biomarkers of interest include C-reactive protein, prostaglandin E2, prostacyclin, thromboxane B1, HMGB1, β2-microglobulin, NGAL, and KIM-1 among others.
Analysis of the RNAseq data will focus particularly on the expression of TNF-α signalling via NFκB, mTORC1 signalling, inflammatory response, P53 pathway, IL-2 signalling and TGF-ß signalling as these were the most upregulated genes in our previous study [7].

ETHICS AND DISSEMINATION

Ethics

This study has been approved by the Health Research Authority (HRA) Health and Care Research Wales (HCRW) Committee (REC 23/WM/0141) and by NHS Blood and Transplant (Ref: Study 148).

Confidentiality

Patient identifiable information will be accessed and handled by members of the research team in compliance with the 1998 Data Protection Act. Only patient unidentifiable data will be transmitted to the sponsor and co-investigators. The data will be stored for 5 years after the end of study. The custodian of the data will be the Chief Investigator.

Adverse events

The risk to participants entering the study is low. Any adverse events directly linked to the intervention will be recorded and reported to the Chief Investigator and Sponsor immediately.

Indemnity

Cambridge University Hospitals NHS Foundation Trust will accept full financial liability for harm caused to participants in the study through the negligence of its employees and honorary contract holders. The University of Cambridge will arrange insurance for negligent harm on account of protocol design and for non-negligent harm arising through participation in the study.

Dissemination

Findings will be published in a peer-reviewed journal and disseminated at scientific conferences. The dataset will be made available on request.

DISCUSSION

Anticipated benefits

To our knowledge, this is the first clinical study to test the safety and feasibility of using CytoSorb® therapy during kidney NMP.

It is anticipated that the analysis will provide novel mechanistic insights. The use of global transcriptomic techniques on post-reperfusion kidney biopsies will elucidate the role played by cytokine signalling in reperfusion injury to the graft. Further, the isolation and characterisation of peripheral blood mononuclear cells and inflammatory mediators from recipient blood samples taken in the days following transplant will provide valuable information about the host systemic inflammatory response in the early postoperative period, and whether this is altered by pre-transplant CytoSorb® therapy.

Whilst not its primary aim, the study will contribute to the evidence base for the safety and feasibility of intermediate durations of kidney NMP (2–6 hours).

Limitations

The study design has some limitations. Firstly, whilst the sample size is large enough to prove feasibility, the study is not sufficiently powered to draw causal inferences about the relationship between the interventions and clinical endpoints. However, the results will inform future efficacy trials. Secondly, randomisation does not account for donor type, therefore there is a small risk of imbalance in the number of DBD/DCD kidneys between the two study arms.
Finally, a clinical and ethical decision was made to take a single renal cortical biopsy post-reperfusion to minimise the risk of bleeding. The lack of a pre-transplant (pre-NMP) biopsy makes it difficult to assess the contribution of baseline inflammation in the donor allograft at the time of organ retrieval, as well as the impact of NMP on the inflammatory status of the donor kidney. To mitigate the impact of this on the analysis, access to Quality in Organ Donation (QUOD) National Biobank renal cortical biopsy samples taken at the time of organ retrieval will be requested retrospectively, where available.

ACKNOWLEDGEMENTS

Author contributions

MM revised and wrote the protocol for publication, designed the figures and edited the manuscript. SH and MLN designed the study and wrote the protocol, co-wrote the published version and edited the manuscript. SH is the guarantor.

Sponsors

This study is sponsored by the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust.

Funding

This study is funded by the National Institute for Health and Care Research (NIHR) Blood and Transplant Research Unit in Organ Donation and Transplantation (NIHR203332), a partnership between NHS Blood and Transplant, University of Cambridge and Newcastle University. The views expressed are those of the author(s) and not necessarily those of the NIHR, NHS Blood and Transplant or the Department of Health and Social Care.

Competing interests

Sarah Hosgood received an honorarium from CytoSorbents Europe GmbH. The other authors have no competing interests to declare.

Figures used in this publication were created using Biorender.com.

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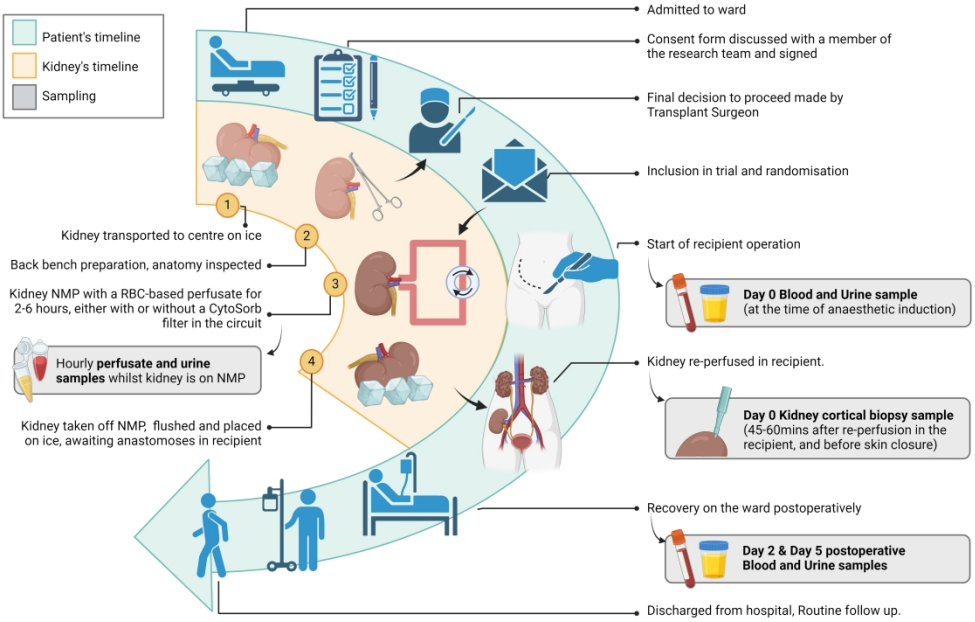
Figure 1: Participant timeline, delineating the patient's clinical care whilst enrolled in the trial (blue panel), the kidney's temporal course (yellow panel) and the key time points for sample collection (grey inserts).

Figure 2: Schematic representation of the XVIVO Kidney Assist perfusion circuit with a CytoSorb® filter incorporated. The kidney is placed in a sterile organ chamber with a lid. A patch clamp is used to connect the arterial end of the tubing to the renal artery. The renal vein and ureter drain directly into the venous reservoir (urine recirculation). Additional component parts (not shown) include a pressure transducer, an arterial flow sensor, temperature sensors and sampling ports.



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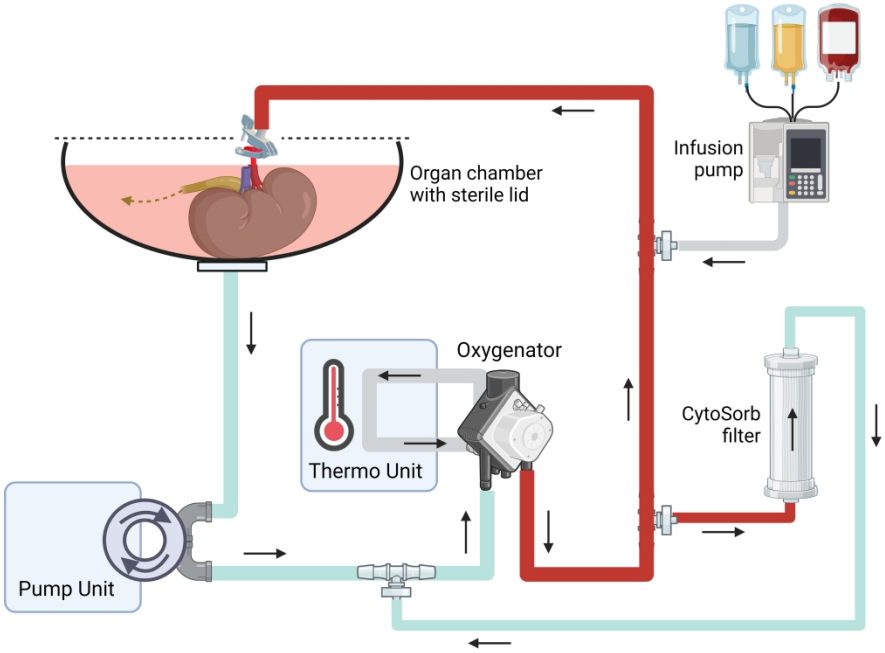

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UNIVERSITY OF CAMBRIDGE

Participant Information Sheet

IRAS: 322728

Sponsor: Cambridge University Hospitals NHS Foundation Trust and The University of Cambridge

Study title

A randomised pilot study to assess the safety and feasibility of adding a Cytosorb filter during kidney normothermic machine perfusion.

Invitation and brief summary

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.
In this research study, we would like to assess whether a new technique of kidney preservation is beneficial.

What is involved?
When kidneys are removed from an organ donor they are normally stored on ice until they are ready to be transplanted. A kidney can be preserved safely at a low temperature in these conditions. However, there is some degree of deterioration and the longer they are left in this condition the more they deteriorate (rather like food that is kept in the fridge). We have developed a technique that may improve the quality of the kidney. This involves placing the kidney on a machine and passing a warmed, oxygen-rich solution containing red blood cells through it. Under these conditions the kidney can start to function again and produce urine. We have trialled this in patients with no adverse effects. From our research we know that whilst being perfused, kidneys release cells that cause inflammation. In a small number of patients, we want to test whether adding a specialised filter to the machine has any beneficial effects.

What would taking part involve?

You will be prepared for surgery in the normal way. Standard practice involves keeping the transplant kidney under cold storage in ice until the time of the transplant operation. If you

consent to take part in this trial then your kidney will be randomly assigned to having your kidney placed on a machine and warmed with an oxygenated blood-based solution. In one group a special filter will be added to the circuit to reduce the risk of inflammation. Should there be any problem with the warm perfusion procedure then the kidney can be quickly removed from the perfusion machine and returned to cold storage in ice before transplantation.
During the transplant operation we will also take a small tissue biopsy from the kidney before after transplantation. Although there is a small risk of causing bleeding from the kidney biopsy site (<5%) your surgeon will be able to repair the bleeding site if this happens.

After your transplant you will receive the normal standard care but will also be asked to provide a few additional blood and urine samples for analysis. Your participation in this study will not affect the way you are followed up after a transplant. The normal follow up involves clinical visits at least twice a week for six weeks and then weekly for a further six weeks.

The data collected will be stored on a secure database and tissue, blood and urine samples within secured laboratories only accessed by the transplant research team. Once the samples have been analysed, they will be disposed as per HTA guidance.

What are the possible benefits of taking part?

This study is being performed to test whether the specialised filter added during warm perfusion can improve the condition of the kidney. The first step is to assess whether this is practical before carrying out a larger trial. We cannot guarantee that this will improve the outcome of your kidney transplant but it will help us to improve future techniques of kidney preservation. This may enable us to transplant more kidneys in the future.

What are the possible disadvantages and risks of taking part?

There are no potential side effects to you. This technique of warm perfusion is applied to the kidney only, before it is transplanted. There is a small risk that the kidney might be damaged during the assessment and therefore could not be transplanted. This has not happened in our experience of 200 cases so far but it remains as a potential risk.

What happens when the research study stops?
At the end of the research study, you will continue to be followed up for your kidney transplant either at Addenbrooke's Hospital or at your local renal hospital.

What if new information becomes available?
If new information becomes available your Transplant Consultant might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

What will happen if I do not want to carry on with the study?
You will be given normal care after the kidney transplant. If you withdraw from the study, we will ask permission to use the data collected up to your withdrawal.

What will happen to the results of the research study?
The results of the research will be published in specialist journals in order to inform other transplant doctors around the world. You will not be identified in any report or publication. You will be able to get a copy of the results by asking the kidney doctors in the follow up clinic.

Will my General Practitioner/Family Doctor (GP) be involved?
Participation in this trial will not affect your treatment and follow-up by your GP after discharge from the hospital.

Who is organising the research?
The research is being organised by the Transplant Research Team, Department of Surgery, University of Cambridge and at the Cambridge Transplant Unit

Who has reviewed the study?
The study has been reviewed by CUH research advisory committee and the Local Research Ethics Committee.

Will my taking part in this study be kept confidential?
We will follow ethical and legal best practice and all information about you will be handled confidentially. If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the research team. They may also be looked at by representatives of the regulatory authority or by those responsible for research and development audit (for monitoring the quality of the research). All have a duty of confidentiality to you as a research participant and will do their best to meet this duty. Our procedure for handling, processing, storage and destruction of data will match the *Data Protection Act 1998*. Your name will not be disclosed outside the hospital. The data collected will be stored and retained securely for 10 years and it will also be disposed of securely. You have the right to check the accuracy of data held about you and correct any errors.

What if there is a problem?
Any complaint about the way you have been dealt with during the trial or any possible harm you might suffer will be addressed. If you have any concerns about any aspect of this trial you should speak to your trial doctor who will do their best to answer your questions.

In the event that something does go wrong and you are harmed by taking part in the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Cambridge University Hospitals NHS Foundation Trust or

the University of Cambridge. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). The University has obtained insurance, which provides no-fault compensation i.e. for non-negligent harm, you may be entitled to make a claim for this.

Obtaining further information
If you have any questions or concerns about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions or to Professor Michael Nicholson (01223 339221)/ Dr Sarah Hosgood (01223 763105)

Complaints and Independent Advice
If you wish to speak to an independent body about any concerns or complaints about any aspect of the way you have been approached or treated during this trial, you can do this through the Addenbrooke's Kidney Patient's Association or the Patient Advice and Liaison Service (PALS) at Addenbrooke's Hospital. The formal NHS complaints procedure is also available to you. Details can be obtained through the hospital.

Complaints
If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure.

NHS based research
If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

Contacts for further information
A) General information about research can be found on www.nres.org.uk; www.addenbrookes.nhs.uk; or www.instituteofclinicalresearch.org.uk

B) For specific information about this research project, contact Professor Michael Nicholson, 01223 339221.

BMJ Open

Protocol for a single centre randomised pilot study to assess the safety and feasibility of adding a CytoSorb® filter during kidney normothermic machine perfusion to remove inflammatory and immune mediators prior to kidney transplantation

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-093001.R2
Article Type:	Protocol
Date Submitted by the Author:	07-Mar-2025
Complete List of Authors:	Mehta, Maithili; University of Cambridge, Department of Surgery Hosgood, Sarah; University of Cambridge, Department of Surgery Nicholson, Michael; University of Cambridge, Department of Surgery
Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Renal medicine, Surgery
Keywords:	Renal transplantation < NEPHROLOGY, Transplant surgery < SURGERY, Transplant medicine < INTERNAL MEDICINE

Protocol for a single centre randomised pilot study to assess the safety and feasibility of adding a CytoSorb® filter during kidney normothermic machine perfusion to remove inflammatory and immune mediators prior to kidney transplantation

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Word count: 3443

Protocol version 1.2, 4th July 2023. Rewritten for publication.

ABSTRACT

Introduction

The introduction of perfusion technologies in kidney transplantation has the potential to improve graft function and survival and increase utilisation. Our previous work demonstrated that kidneys with an enhanced inflammatory and immune response during normothermic machine perfusion (NMP) had significant graft dysfunction after transplantation. The addition of a cytokine filter (CytoSorb®) to the NMP circuit dramatically reduces both circulating inflammatory mediators and inflammatory gene expression but this has not been trialled in clinical practice.

Methods and analysis

This is a randomised phase I pilot study to evaluate the safety and feasibility of using a CytoSorb® filter in clinical NMP to remove inflammatory and immune mediators. Eligible kidney transplant recipients on the waiting list in the East of England will be approached for consent. A total of 20 patients will be recruited and randomised in a 1:1 ratio for the donor kidney to receive either NMP or NMP with a CytoSorb® filter pre-transplantation. The kidney will be transplanted according to standard practice after NMP. The primary endpoint is inflammatory and immune gene expression measured in a cortical biopsy from the kidney 60 minutes post-transplant. Secondary endpoints include rates and duration of delayed graft function, and graft function as assessed by change in creatinine clearance and estimated glomerular filtration rate 2 days, 5 days, 1 month and 3 months post-transplant. Additionally, inflammatory mediators and injury markers will be measured in peripheral blood and urine samples taken pre-operatively and on days 2 and 5 after transplant.

Ethics and dissemination

This study has been approved by the Health Research Authority (HRA) Health and Care Research Wales (HCRW) Committee (REC 23/WM/0141) and by NHS Blood and Transplant (NHSBT) (Ref: Study 148). Findings will be published in a peer-reviewed journal and disseminated at scientific conferences. The dataset will be made available on request.

Trial registration

The study is prospectively registered on the ISCRTN registry (ID: 13698207).

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study uses a highly sensitive and unbiased measure (inflammatory gene expression in post-reperfusion kidney cortical biopsies) as its primary endpoint
- The study is not sufficiently powered to support the inference of causal relationships between NMP and clinical outcomes.
- The study is not double-blinded.

INTRODUCTION

An increasing number of kidneys from extended criteria donors (ECD) and donation after circulatory death donors (DCD) are being used in transplantation due to the shortage of organs. These marginal kidneys offer a survival benefit to transplant patients compared to remaining on dialysis but portend a higher risk of delayed graft function (DGF) and graft loss compared to alternative kidney allografts[1,2]. Prolonged DGF is independently associated with longer hospital stays, increased cost, higher incidence of acute rejection, poorer 12-month graft function and higher rates of graft loss and death[3–5].

Traditional hypothermic kidney preservation techniques suppress cellular metabolism and oxygen requirements during transport; however, these conditions cause gradual depletion of energy substrates culminating in cellular damage. After transplantation, restoration of blood flow to the kidney causes further insult via ischaemia reperfusion injury (IRI), which involves a cascade of inflammatory and immune mediators that can lead to graft dysfunction.

Normothermic machine perfusion (NMP) offers an alternative organ preservation technique that recirculates an oxygenated red blood cell-based solution through the donor kidney at near physiological pressure and temperature[6]. It restores oxidative phosphorylation and cellular function following hypothermic preservation and prior to implantation in the recipient. NMP can potentially reduce injury caused by hypothermic conditions and provides a platform for organ assessment and the delivery of targeted treatments. The safety and feasibility of NMP in clinical practice has been established. The results of a randomised controlled trial of NMP in DCD kidneys showed no benefit of 1 hour of NMP in reducing rates of DGF post-transplant[7]. Longer durations of NMP have since been trialled and shown to be safe in clinical practice[8,9].

Evidence from non-transplanted human kidneys has shown that during NMP inflammatory and immune mediators are also upregulated similarly to the reperfusion response following graft implantation and restoration of blood flow[10]. Correlations with early outcome after transplantation show that kidneys with an enhanced inflammatory profile after NMP are more likely to have prolonged DGF[7,10]. Moreover, systemic inflammation early after kidney transplantation is associated with long-term graft loss[11].

Our group has shown in transplant-declined human kidneys that adding a CytoSorb® filter to the kidney NMP circuit removes the inflammatory and immune mediators from the circulating perfusate and attenuates a DGF-associated inflammatory gene signature in renal tissue[10,12]. CytoSorb® is a

Class IIb medical device made of a bio- and haemo-compatible porous bead-based polymer, which adsorbs hydrophobic molecules up to 55kDa (including cytokines and chemokines) in a concentration-dependent manner.

Clinically, the CytoSorb® filter has been used successfully to treat patients with sepsis, COVID-19 and severe inflammatory response syndrome[13–16]. It has also been used in several clinical trials during cardiac bypass[17]. Experimental studies in lung NMP have shown a beneficial effect after transplantation[18].

The primary aim of this study is to establish the safety and feasibility of adding a CytoSorb® filter in clinical kidney NMP to reduce the inflammatory and immune response within the kidney after transplantation. Secondary outcome measures include rates and duration of delayed graft function, and graft function as assessed by change in creatinine clearance and estimated glomerular filtration rate 2 days, 5 days, 1 month and 3 months post-transplant. Additionally, inflammatory mediators and injury markers will be measured in peripheral blood and urine samples taken pre-operatively and on days 2 and 5 after transplant.

METHODS

Study design

This is a randomised, single-blinded, single centre phase 1 pilot study. The protocol is written in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) guidelines[19]. Figure 1 summarises the study design including the temporal course of events for the patient and the kidney after arrival at the centre and the key time points for sample collection.

Sample size

A total of 20 patients will be recruited. As this is a pilot study, a formal sample size calculation was not performed. The sample size is pragmatic and based on previous experimental work in human kidneys showing that n = 5 per group was able to identify differences in transcriptional changes in human kidneys that were perfused with or with the Cytosorb filter in the perfusion circuit.

Recruitment

Patients will be recruited from a single centre, Cambridge University Hospitals NHS Foundation Trust (Addenbrooke's site) between 01 January 2025 – 01 June 2026. The deceased donor kidney transplant waiting list at Cambridge will be used to identify eligible participants. Participant information sheets will be sent to eligible participants beforehand. The transplant co-ordinators will

contact the research team when the patient is called into the transplant centre after the identification of a suitably matched donation after brain death (DBD) or DCD donor kidney. Once assessed and the inclusion criteria met, the patient will be offered the opportunity to ask any further questions.

Inclusion criteria

Kidney transplant recipients who meet all of the criteria below are eligible to participate.

1. Aged ≥18 years
2. Either on dialysis (any modality) or pre-dialysis
3. Undergoing a first or second kidney transplant
4. Receiving a kidney from a DBD or DCD donor aged ≥50 years
5. Able to provide written informed consent

Exclusion criteria

Kidney transplant recipients who meet any of the criteria below are ineligible to participate.

1. Undergoing a third or subsequent kidney transplant
2. Receiving a multi-organ transplant (e.g. simultaneous pancreas–kidney transplant)
3. Receiving a dual kidney transplant
4. Receiving a paediatric en-bloc kidney transplant
5. Receiving a kidney from a DBD or DCD donor aged <50 years
6. Receiving a kidney with complex vascular anatomy

Consent

Written informed consent (see supplement) will be obtained by a qualified member of the research team on the day the patient is called into hospital and prior to the kidney transplant taking place.

Randomisation

Patients receiving a deceased donor kidney transplant who meet the eligibility criteria and provide informed consent will be randomised in a 1:1 ratio for the donor kidney to receive NMP alone or NMP with the CytoSorb® filter prior to transplantation. Randomisation will be performed after the transplant recipient and kidney have both arrived in the transplant centre and a final decision to proceed with transplantation has been made. The randomisation will be performed by a member of the research team using the sealed envelope™ simple randomisation service. The patient and medical staff caring for the patient after surgery will be blinded to the groups.

Clinical care

Following organ retrieval, the kidney will be transported to the transplant centre on ice as per standard protocol. Bench preparation of the kidney will take place and any anatomical abnormalities

that preclude inclusion in the trial will be noted.

NMP with or without the CytoSorb® filter will then be carried out as described below by members of the research team and clinical staff who are independent to the care of the patient.

The recipient will concurrently be anaesthetised according to local protocols. Anti-microbial prophylaxis, anti-thrombotic prophylaxis and immunosuppressive medication will be given according to local protocols. It is expected that patients will also receive prophylaxis against Pneumocystis jiroveci pneumonia, oral candidiasis and cytomegalovirus.

The kidney will be transplanted using standard techniques into either iliac fossa. The renal artery will be anastomosed to either the common, external or internal iliac arteries, and the renal vein to either the common or the external iliac vein. The ureteric anastomosis will be performed as an extravesical onlay over a double J stent.

A cortical 4mm punch biopsy of the kidney will be taken post-reperfusion (45–60 minutes), and recipient blood and urine samples will be collected at pre-determined time points as described below.

Study groups

Normothermic machine perfusion

Kidneys will be placed on the NMP system (Kidney Assist, XVIVO) and perfused with a red cell-based solution mixed with a priming solution (human serum albumin 5%, ringer's solution, dexamethasone, heparin, meropenem, calcium gluconate 10% and sodium bicarbonate 8.4% to adjust pH within normal range). Infusion pumps will deliver the following:

- 5% glucose 2–4ml/h as required
- Amino acid/electrolyte solution (with 100units of Actrapid insulin, 15ml sodium bicarbonate 8.4% and 5ml Cernevit) 5–10ml/h
- Prostacyclin 0.5mg x1 (i/v infusion 16ml in 100ml 0.9% sodium chloride) 3ml/h as required
- Verapamil 2.5mg as required
- Ringer's lactate solution to replace urine output

Kidneys will be perfused for a minimum of 2 hours and a maximum of 6 hours at a mean arterial pressure of 80–85mmHg and temperature of 35–37.4°C. Renal blood flow (RBF) will be monitored continuously during NMP. Intra-renal resistance (IRR) will be calculated (mean arterial

pressure/RBF) until the end of perfusion. Blood gas analysis will be used to measure the acid–base balance during NMP.

After NMP kidneys will be flushed with cold preservation solution and placed on ice until transplantation.

Normothermic machine perfusion with CytoSorb® filter

Kidneys will be perfused as per the standard NMP protocol but with the CytoSorb® filter (CytoSorbents Europe GmbH) added to the circuit for the duration of perfusion (Figure 2).

Follow up

Patients will be followed up at the transplant clinic or local centre at 1 and 3 months as normal practice after discharge from hospital.

Withdrawal from the study

Withdrawal from the study is likely to be uncommon but may occur on account of withdrawal of consent by the patient or the kidney being deemed untransplantable following final bench surgery.

Patients who are randomised but withdraw before intervention will receive standard clinical care according to the local protocol. If patients undergo the intervention but subsequently withdraw, they will also receive standard clinical care. In the unexpected situation where consent to use data and samples that have already been collected is withdrawn, these will be discarded.

Protocol deviations

One-off protocol deviations will be documented and reported to the Chief Investigator and Sponsor. Frequent deviations will be reported to the Sponsor and REC.

Patient and Public Involvement

This research proposal was presented to the NHIR Blood and Transplant Research Unit in Organ Donation and Transplantation (BTRU in ODT) Patient and Public Research Panel who provided guidance on the protocol design. On completion of the study, the results will be presented to panel members at an annual NIHR BTRU in ODT progress meeting.

OUTCOMES

Primary outcome

The primary outcome measure is inflammatory and immune gene expression measured in post-reperfusion renal cortical biopsies.

Secondary outcomes

The secondary outcome measures are:

1. Rates of DGF, defined as the need for dialysis within the first seven postoperative days
2. Duration of DGF
3. Incidence of primary non-function (PNF), defined as dialysis dependence or creatinine clearance≤20 ml/min at 3 months post-transplant
4. Graft function at 3 months post-transplant as measured by estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI equation
5. Graft survival at 3 months post-transplant
6. Patient survival at 3 months post-transplant
7. Incidence of biopsy-proven acute rejection
8. Complications within 3 months of transplant (infection, re-operation due to bleeding)
9. Length of hospital stay
10. Levels of inflammatory/immune and injury markers in perfusate and urine samples taken during NMP
11. Isolation of peripheral blood mononuclear cells from recipient blood samples pre-transplant and on days 2 and 5 post-transplant to examine the inflammatory/immune response
12. Biomarkers of kidney injury on days 2 and 5 post-transplant.

SAMPLES AND ANALYSIS

Sample collection

- A cortical 4mm punch biopsy of the kidney will be taken from the transplant kidney at 45–60 minutes after reperfusion. Tissue samples will be divided. Half will be fixed in 10% formalin and the other half placed in RNA later.
- Samples of the perfusate and urine (if any) from the kidneys during NMP will be collected at the start and hourly until the end of NMP.
- Peripheral blood (10ml) and urine (10ml) samples from the transplant recipient will be collected at the start of surgery and then at days 2 and 5 post-transplant.

Sample storage

Samples will be processed as required and then stored at –80°C or in liquid nitrogen. Frozen tissue samples will be stored in the research laboratories at the University of Cambridge, Department of Surgery. Fixed tissue will be processed by the tissue bank at Addenbrooke's Hospital and stored within the Department of Surgery laboratories.

Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation and the requirements set out in the 2004 Human Tissue Act. Samples will be labelled in compliance with the 1998 Data Protection Act. On completion of the trial samples will be disposed of in accordance with the Human Tissue Authority's Code of Practice.

Data collection and storage

Patient related data will be collected from computerised health records. Each participant will be allocated a study code to facilitate data anonymisation for storage on a password protected database.

Analysis

- Donor and recipient characteristics, levels of creatinine clearance, eGFR, graft and patient survival, complications, length of hospital stay, incidences of DGF and PNF, episodes of acute rejection and perfusion parameters during NMP will be compared between the two groups using the appropriate statistical tests.
- Cortical kidney tissue will be analysed as follows. RNA will be extracted from RNALater biopsies and bulk sequenced. The data will thereafter be demultiplexed, assessed for quality control using FASTQC, the Fastq files aligned to the human genome using Hisat2, and normalisation and differential gene expression analysis performed using DESeq2 . RNA extraction, library preparation, sequencing and sample analysis will be performed at a laboratory with expertise in this area. Additionally fixed tissue will be used to assess the level of injury and inflammation.
- Samples of perfusate and urine from the kidney during NMP, and of urine from the recipient will be analysed as follows. Levels of cytokines will be measured using a Cytokine Luminex panel. Biomarkers will be quantified using a Proteome Profiler Human Kidney Biomarker Array Kit. Changes in the level of cytokines and injury markers will be calculated and compared between groups using repeated measures and ANOVA analysis.
- Peripheral blood samples from recipients will be analysed as follows: peripheral blood mononuclear cells will be isolated from whole blood using density-gradient separation, and their relative abundance, phenotype and activation status determined using cell sorting, flow

cytometry, transcriptomic analysis and cell culture techniques. Levels of inflammation/immune mediators will be measured using a Luminex panel.

Cytokines and chemokines of particular interest are G-CSF, GM-CSF, IFN-α, IFN-γ, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, IL-18, TNF-α, CXCL2, CXCL3, CCL5 and CXCL4. Biomarkers of interest include C-reactive protein, prostaglandin E2, prostacyclin, thromboxane B1, HMGB1, β2-microglobulin, NGAL, and KIM-1 among others.
Analysis of the RNAseq data will focus particularly on the expression of TNF-α signalling via NFκB, mTORC1 signalling, inflammatory response, P53 pathway, IL-2 signalling and TGF-ß signalling as these were the most upregulated genes in our previous study [7].

ETHICS AND DISSEMINATION

Ethics

This study has been approved by the Health Research Authority (HRA) Health and Care Research Wales (HCRW) Committee (REC 23/WM/0141) and by NHS Blood and Transplant (Ref: Study 148).

Confidentiality

Patient identifiable information will be accessed and handled by members of the research team in compliance with the 1998 Data Protection Act. Only patient unidentifiable data will be transmitted to the sponsor and co-investigators. The data will be stored for 5 years after the end of study. The custodian of the data will be the Chief Investigator.

Adverse events

The risk to participants entering the study is low. Any adverse events directly linked to the intervention will be recorded and reported to the Chief Investigator and Sponsor immediately.

Indemnity

Cambridge University Hospitals NHS Foundation Trust will accept full financial liability for harm caused to participants in the study through the negligence of its employees and honorary contract holders. The University of Cambridge will arrange insurance for negligent harm on account of protocol design and for non-negligent harm arising through participation in the study.

Dissemination

Findings will be published in a peer-reviewed journal and disseminated at scientific conferences. The dataset will be made available on request.

DISCUSSION

Anticipated benefits

To our knowledge, this is the first clinical study to test the safety and feasibility of using CytoSorb® therapy during kidney NMP.

It is anticipated that the analysis will provide novel mechanistic insights. The use of global transcriptomic techniques on post-reperfusion kidney biopsies will elucidate the role played by cytokine signalling in reperfusion injury to the graft. Further, the isolation and characterisation of peripheral blood mononuclear cells and inflammatory mediators from recipient blood samples taken in the days following transplant will provide valuable information about the host systemic inflammatory response in the early postoperative period, and whether this is altered by pre-transplant CytoSorb® therapy.

Whilst not its primary aim, the study will contribute to the evidence base for the safety and feasibility of intermediate durations of kidney NMP (2–6 hours).

Limitations

The study design has some limitations. Firstly, whilst the sample size is large enough to prove feasibility, the study is not sufficiently powered to draw causal inferences about the relationship between the interventions and clinical endpoints. However, the results will inform future efficacy trials. Secondly, randomisation does not account for donor type, therefore there is a small risk of imbalance in the number of DBD/DCD kidneys between the two study arms.
Finally, a clinical and ethical decision was made to take a single renal cortical biopsy post-reperfusion to minimise the risk of bleeding. The lack of a pre-transplant (pre-NMP) biopsy makes it difficult to assess the contribution of baseline inflammation in the donor allograft at the time of organ retrieval, as well as the impact of NMP on the inflammatory status of the donor kidney. To mitigate the impact of this on the analysis, access to Quality in Organ Donation (QUOD) National Biobank renal cortical biopsy samples taken at the time of organ retrieval will be requested retrospectively, where available.

ACKNOWLEDGEMENTS

Author contributions

MM revised and wrote the protocol for publication, designed the figures and edited the manuscript. SH and MLN designed the study and wrote the protocol, co-wrote the published version and edited the manuscript. SH is the guarantor.

Sponsors

This study is sponsored by the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust.

Funding

This study is funded by the National Institute for Health and Care Research (NIHR) Blood and Transplant Research Unit in Organ Donation and Transplantation (NIHR203332), a partnership between NHS Blood and Transplant, University of Cambridge and Newcastle University. The views expressed are those of the author(s) and not necessarily those of the NIHR, NHS Blood and Transplant or the Department of Health and Social Care.

Competing interests

Sarah Hosgood received an honorarium from CytoSorbents Europe GmbH. The other authors have no competing interests to declare.

Figures used in this publication were created using Biorender.com.

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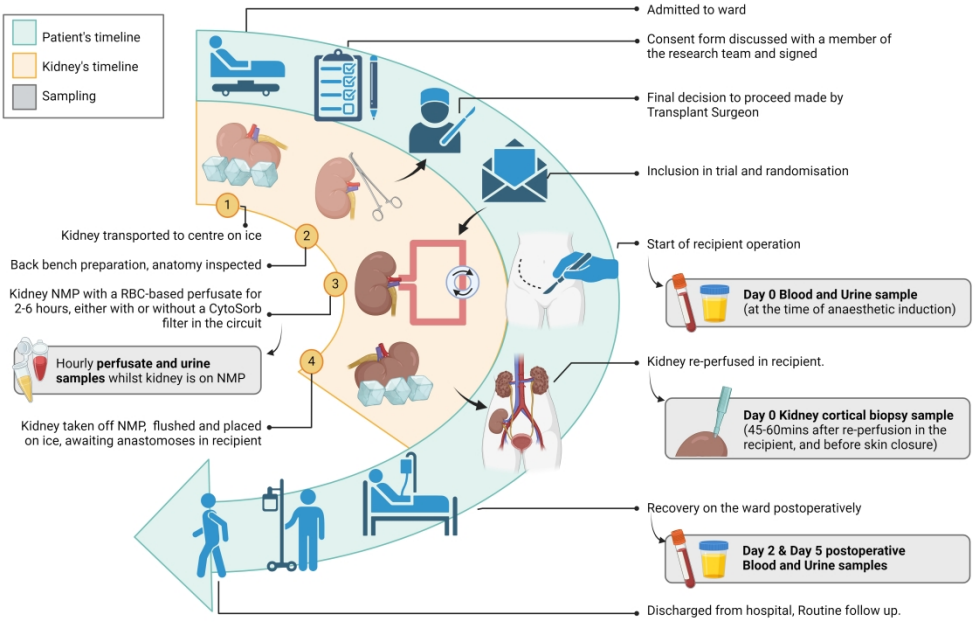
Figure 1: Participant timeline, delineating the patient's clinical care whilst enrolled in the trial (blue panel), the kidney's temporal course (yellow panel) and the key time points for sample collection (grey inserts).

Figure 2: Schematic representation of the XVIVO Kidney Assist perfusion circuit with a CytoSorb® filter incorporated. The kidney is placed in a sterile organ chamber with a lid. A patch clamp is used to connect the arterial end of the tubing to the renal artery. The renal vein and ureter drain directly into the venous reservoir (urine recirculation). Additional component parts (not shown) include a pressure transducer, an arterial flow sensor, temperature sensors and sampling ports.



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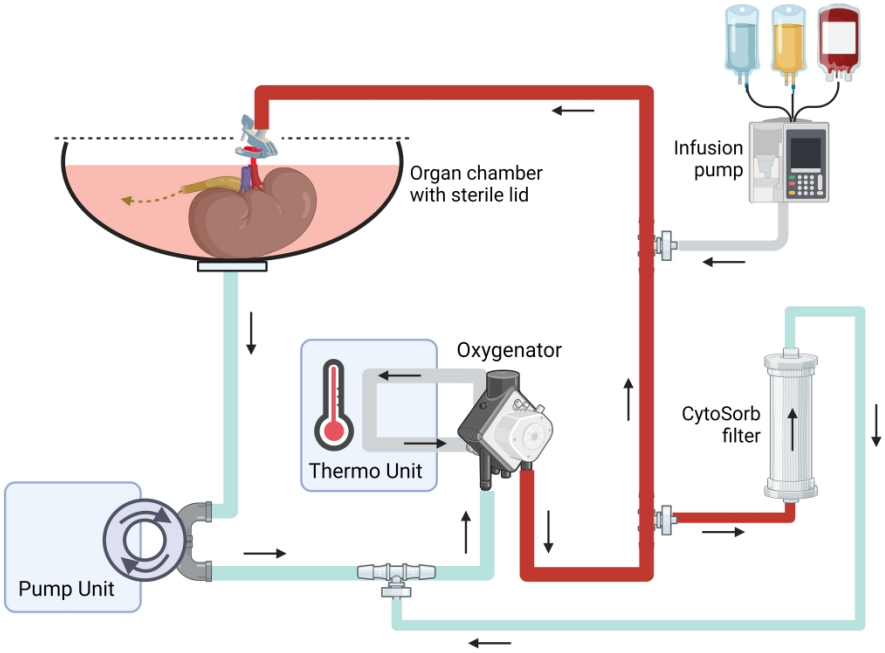

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UNIVERSITY OF CAMBRIDGE

Participant Information Sheet

IRAS: 322728

Sponsor: Cambridge University Hospitals NHS Foundation Trust and The University of Cambridge

Study title

A randomised pilot study to assess the safety and feasibility of adding a Cytosorb filter during kidney normothermic machine perfusion.

Invitation and brief summary

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.
In this research study, we would like to assess whether a new technique of kidney preservation is beneficial.

What is involved?
When kidneys are removed from an organ donor they are normally stored on ice until they are ready to be transplanted. A kidney can be preserved safely at a low temperature in these conditions. However, there is some degree of deterioration and the longer they are left in this condition the more they deteriorate (rather like food that is kept in the fridge). We have developed a technique that may improve the quality of the kidney. This involves placing the kidney on a machine and passing a warmed, oxygen-rich solution containing red blood cells through it. Under these conditions the kidney can start to function again and produce urine. We have trialled this in patients with no adverse effects. From our research we know that whilst being perfused, kidneys release cells that cause inflammation. In a small number of patients, we want to test whether adding a specialised filter to the machine has any beneficial effects.

What would taking part involve?

You will be prepared for surgery in the normal way. Standard practice involves keeping the transplant kidney under cold storage in ice until the time of the transplant operation. If you

consent to take part in this trial then your kidney will be randomly assigned to having your kidney placed on a machine and warmed with an oxygenated blood-based solution. In one group a special filter will be added to the circuit to reduce the risk of inflammation. Should there be any problem with the warm perfusion procedure then the kidney can be quickly removed from the perfusion machine and returned to cold storage in ice before transplantation.
During the transplant operation we will also take a small tissue biopsy from the kidney before after transplantation. Although there is a small risk of causing bleeding from the kidney biopsy site (<5%) your surgeon will be able to repair the bleeding site if this happens.

After your transplant you will receive the normal standard care but will also be asked to provide a few additional blood and urine samples for analysis. Your participation in this study will not affect the way you are followed up after a transplant. The normal follow up involves clinical visits at least twice a week for six weeks and then weekly for a further six weeks.

The data collected will be stored on a secure database and tissue, blood and urine samples within secured laboratories only accessed by the transplant research team. Once the samples have been analysed, they will be disposed as per HTA guidance.

What are the possible benefits of taking part?

This study is being performed to test whether the specialised filter added during warm perfusion can improve the condition of the kidney. The first step is to assess whether this is practical before carrying out a larger trial. We cannot guarantee that this will improve the outcome of your kidney transplant but it will help us to improve future techniques of kidney preservation. This may enable us to transplant more kidneys in the future.

What are the possible disadvantages and risks of taking part?

There are no potential side effects to you. This technique of warm perfusion is applied to the kidney only, before it is transplanted. There is a small risk that the kidney might be damaged during the assessment and therefore could not be transplanted. This has not happened in our experience of 200 cases so far but it remains as a potential risk.

What happens when the research study stops?
At the end of the research study, you will continue to be followed up for your kidney transplant either at Addenbrooke's Hospital or at your local renal hospital.

What if new information becomes available?
If new information becomes available your Transplant Consultant might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

What will happen if I do not want to carry on with the study?
You will be given normal care after the kidney transplant. If you withdraw from the study, we will ask permission to use the data collected up to your withdrawal.

What will happen to the results of the research study?
The results of the research will be published in specialist journals in order to inform other transplant doctors around the world. You will not be identified in any report or publication. You will be able to get a copy of the results by asking the kidney doctors in the follow up clinic.

Will my General Practitioner/Family Doctor (GP) be involved?
Participation in this trial will not affect your treatment and follow-up by your GP after discharge from the hospital.

Who is organising the research?
The research is being organised by the Transplant Research Team, Department of Surgery, University of Cambridge and at the Cambridge Transplant Unit

Who has reviewed the study?
The study has been reviewed by CUH research advisory committee and the Local Research Ethics Committee.

Will my taking part in this study be kept confidential?
We will follow ethical and legal best practice and all information about you will be handled confidentially. If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the research team. They may also be looked at by representatives of the regulatory authority or by those responsible for research and development audit (for monitoring the quality of the research). All have a duty of confidentiality to you as a research participant and will do their best to meet this duty. Our procedure for handling, processing, storage and destruction of data will match the *Data Protection Act 1998*. Your name will not be disclosed outside the hospital. The data collected will be stored and retained securely for 10 years and it will also be disposed of securely. You have the right to check the accuracy of data held about you and correct any errors.

What if there is a problem?
Any complaint about the way you have been dealt with during the trial or any possible harm you might suffer will be addressed. If you have any concerns about any aspect of this trial you should speak to your trial doctor who will do their best to answer your questions.

In the event that something does go wrong and you are harmed by taking part in the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against Cambridge University Hospitals NHS Foundation Trust or

the University of Cambridge. The normal National Health Service complaints mechanisms will still be available to you (if appropriate). The University has obtained insurance, which provides no-fault compensation i.e. for non-negligent harm, you may be entitled to make a claim for this.

Obtaining further information
If you have any questions or concerns about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions or to Professor Michael Nicholson (01223 339221)/ Dr Sarah Hosgood (01223 763105)

Complaints and Independent Advice
If you wish to speak to an independent body about any concerns or complaints about any aspect of the way you have been approached or treated during this trial, you can do this through the Addenbrooke's Kidney Patient's Association or the Patient Advice and Liaison Service (PALS) at Addenbrooke's Hospital. The formal NHS complaints procedure is also available to you. Details can be obtained through the hospital.

Complaints
If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure.

NHS based research
If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms will be available to you.

Contacts for further information
A) General information about research can be found on www.nres.org.uk; www.addenbrookes.nhs.uk; or www.instituteofclinicalresearch.org.uk

B) For specific information about this research project, contact Professor Michael Nicholson, 01223 339221.