Cambridge Transplant Research Group.

Clinical Study Protocol

Full Study Title:	A study to evaluate the effects of different perfusion conditions during *ex situ* liver perfusion on RNA transcription
Short title	Investigating different liver perfusion conditions
Protocol Version	3.1
Protocol date:	7th March 2023
IRAS project number	295373
Research Ethics reference	21/EE/0177
Clinical trials registry:	ISRCTN36453355
CUH R&D number:	A095917

Chief investigator:	Mr Andrew Butler
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Study Location:	Cambridge University Hospitals Transplant Unit
Study Joint Sponsors:	Cambridge University Hospitals NHS Foundation Trust and University of Cambridge

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1 Study Synopsis

Title of clinical trial	A study to evaluate the effects of different perfusion conditions during *ex situ* liver perfusion
Sponsor name	University of Cambridge and Cambridge University Hospitals Foundation Trust
Trials registry reference number	*awaited*
CUH R&D number	A095917
IRAS number	295373
Medical condition or disease under investigation	Liver Transplantation; ischaemia reperfusion injury
Purpose of clinical trial	To evaluate different conditions for perfusing *ex situ* liver perfusion
Primary objective	To determine the optimal perfusate for *ex situ* liver perfusion
Secondary objective (s)	To evaluate transcriptomics as a means of assessing changes in perfusion conditions
Study Design	Single centre, multiple group, randomised study
Study Endpoints	Transcriptomic changes Reperfusion injury markers Graft survival Cholangiopathy
Sample Size	6-10 per study group
Summary of eligibility criteria	All adult patients undergoing liver transplantation
Duration of subject follow up	12 months

2 General information

2.1 Sponsor details

Cambridge University Hospitals NHS Foundation Trust and the University of Cambridge

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2.3 Site investigators

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2.4 Laboratories

MRC Laboratory of Molecular Biology for RNA seq

Department of Surgery for ELISAs

2.5 Trial Manager

None

2.6 Protocol amendments

2.6.1 Following research ethics committee review. Protocol version 2.0, 10-08-21

A statement regarding contacting the subject if any new information arises that may affect their participation has been added. This is in section 9.5

2.6.2 Amendment 1. Change of Chief investigator to Andrew Butler

Change of patient information sheet to reflect changes

2.6.3 Amendment 2. Explicit addition of metabolic studies

Assessment of the perfusate samples to include metabolomic profiling and pharmacokinetics of antibiotics

Protocol version 3.1, date 07-03-2023

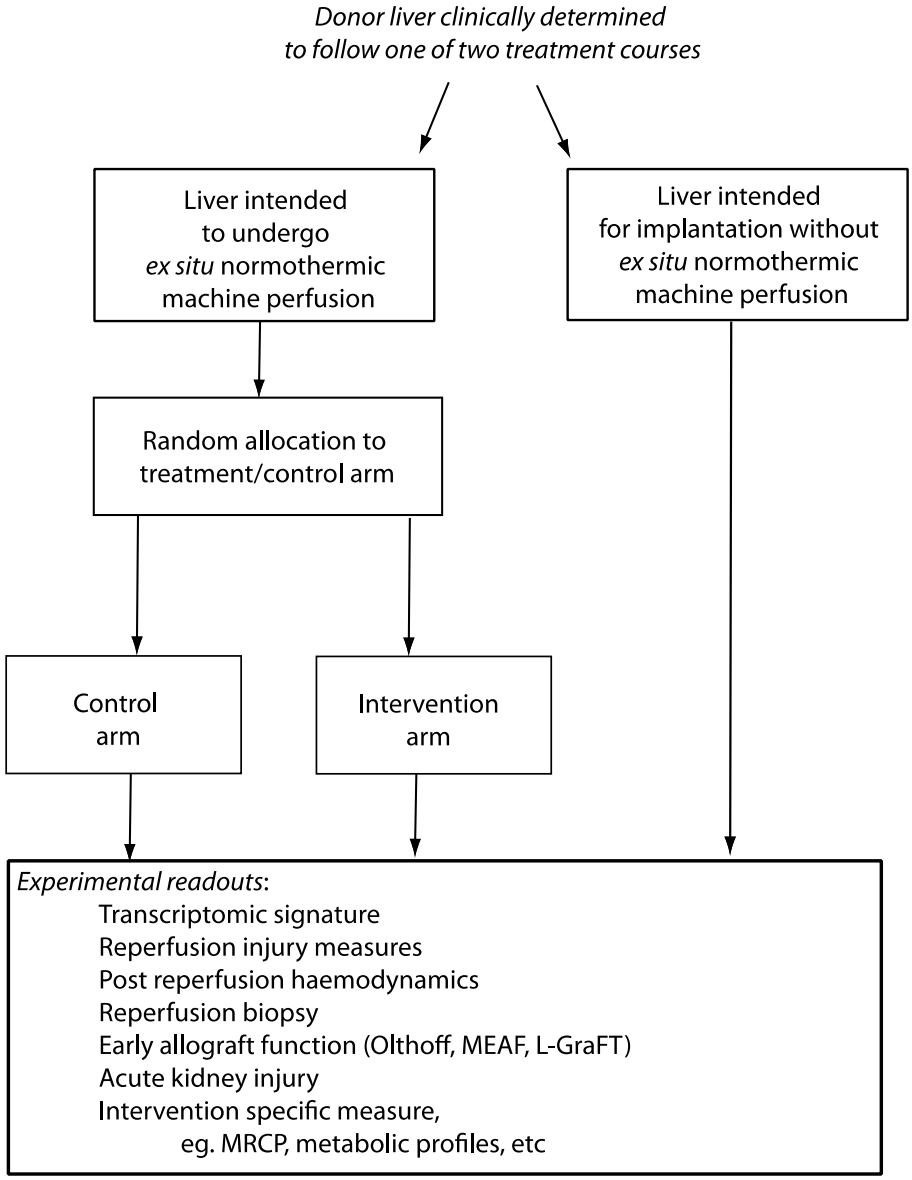
2.7 Abbreviations

°C	degrees Celsius
ALT	Alanine transaminase
AST	Aspartate transaminase
CBD	Common bile duct
CHD	Common hepatic duct
DBD	Donation after brain death
DCD	Donation after circulatory death
FFP	Fresh frozen plasma
GLUT2	Glucose transporter 2
H&E	Haematoxylin and eosin, a histology stain
HAS	Human Albumin solution
HMGB-1	High mobility box group-1
HOPE	Hypothermic oxygenated perfusion
ICH	International Conference on Harmonisation
ICJME	International Committee of Medical Journal Editors
INR	International normalised ratio
L-GraFT	Liver Graft Assessment following transplantation
LDH	Lactate dehydrogenase
MEAF	Model for Early Allograft Function
MRCP	Magnetic resonance cholangiopancreatography
MSB	Martius Scarlet Blue, a histology stain
NAS	Non anastomotic biliary strictures
NESLiP	Normothermic *ex situ* liver perfusion
pH	$-\log_{10}[H^+]$
REC	Research ethics committee
RIFLE	Risk, Injury, Failure, Loss, and End-stage kidney disease
RNA	Ribonucleic acid
ROS	Reactive oxygen species
TBARS	Thiobarbituric Acid Reactive Substances
TPA	Tissue plasminogen activator
UW	University of Wisconsin Organ preservation solution
WGCNA	weighted correlation network analysis

2.8 Trial summary

Liver transplantation is a life-saving treatment that is limited by shortage of organs. *Ex situ* machine perfusion has being introduced as a way of assessing livers pre-transplant to ensure they will work post transplant. In spite of initial evidence as to its efficacy, the optimal perfusion conditions remain to be determined. This study will use the OrganOx *metra* device to perfuse livers and will introduce modifications either to the perfusate or the perfusion conditions, and use standard clinical markers together with analysis of the transcriptomic signature to determine which is best. At the same time the study will enable identification of transcriptomic markers during perfusion which determine the post transplant outcomes.

3 Study Flow Chart



4 Background

4.1 Introduction

In 2019/20, around a third of deceased donor livers were not used for transplantation[1] and while for some there would be unmodifiable reasons such as cirrhosis in the donor liver, for many it was because the surgeon was not confident that the liver being offered would function satisfactorily in the intended recipient. This, in part, accounts for why over 10% of patients listed for a replacement liver will not survive long enough to undergo transplantation in spite of a surplus of deceased donor livers apparently being available [1]. This figure is likely to increase as a consequence of COVID due to the reduced number of donations. In addition, access to the waiting list is restricted due to the perceived shortage of donor livers[2].

Normothermic *ex situ* liver perfusion (NESLiP) is a new technique for preserving the function of a liver outside the body before transplantation. Importantly it affords the ability to check the viability of a liver [3], and as such has enabled livers that would previously have been turned down to be transplanted by removing the uncertainty at the time of offering [4,5]. The UK is a pioneer in the use of NESLiP, having developed the technology used today two decades ago [6].

Following the initial first in man studies in 2013[7], clinical research programmes in NESLiP began in Birmingham and Cambridge in 2015 [4,8]. Some elements of NESLiP, such as the circuit design and need for bile salts in the perfusate, had been defined in preclinical models[6,9,10]. However, many of the perfusate elements involved in the *ex situ* perfusion of a human liver were not optimised before the technique entered the clinic, and it is likely that the current technique does not provide the optimal perfusion conditions.

Variations in perfusion conditions have been explored in livers deemed not to be fit for transplantation, with evidence that many different variations in protocol and perfusate composition were permissive for *ex situ* liver function, and many discarded livers could be transplanted safely with excellent results [11].

By virtue of the heterogeneous nature and poor quality of most of the livers deemed unsuitable to transplant, and an inability to translate *ex situ* metabolic function into successful perfusion *in vivo,* it has not been possible to fully evaluate the effects of different perfusion conditions in the laboratory using discarded livers. For this reason we propose a study in livers which are intended for transplantation, which should be a less heterogenous cohort.

Undertaking a study powered on graft survival or early function parameters alone would require huge numbers of livers for each small alteration in perfusion protocol. Instead we propose to look for transcriptomic readouts of efficacy, judging livers by transcriptomic signatures that have been previously shown to be associated with poor or non function, or excellent function, as well as transcriptomic markers of immune activation. This should enable much smaller numbers of perfusions to be undertaken and early identification of good candidates to subject to bigger randomised studies with graft survival as an outcome.

4.2 Transcriptomic signatures of reperfusion injury

RNAseq is a technique to assess which genes in a tissue are activated at a given time point. It has been used to evaluate discarded human kidneys and livers perfused under varying conditions to determine the typical perfusion transcriptomic signature [12], and how different interventions change it[13]. Ferdinand et al have demonstrated that addition of a leucocyte filter into the kidney perfusion circuit reduces the inflammatory RNAseq signature, something

previously shown in pig kidney perfusions[14]; They also demonstrated the beneficial effects of incorporation of a Cytosorb "cytokine filter" to remove circulating cytokines (figure 1) [12].

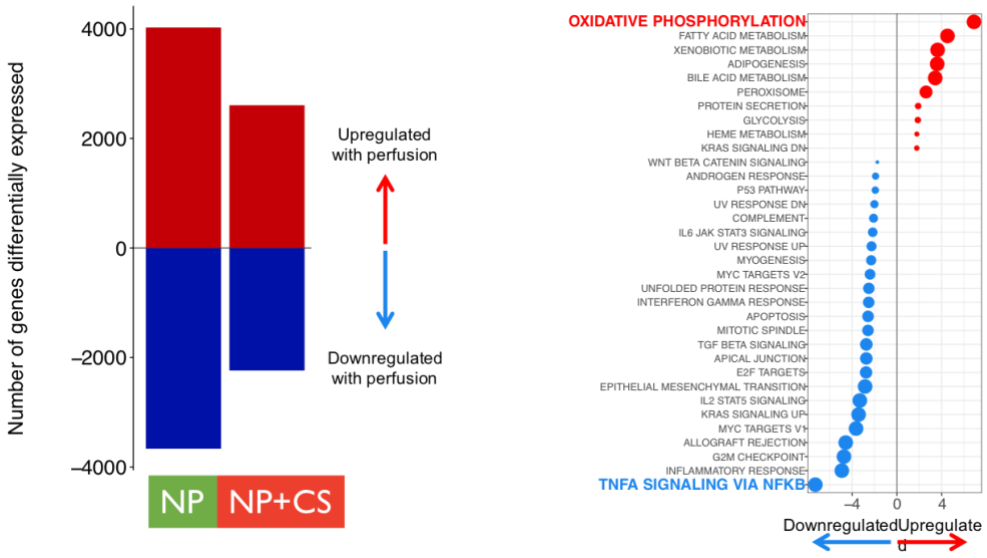


Figure 1. Changes in gene expression by the addition of a Cytosorb cytokine filter (NP+CS) to the circuit of kidneys undergoing one hour of normothermic perfusion (NP) [13].

We propose to extend this work in our study by looking at livers intended for transplantation which are subjected to slight modifications in the perfusion conditions that we have previously shown to be metabolically safe in "research livers" using our own published and widely accepted, viability readouts [11]. In addition we have shown that the transcriptional changes induced by normothermic perfusion are similar across kidneys and livers and lungs (figure 2) [12]. This transcriptional response relates not only to normal metabolic activity, but also to the response to reperfusion after ischaemia.

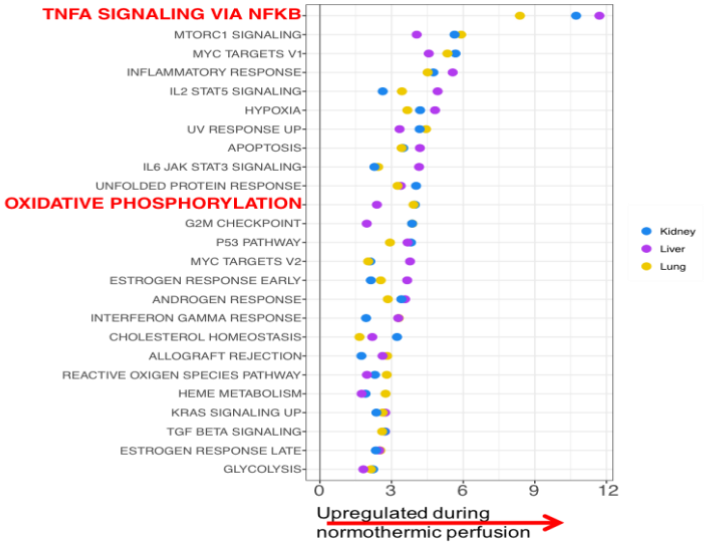


Figure 2. Gene expression during normothermic perfusion of livers, kidneys and lungs showing similarities in transcriptional response [12].

4.3 Transcriptomic signature of viability

In parallel to this study, two separate studies are currently being undertaken to identify a transcriptomic signature of viability.

4.3.1 QUOD pre-perfusion biopsy study

The first is being done using biopsies from the Quality in Organ Donation (QUOD) biorepository. The QUOD biorepository holds biopsies taken at the time of organ retrieval from the donor from around 80% of livers transplanted in the UK since it was established over 5 years ago. Biopsies have been stored in RNAlater for analysis by research teams.

The transcriptomic signature for "good" and "bad" livers, is being defined from QUOD biopsies from donor livers that worked well and those that did not work well, selected as follows:

a) Livers that suffered primary non function (i.e. never worked post transplant) and underwent early retransplant;
b) Livers identified from our own patients as suffering poor initial function using the Model for Early Allograft Function (MEAF) score [15]
c) Livers with good immediate function identified from our own database.

4.3.2 Pre and post perfusion biopsy evaluation

Using the same QUOD biorepository it has been possible to define a transcriptomic signature of kidneys that did and did not suffer delayed function following transplantation, by looking at the gene expression after a 1 hour period of normothermic perfusion; upregulation of pro-inflammatory genes was the predominant association with delayed graft function (figure 3) [13].

Using biopsies from livers with poor and good early graft function in this study, defined by their MEAF and L-Graft scores, a transcriptomic signature of early liver graft function will be sought.

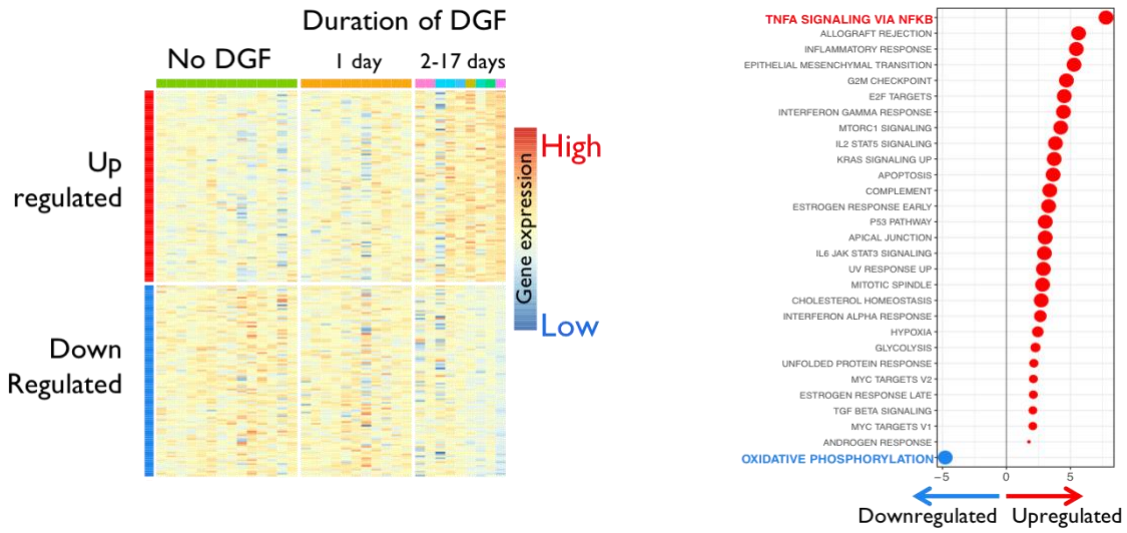


Figure 3. Gene expression in kidneys with and without delayed graft function (DGF). Genes associated with an inflammatory response are upregulated more in kidneys with DGF [13].

4.4 Rationale for Study

This project aims to look at a number of different, often subtle changes in perfusion conditions and use clinical, metabolic, transcriptomic and histological readouts, in addition to transplant outcomes, to assess which techniques are associated with the most favourable outcomes on all parameters.

4.5 Current liver perfusion practice

Currently the preferred machine for performing NESLiP is the OrganOx *metra,* which has a CE mark and is currently being considered by the FDA in the USA for licensing. The currently used perfusate composition is listed below. The device has an internal algorithm that controls oxygen delivery, temperature and perfusion flows. In previous work on research livers using a different CE marked device (Liver Assist, Organ Assist, Groningen) which did not have an internal algorithm for regulating perfusion, it was possible to manipulate flow rates, pressures, oxygenation and temperature, as well as using different perfusate components. However this machine is not as conducive to perfusing livers that will be transplanted.

The current perfusate composition in Cambridge includes the following (starred items are not specified by the company):

- 3 units *washed** packed red cells (PRBCs)
- 500ml 5% Human Albumin solution
- Heparin 10000u
- Magnesium Sulphate 50%: 4mmol (2mls)*
- Sodium bicarbonate 8.4% 20mmol (20ml)
- Fluconazole 100mg (anti-candidal antimicobial)*
- Meropenem 100mg (broad spectrum antibacterial)*
- Hydrocortisone: 100mg (anti-inflammatory)*
- N-acetylcysteine (Parvolex) 400mg (antioxidant)*
- Aminoven 10mL (amino acid mixture)*

At 15 minutes, 5 mmol of calcium chloride is added and pH is actively corrected until pH>7.2.

In addition, the following infusions are delivered throughout the perfusion, as per manufacturer's instruction:

- Epoprostenol, 1.7µg/h
- Heparin, 833u/h
- Insulin, 6.7u/h
- Bile salts (sodium taurocholate) 1ml/h

4.6 Data from non-clinical studies

In a porcine model of DCD liver transplantation, Schön et al[16] showed that livers undergoing NESLiP experienced less reperfusion injury following transplantation than livers stored in cold UW solution, or even livers transplanted immediately, suggesting that NESLiP somehow ameliorated the reperfusion injury following transplantation. Similar observations have been made in a dog kidney perfusion model[17].

Perfusion variations have been studied, or adopted as part of standard protocols, based on animal and human liver perfusions [9,18-23].

4.7 Data from clinical studies

Normothermic perfusion of the liver has been shown to be associated with better early allograft function in randomised European studies [7,24]. Other studies have defined viability parameters [3,25], and often involved different perfusion protocols [11,26-28].

There are no data concerning comparative effects of different perfusion conditions in man.

4.8 Risks and benefits

It is not clear whether the current perfusion protocols are optimal, but animal data suggest that there are several aspects of the current clinical perfusion protocol that are not; slow rewarming with low oxygen tensions, and the use of an albumin based perfusate rather than a gelatin based perfusate being two examples.

The risks of liver transplantation include:

- Primary non function, requiring urgent retransplantation in the first week: 4% of DCD livers, 0.8% of DBD livers;
- Early allograft dysfunction (Olthoff definition [29]): 22%
- Acute kidney injury [30]: 32% DBD, 54% DCD
- Death in first year [1]: 6%
- Retransplantation: 11% DBD, 17% DCD
- Cholangiopathy in DCD livers [31]: anastomotic leak 10%, stricture 27%, ischaemic type biliary lesions 27%

The risks of normothermic perfusion interventions includes introduction of infection. To counter this the perfusate contains antibiotics and an antifungal agent, and in over 100 perfusions we have identified an infection once, which did not manifest in the recipient but which required treatment (candida glabrata). One centre using OrganOx's recommended antibiotic, cefuroxime, have reported overwhelming post perfusion sepsis relating to an organism resistant to cefuroxime.

During the perfusion the liver is constantly being assessed to assure function before implantation. Any intervention that affected this should be apparent during the monitoring phase before committing to transplant the liver. All the interventions have either already, or will be, studied on discarded livers; at the time of writing only the ciclosporin, allopurinol and vitamin C and E protocols await testing in discarded livers.

4.9 Population

Adult patients undergoing liver transplantation as part of their routine clinical care will be approached to participate

5 Trial objective and purpose

The primary objective of the study is to define an optimum perfusion protocol for livers undergoing NESLiP.

5.1 Study objectives

5.1.1 Primary objective

To determine the optimal perfusate for *ex situ* liver perfusion

5.1.2 Secondary objective

To validate transcriptomics as a means of assessing changes in perfusion protocol

6 Trial Design

6.1 Perfusion variables to be studied

A number of different alterations in perfusion protocol will be studied, as illustrated in Table 1 and described in more detail below. The aim would be to assess different interventions sequentially, groups

6.1.1 Starting pH

After adding all the constituents of the perfusate the pH is adjusted to between 7.2 and 7.4 by the addition of sodium bicarbonate before the liver is placed in circuit. Initial liver perfusion results in a fall in pH to around 7.0, and often lower in the case of DCD livers. Current practice is not to correct this until 15 minutes have elapsed, with the availability of the first perfusate gas reading. The theory underpinning this is that mitochondrial injury may be minimised in an acidic environment, with a lower pH opposing opening of the mitochondrial permeability pore [32,33]. In contrast early work on cardiac perfusion in dog hearts suffering 60 minutes of hypothermic arrest showed that initial reperfusion with blood buffered to pH7.8 was associated with better left ventricular function on reperfusion [34,35]. The argument in favour of such rapid pH correction is that cellular metabolism needs normal extracellular pH to resume normal function [36]. Given the lower volume of this circuit, a target prime pH of 7.6 would probably achieve the same initial normalisation of pH.

Protocol intervention: This experiment subgroup will start with a perfusate pH of 7.6, and will be checked at 5 minutes and further corrected if required to achieve a perfusate pH >7.2.

6.1.2 Circuit design: Leucocyte filter

Leucocytes are one of the mediators of reperfusion injury, and the liver contains a large amount of different leucocyte lineages. These are activated and mobilised during reperfusion, but can be removed from the perfusate using a leucocyte filter and their recirculation blocked. The current circuit possess no leucocyte filter. Research in *ex situ* kidney perfusion [12,37] and in cardiac reperfusion [38] suggests that removing leucocytes at the time of reperfusion results in less reperfusion injury by minimising ROS production and immune activation.

Protocol: A specially designed addition to the circuit including a Pall Leukoguard-6 leucocyte filter will be connected to the existing circuit between the pump and the oxygenator to facilitate leucocyte depletion.

Table 1. Interventions to be examined

No	Intervention subject	Current protocol	Variant(s) to be examined		Rationale
	Machine set up				
1	Starting pH	pH >7.2 before reperfusion, correction to >7.2 at 15 mins	Change initial pH to start at 7.6		To achieve a physiological pH on reperfusion, rather than at 15 mins.
	Circuit design				
2	Leucocyte filter	No leucocyte filter	Adding a leucocyte filter into circuit	Adding a Cytosorb cytokine filter	Remove circulating leucocytes or cytokines thus reducing immune activation
	Pre-treatment				
3	Hypothermic oxygenated perfusion	DCD livers placed on machine directly	Initial 1 hour of hypothermic oxygenated perfusion on the Liver Assist		HOPE is said to restore ATP without generating harmful reactive oxygen species
	Perfusate				
4	Thrombolysis in DCD livers	Nil	FFP plus TPA (50mg, 10mg as bolus, then 30mg/h infusion)		Cholangiopathy is due to fibrin thrombi in small vessels around bile ducts
5	Plasma substitute	500ml 5% Human Albumin solution	500ml Gelofusine	500ml (2 units) of fresh frozen plasma (FFP)	Comparing the two currently used "plasma" substitutes with plasma and trauma whole blood. HAS is reported to bind ROS.
6	Amino acids	10mls Aminoven bolus	10mls Aminoven + infusion of 1ml/h	10mls Aminoven + L-Arginine infusion	Aminoven is a mixture of most amino acids; Arginine is rapidly metabolised by livers on the machine
7	Insulin	Insulin infusion 8.3u/h	No insulin		The liver does not need insulin to incorporate glucose into glycogen, but insulin may promote unwanted fatty change
8	Steroid	100mg hydrocortisone	No steroid	3.3mg dexamethasone	More glucocorticoid activity to suppress reperfusion injury
9	Ciclosporin	Nil	Ciclosporin 4mg added to perfusate		Blocks mitochondrial transition pore opening, blocking ROS damage
10	Allopurinol	Nil	Allopurinol added to perfusate.		Present in cold storage solutions to block purine breakdown and thus reduce reperfusion injury
11	Vitamins C and E	Nil	Vitamin C (ascorbic acid) Vitamin E (alpha tocopherol)		Vitamins C & E have anti-oxidant properties and are in some unit protocols for DCD livers
	Subsequent protocols will incrementally combine good elements				

6.1.3 Circuit design: Cytokine filter

Reperfusion injury triggers the release of pro-inflammatory products from leucocytes and hepatocytes resulting in production of high levels of cytokines (figure 4). The Cytosorb® cytokine filter has been shown to reduce the concentration of circulating cytokines in perfusions of discarded livers more effectively than a leucocyte filter, although it is possible

that this may also be removing beneficial as well as harmful cytokines. The aim of both this intervention and the leucocyte filter is to stop the propagation of an immune response to reperfusion.

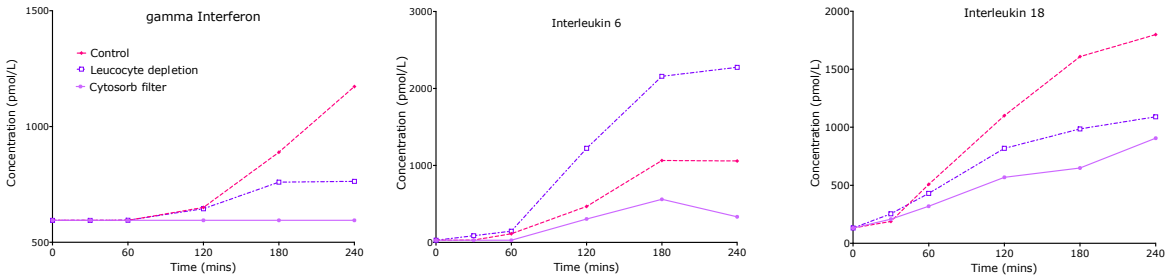


Figure 4. Perfusate concentrations of γinterferon, IL6 and IL18 during *ex situ* perfusions of discarded human livers with the addition of either a leucocyte filter or a cytokine filter or neither (control).

6.1.4 Prior hypothermic oxygenated perfusion of DCD livers

Hypothermic oxygenated perfusion (HOPE) of DCD livers before implantation is claimed to be associated with minimal cholangiopathy and excellent function [39-42]. Its advocates cite the ability to generate ATP in the cold without stimulating reverse electron transfer at mitochondrial complex 1 [43]. HOPE requires the use of the Liver Assist device, with 3L of Belzer Machine Perfusion UW solution as perfusate for 1 to 2 hours before implantation. In order to examine this further it liver could be subjected to a period of HOPE before undergoing normothermic perfusion.

Protocol: A period of 1 hour of HOPE before transfer to the OrganOx metra for a period of normothermic perfusion and evaluation before transplantation.

6.1.5 Perfusate: Thrombolysis in DCD livers

Transplant cholangiopathy (also called ischaemic type biliary lesions, ITBL) is common in livers from DCD donors, with reported incidences over 30% in some series. Examination of bile ducts from livers that have undergone normothermic perfusion has shown evidence that necrosis of the duct is a process that starts within the stroma and is associated with the presence of aggregates of red cells in fibrin. This is either a result of *in situ* clots forming at the time of treatment withdrawal or asystole, or fibrin forming *de novo* as a result of ischaemia, a phenomenon that has been noted recently in kidney tubules [44]. We have recently shown that addition of tissue plasminogen activator (alteplase), together with fresh frozen plasma as a source of plasminogen, is associated with no such biliary infarcts and no fibrin/red cell clumps.

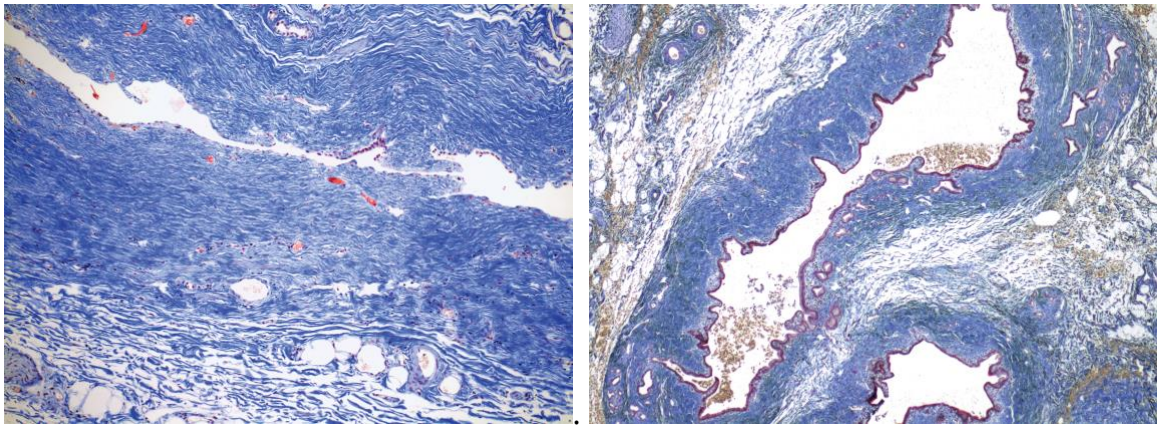


Figure 5. Martius yellow, Scarlet, Blue (MSB) stain of bile ducts of livers undergoing 4 hours of NESLiP; the left panel showing red staining fibrin plugs in the peri-biliary arteries the one on the right, which had also been treated with FFP and TPA, showing no fibrin plugs.

Suggested intervention: A bolus of 10mg TPA (alteplase) will be added to the perfusate with 50mls FFP, with an infusion of 40mg TPA given over 80 mins (30mg/h) together with an infusion of one unit of FFP given over 80 mins (~150mls/h) through the arterial cannula (possible alternative routes to be confirmed). This protocol will be undertaken in DCD livers only, and the endpoint will be clinically significant cholangiopathy on MRCP. The efficacy of urokinase is also being explored, and this may be used instead of TPA if effective

6.1.6 Perfusate: Plasma substitute

At least three different "plasma" substitutes are used for normothermic liver perfusion. In the UK the most common is one based on a gelatin solution (Gelofusine, B Braun, Germany), [24]. In the USA, bovine gelatin derivatives are not licensed, so 5% human albumin solution has been used in its place in the clinical trials of NESLiP. In Toronto, Steen solution (Xvivo, Sweden), which is based on Human Albumin with addition of dextrans and some electrolytes, is the preferred substance[45], and has been shown to be superior to gelatin-based perfusates in a pig model[19]. Human albumin has theoretical advantages since it can act as a free radical scavenger [46], which is why it is the preferred substance used in Cambridge since 2018.

One alternative to both these is to use fresh frozen plasma which, when combined with packed red cells, will produce a perfusate with many of the properties of blood. The disadvantages are the theoretical presence of complement in the plasma which may facilitate reperfusion injury. This does not appear to be a problem [47], and indeed may be preferable due to the presence of endogenous anti-oxidants such as transferrin in fresh frozen plasma.

Protocol: 500mls of a gelatin based perfusate (e.g. Gelofusine) will be used instead of 500mls of Human Albumin solution, and in a third arm, 2 bags (~500mls) human fresh frozen plasma will be used.

6.1.7 Addition of an infusion of Aminoven to the perfusate

Aminoven is a mixture of amino acids used in parenteral nutrition. It contains a number of amino acids which are theoretically beneficial to an organ undergoing normothermic perfusion, such as the -SH donors cysteine and methionine, glycine to suppress Kupffer cell function, and taurine as an anti-oxidant [48,49].

The OrganOx protocol for ESLiP is to introduce an amino acid / glucose infusion only when the perfusate glucose falls to below 10mmol/L. This means that for the first period of perfusion, where the perfusate glucose is typically high, and possibly for the entire perfusion

period, livers will be without any form of amino acid supplementation, the significance of which is not clear. In unpublished studies we have shown a fall in circulating concentrations of arginine, alanine, glycine, histidine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and valine suggesting supplementation may be at least helpful, if not essential. The Cambridge protocol currently uses a bolus of 10mls aminoven in the perfusate at the beginning of perfusion.

Suggested intervention: 10mls aminoven in initial perfusate with 1ml/h infusion.

6.1.8 Perfusate addition of an infusion of L-Arginine

Our unpublished work on amino acid metabolism during ESLiP has shown that L-Arginine is the most rapidly removed amino acid from the circulation and is not measurable in perfusate after 20 minutes; it is the only amino acid to be cleared in this way. This suggests that it is either important and being consumed, or is broken down rapidly possibly by an enzyme leaking into the plasma. It is also metabolised to nitric oxide, a powerful vasodilator and second messenger.

In animal work L-arginine has been shown to ameliorate reperfusion injury [50-53]. Experimental perfusions of discarded livers with L-arginine infusions did not appear deleterious, but neither was any clear benefit seen, but no detailed analysis of ROS production was undertaken.

Suggested intervention: L-Arginine 400mg bolus and 20mg/h infusion

6.1.9 Perfusate: Insulin

Experimental organ perfusions have included insulin to permit glucose entry in the cells. This has been carried over to clinical liver perfusions. GLUT 2 is the principal glucose transporter responsible for facilitating glucose movement from blood into hepatocyte. Unlike other glucose transporter proteins it is not insulin dependent, something we have confirmed in our *ex situ* research perfusions [11].

The OrganOx metra delivers insulin at around 8.3u/h, equivalent to 200u/day which is far in excess of the average insulin dose required for the whole body. Insulin has many effects on the liver, including promoting glycogenesis (something that a glucose excess also does) and stimulating the synthesis of fatty acids which are exported as lipoproteins. Insulin also inhibits lipolysis, although whether this affects lipid stored within hepatocytes is not clear. Work in Oxford suggests that avoiding insulin during perfusion is associated with a reduction in hepatic steatosis during perfusion (Ceresa CDL, DPhil thesis).

Protocol: Omit continuous insulin infusion from perfusions.

6.1.10 Steroid: Omission, or addition of hydrocortisone with dexamethasone

Hydrocortisone was included in the perfusate for its anti-inflammatory properties. It is not the most potent glucocorticoid in this respect. Instead it will be replaced with dexamethasone, at an equivalent dose, or omitted altogether

Suggested intervention: 3.3mg dexamethasone instead of 100mg hydrocortisone, or steroid omitted altogether.

6.1.11 Perfusate addition of a Ciclosporin

Ciclosporin is a powerful immunosuppressant which also combines with intracellular cyclophilins to block the mitochondrial permeability transition pore. Blockade prevents ROS

damage to the mitochondria [54]. In previous animal work it has been shown to ameliorate reperfusion injury [55,56]. It has also been used clinically in studies of patients undergoing myocardial reperfusion following infarction. In preliminary studies, 2.5mg/kg ciclosporin was shown to reduce infarct size on MR and creatinine kinase release [57], observations that were not seen in a larger follow up studies [58,59], although there may be pharmacological reasons for this relating to delayed delivery to the ischaemic area [60]. In the successful studies circulating ciclosporin concentrations at 1 min were a mean 6000ng/ml. Ciclosporin is very lipid soluble, and 35-50% is bound to red cells; it is metabolised by the liver. By adding ciclosporin to the perfusate immediately before reperfusion the concentration of free ciclosporin will be high, but fall as it is taken up by red cells and binds to plastic. Its duration of action need only be the first few minutes of reoxygenation when ROS are generated.

Protocol: Addition of 5mg ciclosporin (as Sandimmun for infusion, initial concentration approx. 4mg/L) to perfusate 5 minutes before reperfusion of the liver.

6.1.12 Perfusate addition of Allopurinol

Allopurinol blocks xanthine oxidase. During ischaemia ATP is consumed and converted to ADP, and two ADP molecules are converted to ATP and AMP. AMP accumulates in the cell and is deaminated to inosine monophosphate and eventually to xanthine and uric acid by xanthine oxidase. Allopurinol is a key constituent of many cold storage solutions designed to facilitate preservation of organs and is used to slow down the degradation of adenine nucleotides. In UW solution, the standard organ preservation solution used in the UK, 1mM allopurinol is used.

Experimental work suggests allopurinol protects the liver from reperfusion injury[61,62], although the mechanism for this is not clear but may involve maintaining a purine pool for ATP generation[63]. The safety profile and dose ranging work on allopurinol in *ex situ* liver perfusion is yet to be completed Allopurinol has a molecular weight 136g; assuming a 1.5L circulating volume, a 1mM solution would require 200mg allopurinol.

Protocol: 200mg Allopurinol will be added to the perfusate.

6.1.13 Red cell quality

In addition to modifications of the perfusate a parallel study of the red cell component will being conducted by Dr Rebecca Cardigan of NHSBT. Quality red cell parameters will be studied by the NHSBT Components Laboratory using 4 aliquots of 20mls from some livers perfused in the study using different perfusates (livers selected based on laboratory availability). Aliquots will be taken as follows:

- Sample from blood bag before addition to circuit
- Baseline, from primed circuit
- 2 hours into perfusion
- 4 hours into perfusion
- End of perfusion

This will not affect the interpretation of data for the main study, but will inform us further on what happens to red cells during perfusions. In particular the following will be studied:

- Haemolysis
- Deproteinisation using perchloric acid
- ATP content
- Oxygenation kinetics

6.2 Statement of design

This is to be an open label, randomised, controlled study of different perfusion protocols. The study will be structured into sequential comparisons as outlined in table 1, with livers randomised between groups in each series:

At each stage the control will adopt the previous best performing strategy. It is envisaged that this will be a slow process of recruitment, in part limited by available grant money and in part by the liver transplant activity

6.3 Livers being studied

Current practice is for livers to electively undergo *ex situ* perfusion if they need extended periods of extracorporeal storage or where viability needs to be confirmed:

- Donor reasons
    - Any DCD donor liver that has not previously undergone *in situ* normothermic regional perfusion;
    - Any liver from a brain death donor where there is concern regarding its viability, for example when it appears steatotic;
    - When a biopsy is necessary to evaluate either the liver, a lesion within the liver, or a lesion found elsewhere in the donor, such that the resultant storage time would compromise the function of the liver were it not to undergo normothermic perfusion.
- Logistical reasons, e.g. when two livers have been accepted at once such that one needs prolonged storage.
- Recipient reasons
    - Prolonged surgery anticipated, such as in difficult re-transplants
    - Where a smooth reperfusion is necessary, such as in the presence of fulminant liver failure;
    - Where intra-operative events dictate the need for an extended period of storage (although livers meeting this criteria will not be considered since it will not be possible to obtain prior informed consent).

These livers will be perfused on the machine using the protocol manipulations described above. It is not the intention to deliberately put livers on the machine for the purposes of the study. In our current practice 75% of the livers undergoing NESLiP have been from DCD donors. Before COVID 20 to 30% of livers underwent NESLiP; during COVID that rose to 80% due to the additional delays inherent in screening recipients for COVID and the additional precautions necessary.

6.4 Outcome Measurements

6.4.1.1 Perfusion parameters

The standard Cambridge perfusion parameters, both liver weight-adjusted and unadjusted, will be recorded. At present these are:

- peak rate of lactate fall,
- rate of glucose fall once in steady state,
- ALT at 1, 2, and 4 hours
- amount of bicarbonate required in the first 4 hours to maintain a pH>7.2,
- bile production at 2 and 4 hours,

- bile: perfusate ratios of H+ and glucose.
- Perfusate lactate at 4 hours

In addition, vascular resistance and oxygen consumption will be monitored. Metabolomic and proteomic studies of the perfusate and bile will also be performed on stored samples, and pharmacokinetic studies on the metabolism of antimicrobials during perfusion will also be performed on stored perfusate samples.

6.4.1.2 Reperfusion injury

Perfusate samples will be taken at intervals during perfusion, more frequently in the early period, spun down and frozen for subsequent analysis. Measures of reperfusion injury will include :

- Damage markers: e.g. liver enzymes (AST, ALT, LDH), change in potassium;
- Adenine metabolism: e.g. Uric acid, also a marker of reperfusion injury;
- Endothelial injury: e.g. Hyaluronic acid concentration;
- ROS production measure: e.g. Thiobarbituric Acid Reactive Substances (TBARS) / Malonaldehyde levels, which reflect lipid peroxidation due to ROS;
- Immune activation, e.g. High mobility box group-1 (HMGB-1) levels, released by activated immune cells.

6.4.1.3 Transcriptomics

13mm long core liver biopsies (or part of biopsy where half goes for histopathology) will be taken pre-perfusion (and pre-HOPE), at 4 hours, at the end of perfusion (if longer than 6 hours) and 1 to 2 hours after reperfusion in the recipient (after completion of the arterial anastomosis (half goes for routine histology). In total 4 biopsies will be taken for most livers, and 5 for those undergoing HOPE. Currently livers undergoing NESLiP undergo 2 biopsies; this study will entail 3 more. The average liver weight between 1 and 1.5kg; the average biopsy weights less than a gram.

RNA will be extracted from the biopsies and the resulting material analysed using next gen sequencing. Following sequencing the raw data will be aligned to the human genome and the number of reads aligned counted to give measure of gene expression. This will yield approx. 16-22,000 genes per sample. To investigate differential expression the gene counts will be normalized across samples and an appropriate liner model fitted to test for differential gene expression using a program such as DESeq2. Changes occurring during perfusion within an organ will be tested and compared across organs and also compared with the transcriptional state of the tissue both pre and post perfusion across groups. In addition to investigating individual genes we will also look at the movement of whole known pathways using approaches such as genes set enrichment analysis and also attempt to identify novel modules of coregulated genes using weighted correlation network analysis (WGCNA). From a combination of these approaches we aim to identify individual and / or groups of marker genes which can be linked to prognosis.

6.4.1.4 Histology

Part of the biopsies taken pre-perfusion and post reperfusion in the recipient will be sent for histopathological analysis. In addition, any biopsies performed for cause will be reviewed.

6.4.1.5 Post reperfusion injury

Post reperfusion injury is a measure of the haemodynamic response to reperfusing a liver at the time of transplantation. It has been defined as a fall in systolic blood pressure of 30% lasting at least one minute in the first 5 minutes post perfusion

6.4.1.6 Early allograft function

Early allograft function in the recipient will be measured using the MEAF score and the 7- or 10-day L-GraFT score[15,64,65], based on routine post transplant blood tests (INR, ALT, bilirubin, and platelets) in the first week post-transplant. In addition, serum creatinine will be monitored for acute kidney injury (peak creatinine in first 7 days divided by baseline creatinine ≥2.0); episodes of haemofiltration will be recorded.

6.4.1.7 Cholangiopathy

i) Cholangioscopy: Cholangioscopy of the donor bile ducts is often performed at the end of perfusion on DCD donor livers. If performed appearances should be recorded detailing the amount of circumferential erythema and bile staining separately for CBD, CHD, and 1st and 2nd order ducts.

ii) Magnetic resonance cholangiopancreatography (MRCP): Current clinical practice is for DCD liver recipients to have MRCPs at around 6 months (or before if clinically indicated), and this should be sufficient in this study. This will be particularly important where DCD livers are being specifically investigated, such as the TPA/FFP protocols.

6.4.1.8 Red cell physiology

The following parameters of red cells will be studied in a subset of liver perfusions:

- Haemolysis
- Deproteinisation using perchloric acid
- ATP content
- Oxygenation kinetics

This will be done in the laboratories of NHSBT.

6.4.1.9 Others

- d-dimers will be measured in the TPA/FFP and FFP control groups.
- Ciclosporin concentrations will be recorded in the ciclosporin group.

6.5 Number of Centres

This will start as a single centre study. At the same time funding will be applied for to extend this to a multicentre study looking all the other elements

6.6 Number of Subjects

The initial plan is for around 150 to 250 liver transplants, but once improved protocols are identified these will be adopted successively.

6.7 Sample size determination

Each study group will comprise 6 to 10 perfusions.

This has been determined according to the power afforded by the transcriptional analysis of multiple genes, plus the sequential biopsies of livers enabling identification of changes in gene expression. Pathway analysis looks at the movement of thousands of genes, so is less noisy than if one was looking at a single gene or biomarker protein. The data shown in the introduction in this protocol comparing livers, kidneys and lungs was performed using just 5 organs per group.

6.8 Randomisation

Liver perfusions will be conducted in stages, and randomised between interventions within each subgroup identified above (section 6.1).

6.9 Study duration

The study intervention occurs before the liver is transplanted, and the last study related sample is the post reperfusion biopsy taken in the operating theatre.

Subjects will be followed up for 1 year, recording the following

- Graft survival
- Patient survival
- Creatinine (eGFR) at 1, 2, 3, 4, 6, and 12 months
- MRCP results (where done)
- Incidence of acute rejection

6.10 Study endpoints

6.10.1 Primary endpoint

Reduction in pro-inflammatory transcriptomic signature

6.10.2 Secondary endpoints

6.10.2.1 Transcriptomics

- Changes in transcriptomic signature to one favouring a low incidence of early allograft dysfunction

6.10.2.2 Post transplant function

- Post reperfusion syndrome: fall in mean blood pressure by 30% in the five minutes post reperfusion of the liver
- Early allograft dysfunction (as measured by MEAF, L-GraFT$_7$, and Olthoff scores)
- Cholangiopathy: incidence of anastomotic and non-anastomotic structures in DCD livers
- Acute kidney injury: $^{\text{peak creatinine d1-7}}/_{\text{baseline}}$

6.10.2.3 Perfusate chemistry changes

- TBARS at 30mins and 4 hours
- HMGB-1 at 30mins and 4 hours
- Hyaluronic acid levels at 30 mins and 4 hours
- Uric acid at 1, 2 and 4 hours
- D-dimer concentration (FFP and TPA groups)

6.10.2.4 Liver probe readings

Readings on each liver will be taken from prescribed sites on segments 2, 3, 5, and 6. The liver fat probe was previously developed in the University Engineering Department and shown to give readings that correlated well with early liver function [66].

6.11 Criteria for Discontinuation

Evidence of a possibly superior protocol in other published studies may result in change in the baseline control perfusate, which will be measured against the Cambridge standard according to the study protocol above.

For individual perfusions, livers will not be transplanted if the perfusate parameters are not satisfactory. That will be decided by Prof Watson, Mr Butler and/or Mr Gaurav.

Dr Michael Allison (consultant hepatologist) and Mr Neil Russell (consultant surgeon) will act as independent reviewers of the progress of the study. After the first and third perfusion of a group they will review the data, and after any graft loss or death or any other event they deem appropriate. Typically 5% of liver recipients die in the first year, and 11% of DBD and 17% of DCD recipients undergo retransplantation, so it is likely that these events will occur.

7 Selection (eligibility) of subjects

7.1 Inclusion Criteria

- Adults aged 18 years and older
- Consented to undergo liver transplantation
- Able to understand informed consent for study without encephalopathy at time of consent.

7.2 Exclusion Criteria

- Liver requiring perfusion once the transplant is underway due to intra-operative difficulties
- Recipient unable to give informed consent

8 Randomisation and enrolment

8.1 Assignment and Randomisation Number

Randomisation will be by random number allocation with allocation in sequentially numbered envelopes for each of the study intervention groups

8.2 Method of Blinding

This is not a blinded study.

The perfusionist will be responsible for the different drug additives; it will not be possible to blind the surgeon from the use of the Liver Assist for HOPE. It is unlikely that there will be a clear difference in perfusion parameters during perfusion, with the real difference being seen in the transcriptomic signature.

8.3 Subject withdrawal criteria

Subjects can withdraw from the study at any time. Since the interventions are at the start of the study before the liver is transplanted, subject withdrawal will in reality be withdrawal

from data usage and future data collection. There are no study related investigations undertaken beyond the post implant biopsy, with the variables that are recorded being part of the standard patient management.

9 Study procedure and assessments

9.1 Informed consent

All patients listed for a liver transplant will be approached with respect to the study and asked to give their informed consent to participate.

The approach may start with study information posted out to them, with consent being taken the next time they visit the hospital. The aim will be that most patients are approached before they are called in for a transplant, although there will be occasions where they are called in before there has been chance to approach them about the study, in which case they will be approached on the ward.

9.2 Screening evaluation

Screening at the time of transplant will determine whether the liver will be placed on the machine electively. Where the liver is not subject to perfusion, this group will act as a further comparator.

Emergency placement of the liver on the machine due to intraoperative difficulties with a transplant where use of the machine was previously not thought to be required will be an exclusion for participation.

9.3 Baseline data

The following are to be recorded on the recipient from their routine admission bloods and data:

a) Age of recipient at transplant
b) Underlying liver disease
c) Indication for transplant
d) Platelet count
e) INR
f) Bilirubin
g) ALT
h) Urea
i) Creatinine
j) Sodium

9.3.1 The following are to be recorded on the donor

a) DBD or DCD
b) Donor age
c) Donor variables for calculating the US donor risk index and the UK donor liver index
d) Donation timings – agonal period, asystolic period, cold ischaemic time, extraction time, bench time
e) Donor liver weight

9.3.2 The following are to be recorded during perfusion

a) Timing of perfusion (start and stop)

b) Perfusate protocol
c) Perfusate gases (standard measurements) at baseline, 15, 30, 45, 60, 90, 120, 150, 180, 240, 360 and every 120 mins thereafter
d) Biochemistry (standard measurements) (ALT, AST, LDH, Uric acid) at 1, 2, 4 hours
e) Perfusate samples for T-BARS and other analyses at 30, 60, 120, 180, 240, 360 mins
f) Liver fat probe readings

9.3.3 Parameters during transplantation

a) Post reperfusion syndrome, defined as a fall in mean blood pressure of 30% in the first 5 minutes following reperfusion or the need for adrenaline
b) Post reperfusion administration of adrenaline or addition of an inotrope (noradrenaline, vasopressin) on top of pre-reperfusion drugs

9.3.4 Post transplant parameters:

a) ALT, ALP, Bilirubin, Platelet count, urea, creatinine daily from transplant to 7 days (sample taken nearest to 06:00h)
b) Creatinine at 30±10, 60±10, 90±15, 120±15, 180±45, 365±60 days post transplant
c) MRCP
d) Any liver biopsies taken for clinical reasons in first year
e) Cause of graft loss
f) Cause of death

9.4 Study assessments

There will be no study related assessments once the patient has returned from the transplant operation. Data required will come from routine follow up clinics and blood tests.

9.5 New information affecting participation

Should any new information arise during the course of the research that may affect continued participation of the participant in the research then a member of the research team will contact the participant to discuss this. Where possible this will be at one of the routine post transplant follow up clinics.

10 Statistics

Dr John Ferdinand and colleagues in the Laboratory for Molecular Biology in Cambridge will be responsible for the bioinformatics in analysing the transcriptomic data.

The research team will be responsible for analysing the other data.

10.1 Number of subjects to be enrolled

The number of subjects depends on the outcomes of the groups as the study progresses. It is likely that at least 150 subjects will be enrolled, but if interventions are beneficial then these will be combined to see if the benefits are additive/synergistic.

10.2 Sample size considerations

The choice of 6 to 10 subjects per study group is a pragmatic one aided by the use of transcriptomics as a read out whose power enables few numbers needed to treat.

This is not a sample size that will enable us to say anything about graft survival or function. The transcriptomic and other biological data may inform the best strategy to go forward into larger clinical trials with graft survival and graft function as endpoints. Given the excellent results of liver transplantation such a study would need 200 – 500 subjects, depending on the magnitude of the anticipated effect.

10.3 Criteria for the termination of the trial

Each study arm will be continued for 6 livers, possibly extended to 10 if there are mitigating circumstances meaning not all data/samples were collected from any of the study livers, or the donor liver was suboptimal before being placed on the machine, or fewer than 6 livers have been transplanted.

10.4 Interim analyses

Interim analyses will be performed after each series of perfusions as defined in table 1.

10.5 Trial supervision

The trial will be overseen by the liver transplant unit at CUH. At the end of each series data will be presented and discussed at liver transplant monthly meetings

11 Assessment of Safety

11.1 Definitions

The investigators have contacted the Medicines and Healthcare products Regulatory Agency (MHRA) who confirm this study does not count as a clinical trial of an investigational medicinal product. In the response to enquiries, the MHRA stated:

"***A healthcare establishment uses a device for a purpose not intended by the manufacturer (as stated in the data supplied by the manufacturer, on the labelling, instructions for use and/or the promotional material), without the knowledge of the manufacturer.*** *Generally, a healthcare establishment will not be treated as a manufacturer of a device for the purposes of the UK MDR 2002 because it uses that device for a purpose not intended by the manufacturer. This is because the UK MDR 2002 do not cover the user. Similarly, where a healthcare establishment uses a device for a purpose other than that stated by the manufacturer on a trial basis* ***on their own patients,*** *the MHRA would not treat that as a clinical investigation unless the intention of the healthcare establishment was to seek commercialisation of the device for the new intended use.*

Since this study is being conducted in a single healthcare establishment without intention to commercialise the device the MHRA does not consider this as a clinical investigation.

The following definitions apply to clinical trials of investigational medicinal products (CTIMPs) and, although this is not a CTIMP, we will use the terms adverse events/reaction for incidents occuring in connection with this research.

11.1.1 Adverse event

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an

investigational medicinal product, whether or not considered related to the investigational medicinal product

11.1.2 Adverse reaction of an investigational medicinal product (AR)

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression "reasonable causal relationship" means to convey in general that there is evidence or argument to suggest a causal relationship

11.1.3 Unexpected adverse reaction

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information.

When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

The term "severe" is often used to describe the intensity (severity) of a specific event. This is not the same as "serious," which is based on patient/event outcome or action criteria.

11.1.4 Serious adverse event or serious adverse reaction

Any untoward medical occurrence or effect that:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect.

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

11.2 Expected Serious Adverse Events

Liver transplantation is a life-saving procedure but is associated with serious life threatening complications which would be expected during the study and which we do not propose to report as AEs or SUSARs

- Peri-operative death (within 48h) ~ 2% in 2019
- Primary non function: 0.8% in DBD, 4.0% in DCD livers
- Early allograft dysfunction (Olthoff definition): 25% DBD, 40% DCD
- Hepatic artery thrombosis: 4-15%, depending whether anomalous arterial supply required reconstruction or not
- Post reperfusion syndrome: 30%, more common in DCD than DBD
- Biliary anastomotic breakdown in 10%, more common in DCD
- Post operative haemorrhage requiring reoperation: 10%
- Acute rejection: 20%

- Acute kidney injury: 25% in DBD, 50% in DCD
- Chest complications: sepsis; effusion; paralysed right hemidiaphragm: common
- Ascites: universal if pre-existing ascites, common if not pre-existing
- Line sepsis
- Biliary anastomotic stricture: 10%
- Ischaemic cholangiopathy: 4% DBD, 20-25% DCD
- Retransplantation in first year: around 11% for DBD, rising to 17% for DCD
- Death from graft failure or sepsis: 5%

11.3 Recording and evaluation of adverse events

Individual adverse events should be evaluated by the investigator and, where indicated, they should be reported to the sponsor for evaluation. This includes the evaluation of its seriousness and the causality between the investigational treatment and the adverse event.

The sponsor has to keep detailed records of all AEs reported to him by the investigator(s) and to perform an evaluation with respect to seriousness, causality and expectedness.

11.3.1 Assessment of seriousness

Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated

Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity

Severe: Significant impairment of functioning; the subject is unable to carry out usual activities and / or the subject's life is at risk from the event.

11.3.2 Assessment of causality

Probable: A causal relationship is clinically / biologically highly plausible and there is a plausible time sequence between onset of the AE and intervention as part of the study.

Possible: A causal relationship is clinically / biologically plausible and there is a plausible time sequence between onset of the AE and intervention as part of the study.

Unlikely: A causal relation is improbable and another documented cause of the AE is most plausible.

Unrelated: A causal relationship can be definitely excluded and another documented cause of the AE is most plausible.

11.4 Reporting adverse events

The sponsor is responsible for the prompt notification to all concerned investigator(s) and the Research Ethics Committee of findings that could adversely affect the health of subjects, impact on the conduct of the trial or alter the authorisation to continue the trial.

11.5 Reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs)

All suspected adverse reactions that are both unexpected and serious (SUSARs) are subject to expedited reporting.

11.5.1 Who should report and whom to report to?

The Investigator should report all the relevant safety information previously described to the sponsor and to the Ethics Committee concerned. The sponsor shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.

11.5.2 When to report?

11.5.2.1 Fatal or life-threatening SUSARs

The Research Ethics Committee should be notified as soon as possible but no later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting.

In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to the MHRA and the Ethics Committee within an additional eight calendar days.

11.5.2.2 Non fatal and non life-threatening SUSARs

All other SUSARs and safety issues must be reported to the Ethics Committee as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. Further relevant follow-up information should be given as soon as possible.

11.5.3 How to report?

11.5.3.1 Minimum criteria for initial expedited reporting of SUSARs

Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted within the time limits as soon as the minimum following criteria are met:

a) an identifiable subject (e.g. study subject code number),
b) an adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship,
c) an identifiable reporting source,

11.5.3.2 Follow-up reports of SUSARs

In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. The sponsor should report further relevant information after receipt as follow-up reports.

In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.

12 Data handling and record keeping

The intention is for data to be stored on the CUH hospital server in a dedicated database. If that is not possible then a password controlled database held in an encrypted partition of a researcher's computer.

Participation will be recorded in the patient's notes on EPIC.

13 Direct access to source data / documents

Source data will be stored in the Trial Master File.

The files will be maintained contemporaneously to enable access for monitoring, audit, REC review, and regulatory inspections.

14 Publications policy

Publications should include the names of the study team plus any others making substantial contributions, according to the guidelines published by the International Committee of Medical Journal Editors.

15 Finance

OrganOx disposable sets: These will be clinical sets and not funded as part of the study.

The following will be borne out of existing research funds held by Professor Watson (1363), together with contributions from the NIHR Cambridge Biomedical Campus monies and funding as part of the NIHR NHSBT Blood and Transplant Research Unit.

- Initial cost of perfusate components
- Research histological analysis
- RNA seq and other *in vitro* assays
- The Liver Assist disposables for HOPE
- Leucocyte filter circuits
- Cytosorb filter circuits.

At this time additional funds will be sought to complete the preclinical evaluation of ciclosporin, allopurinol, and vitamins C and E together with the purchase of 7 Liver Assist sets and 10 Cytosorb circuits, together with funding for transcriptomic and *in vitro* analysis.

16 Ethical considerations

16.1 Consent

All patients will freely give their informed consent to participate in the study. A patient may decide to withdraw from the study at any time without prejudice to their future care. However, given the nature of the study being at the time of transplant, with all other tests being part of the routine care of the patient, withdrawal from the study will in effect be withdrawal from collection of follow up data

16.2 Ethical committee review

The study protocol will be reviewed by an appropriately constituted research ethics committee. Copies of the letters of approval will be filed in the study master file

16.3 Declaration of Helsinki and ICH Good Clinical Practise

The study is to be carried out in conformation with the spirit and the letter of the declaration of Helsinki, and in accord with the ICH Good Clinical Practice Guidelines

17 Regulatory approval

MHRA approval is not required as this intervention does not involve treatment the patient.

18 Indemnity / compensation / Insurance

Cambridge University Hospitals NHS Foundation Trust, as a member of the NHS Clinical Negligence Scheme for Trusts, will accept full financial liability for harm caused to participants in the study caused through the negligence of its employees and honorary contract holders. There are no specific arrangements for compensation should a participant be harmed through participation in the study, but no-one has acted negligently.
The University Insurance Section has advised that subject to the study being approved by the relevant Ethics Committees there should be no difficulty in arranging insurance cover for negligent and non-negligent harm to research subjects under the University's Clinical Trials and/or Human Volunteer Studies policy.

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20 Appendices

20.1 Appendix 1. Patient and Public Research panel assessment

The NIHR NHSBT Blood and Transplant Research Unit Public and Patient Research panel were asked for feedback about this study in May 2020, along with feedback on a separate study using research biopsies from the QUOD biorepository. . Their pinion, together with the request, is printed below.

Research summary:

Background:

Livers from deceased donors have traditionally been cooled when removed from the donor by flushing with a special preservation solution, then placed in a box with ice for transport to the recipient hospital where it remains until the recipient is ready to have it implanted.

In the last few years machines have been made that allow us to pump a blood substitute through the liver outside the body, to keep it warm and functioning while awaiting transplantation. This has several benefits, including being able to test the liver to see how well it is working, as well as to keep it stored for longer.

The fluid that is pumped into the liver is called the perfusate; the process of pumping a fluid into an organ is called perfusion.

The most obvious perfusate to use would be human blood. However this contains substances such as white cells (leucocytes) that we know can cause damage to a liver that has been stored on ice for some time, a process called reperfusion injury. So instead of blood an artificial perfusate is used, which contains red blood cells to carry oxygen, suspended in an artificial "plasma". The machine pumps the perfusate through an oxygenator, which acts like the lungs to replenish the oxygen stores of the red blood cells before they are pumped into the liver.

Clinical Problem:

Currently we use a perfusate which comprises red blood cells from blood donors, mixed with another solution which acts as a plasma substitute. In the UK and Europe that solution is typically Gelofusine, in the USA they use an Albumin solution. The original research with the machine was done in the UK with Gelofusine, hence that is most commonly used. However this is not available in the USA, hence they use a different solution. We don't know which is best, and there are theoretical arguments in favour of both.

There are other modifications to the composition of the perfusate, the artificial blood we pump through the livers, and to the perfusion conditions that need to be examined to see which is best. For example:

i) Should we use human plasma instead of an artificial plasma like Gelofusine?

ii) Should we remove all the white cells from the perfusate as they come out of the liver – white cells are responsible for triggering some of the reperfusion injury to organs following transplantation, and the liver contains lots which come out on the machine and circulate to cause damage. In research models removing them does not appear to cause harm, and there is evidence that it may be beneficial

iii) The current machine starts the perfusion using high levels of oxygen, yet research in animals suggests that a lower starting level may be better.

iv) The current machine starts perfusing the organ at body temperature, 37°C, but the liver is still cold then (around 4°C); there is some research to suggest that slow rewarming over 20 minutes is better than abrupt warming to 37°C

v) The current machine includes insulin being given to the liver, but we have shown in research livers that insulin is not necessary and may theoretically be harmful.

There are many similar questions such as these, where we have research in unused human livers that we have perfused under different conditions, or where there is good animal research, to suggest there may be a better way. However to examine lots of different variations we will need a smart way to assess function, rather than performing hundreds of transplants under each different condition.

What we already know:

Work in kidneys in Cambridge has shown that it is possible to measure how active particular genes are in kidneys (RNAseq) and identify a set of genes that correspond to kidneys that are going to work well after transplantation. If the perfusion conditions are changed this is reflected in the how many of the good genes are active thus allowing you to measure the impact of any changes. Thus it seems possible to make changes to perfusion conditions and see how the organs respond using just a few organs and patients, rather than have a lot of patients undergoing a trial.

What we hope to find out:

1. What are the genes that predict good function after liver transplantation.
2. What is the best perfusate solution and method to perfuse a liver outside the body

How we will do this:

There are two parts to the study.

The first part of the study involves looking at biopsies (samples of livers) that have been stored in a research laboratory in Oxford (The QUOD bioresource) taken from donor livers at the time the liver was retrieved and which we know from our own records had either worked well when transplanted or had worked poorly or not at all. From this we hope to

identify a pattern of genes that are active in good livers, in a similar way to how it was done in kidneys.

The second part of the study involves a series of liver perfusions done for normal clinical reasons (typically testing the function of livers were doubt exists, or to enable the liver to be stored for longer). The liver perfusions will be divided into groups of 10. Each group will examine a slightly different perfusion condition, such as comparing the USA and English plasma substitutes (Albumin and Gelofusine). Biopsies will be taken from the livers before and at the end of perfusion, as well as at the end of the transplant, to allow us to look at which genes are activated and so measure any differences in perfusion conditions. A biopsy is a small core of liver, approximately 2mm across and 15mm long, and weighing less than a gram; a typical liver weighs 1600 to 1800 grams

All the different perfusion conditions that we will examine have already been tried on research livers and known not to be harmful; we just don't know which is best. Research livers are not ideal to say which is best because they are inherently poor livers due to the circumstances around death (that is why they are not used).

Patients on our liver transplant waiting list will be invited to participate at the time they join the waiting list or when they are in hospital before the transplant, so that if they were to receive a liver that underwent machine perfusion they could be included.

Why this is important:

Liver perfusion machines are now installed in every UK liver transplant centre, and a handful of European and American centres. No centre has yet defined the best conditions for perfusing the liver. By developing a method to test different conditions on a handful of transplanted livers, we hope to be able to screen a lot of different conditions and identify those which appear to be more promising to explore further.

COMPLETE AFTER PANEL REVIEW

Summary of panel feedback

The Panel asked for further information on the following aspects:

International approaches: The Panel were interested in the difference between US and UK. The Panel were interested to hear about the UK's strengths in this field especially Cambridge, Birmingham and Oxford.

One Panel further question: "I'd love to know the difference between Gelofusine & Albumin ..why USA uses the Albumin?"

Genetic marker: The Panel think that this is an excellent study aim and support the collaboration with QUOD and think that trying to identify genetic markers is a sensible and efficient way to further understanding in the field of transplantation.

Cost dimension: The Panel wanted to know if there is a cost difference between the perfusate solutions and if this will have any impact upon implementation?

Benefits to patients: The Panel think that is important that expectations are managed for the patient and that clinical team and person taking the consent explains that all perfusion processes within the trial have been shown to do no harm.

Patient Consent: The Panel think that the plan to consent patients on to the trial when they join the waiting list is the optimum arrangement. However, they understand that due to a range of circumstances that this may not be possible (e.g. blood and tissue matching, time on waiting list, urgency etc) and therefore recommend that care is taken to explain that the perfusion process is not harmful; likely to be beneficial, and is an important part of enhancing transplantation in the future for other patients.

The Panel support the study and the two dimensions of it. The Panel think that this study will be valuable in (A) enhancing the transplantation process and (B) look forward to seeing its potential for understanding genetic markers for function of livers post-transplant which they could think could be transformational.

Other comments on the research:

"So easy to understand. So well set out. Well worth research"

"Thought it very worthwhile. Must be so much information & know how from around the world ..needs a pooling of ideas, experience & results".

"Model of clarity. Any researcher should read this & learn how it's done if you want to involve the general public."

Prepared by PPIE Lead Jenny Hasenfuss 18/05/2020