Mechanisms Underlying the Efficacy of Normothermic Machine Perfusion in Human Liver Transplantation

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Abstract

Introduction:

By perfusing a liver with oxygenated blood, medications and nutrients at 37°C, normothermic machine perfusion (NMP) may improve outcomes after liver transplantation when compared with conventional static cold storage (SCS). Herein is reported the first randomised controlled trial (RCT) comparing continuous NMP with SCS in human liver transplantation. Additional work exploring the mechanisms behind the effects of NMP is also described.

Methods:

This multinational RCT was initiated by the Consortium for Organ Preservation in Europe (COPE) and involved seven European transplant centres. Adult DBD and type III DCD livers were randomly assigned (1:1) to continuous NMP or SCS. The primary end point was the difference in peak-AST, requiring 220 transplants (90% power). Secondary endpoints included: organ utilisation, preservation time, early allograft dysfunction (EAD), six month graft and patient survival and ischaemic cholangiopathy on MRCP. During NMP, perfusate and bile samples were collected and subsequently analysed to provide information regarding bile salt utilisation and identify markers that may indicate organ quality.

Results:

272 livers (135 SCS, 137 NMP) were enrolled, consisting of 194 DBD and 78 DCD organs. 48 livers were discarded (32 SCS [15 DBD, 17 DCD] vs 16 NMP [10 DBD, 6 DCD]; p=0.01). NMP livers experienced significantly longer preservation times than SCS (7hr 21min vs 11hr 39min; p< 0.01). Despite this, better early graft function was observed in the NMP group with regards to peak AST (974 IU/L SCS vs 485IU/L NMP; p< 0.001) and EAD (29.9% SCS vs 12.6% NMP; p=0.002) with the magnitude of these effects being greater for DCD organs (p=0.02). No measurable difference was found in radiological rates of ischaemic cholangiopathy with no demonstrable correlation between MRCP findings and clinically relevant strictures. NMP livers were found to utilise bovine bile salts effectively with bile production and several other biochemical parameters found to correlate with graft quality.

Discussion:

NMP livers show better early graft function than SCS in terms of peak-AST and EAD, both of which are surrogates for long-term graft outcomes. This is despite better organ utilisation and longer preservation times in the NMP group. NMP can be used to predict organ quality although it is not possible to draw conclusions about markers that may predict viability. If translated to clinical practice, these results would have a major impact on liver transplant outcomes and waiting list mortality. The fact that the study has definitively met its primary endpoint should now enable the exploration of the technology's wider potential.

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Contents

Abbreviations

ADE	Adverse Device Effect
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransaminase
AS	Anastomotic stricture
AST	Aspartate Aminotransaminase
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BSDF	Bile Salt Dependent Flow
BSEP	Bile Salt Export Pump
BSIF	Bile Salt Independent Flow
Ca^{2+}	Calcium
CA	Cholic acid
CBD	Common Bile Duct
CDCA	Chenodeoxycholic acid
COD	Cause Of Death
COPE	Consortium for Organ Preservation in Europe
CRF	Case Report Form
CRN	Clinical Research Network
CVA	Cerebrovascular Accident
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DHC	Dihydroxycholanoic acid
DMC	Data monitoring committee
DPMP	Donors per Million Population Per Year
DRI	Donor Risk Index
DSO	Deutsche Stiftung Organtransplantation (German Organ Transplantation Foundation)
EAD	Early Allograft Dysfunction
EC	European Commission
ECD	Extended Criteria Donor
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ER	Endoplasmic Reticulum
ESI	Electrospray Ionisation
ESI-MS	Electrospray Ionisation-Mass Spectrometry
ESOT	European Society for Organ Transplantation
ET-DRI	Euro-Transplant Donor Risk Index
ETC	Electron Transport Chain
FAD	Flavin Adenine Dinucleotide
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GST	Glutathione S-Transferase
HA	Hepatic Artery
HCC	Hepatocellular Carcinoma

HD	Haemodialysis
HDF	Haemodiafiltration
HDU	High Dependency Unit
HF	Haemofiltration
HMP	Hypothermic Machine Perfusion
HOPE	Hypothermic Oxygenated Perfusion
IC	Ischaemic Cholangiopathy
IFU	Instructions for Use
INR	International Normalised Ratio
IPF	Initial Poor Function
IRAS	Integrated Research Application System
IRB	Institutional Review Board
IRI	Ischaemia-Reperfusion Injury
ITT	Intention to treat
ITU	Intensive Care Unit
IUD	Intrauterine Device
IVC	Inferior Vena Cava
K^+	Potassium ion
KC	Kuppfer Cell
m/z	Mass-to-charge Ratio
MAP	Mean Arterial Pressure
MELD	Model for End Stage Liver Disease
MHRA	Medicines and Healthcare Products Regulatory Authority
MPTP	Mitochondrial Permeability Transition Pore
MRCP	Magnetic Resonance Cholangiopancreatography
MS	Mass Spectrometry
NADH	Nicotinamide Adenine Dinucleotide
NAS	Non-anastomotic strictures
NHSBT	National Health Service Blood and Transplant
NIHR	National Institute for Health Research
NMP	Normothermic Machine Perfusion
NREC	National Research Ethics Committee
NTCP	Sodium-Taurocholate Co-transporting Polypeptide
OATP	Organic Anion Transporting Polypeptide
PI	Principal Investigator
PIL	Patient Information Leaflet
PNF	Primary Non-Function
PP	Per protocol
PSF	Persufflation
PV	Portal Vein
QUOD	Quality in Organ Donation
R&D	Research and Development
REC	Research Ethics Committee
ROS	Reactive Oxygen Species
SADE	Serious Adverse Device Effect

SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SEC	Sinusoidal Epithelial Cell
SITU	Surgical Intervention Trials Unit
SME	Small and Medium-sized Enterprises
SnMP	Sub-normothermic Machine Perfusion
THC	Trihydroxycholanoic acid
TMC	Trial Management Committee
TT	Transplant Technician
UK-DRI	United Kingdom Donor Risk Index
UNOS	United Network for Organ Sharing
USADE	Unanticipated Serious Adverse Device Event
UW	University of Wisconsin solution
WP	Work Package

1 Introduction

1.1 Challenges in Liver Transplantation

1.1.1 Background

According to the 2014 report of the all-party Parliamentary Hepatology Group, deaths from liver disease in England have risen 40% between 2001 and 2012 [1]. For patients with liver failure, techniques for supporting liver function provide only limited and temporary benefit as a bridge to transplantation or, in the case of acute liver failure, liver regeneration. In contrast to kidney dialysis support in renal failure, there is no artificial means to support a patient with liver failure for an extended period.

Liver transplantation is the only effective treatment for many patients with liver disease. Since the first clinical case in 1963, it has evolved from a pioneering technique to the gold-standard treatment for end-stage liver failure. Advances in surgical expertise, improved selection of donors and recipients, medical and anaesthetic management, developments in antimicrobial therapy and immunosuppression have all combined to dramatically improve outcomes after surgery. One year survival exceeds 90% in many units with 5 year survival commonly exceeding 70% [2].

1.1.2 Liver failure epidemiology

In European countries cirrhosis is the most common indication for liver transplantation (68%). Other indications include cancer (14%), and acute hepatic failure (8%) [www.eltr.org]. The main causes for cirrhosis in Europe are virus related cirrhosis and alcoholic liver disease [www.eltr.org]. In the UK it is predicted that HCV-related cirrhosis and deaths from hepatocellular carcinoma (HCC) will increase substantially during the next decade. Similarly the incidence of liver disease related to both obesity and alcohol consumption continues to increase despite health campaigns [1]. It is very likely that the demand for donor livers suitable for transplantation will continue to rise exacerbating the existing organ shortage.

1.1.3 Liver transplant surgery

Liver transplantation is now the gold standard treatment for many causes of liver failure, congenital liver disease as well as some types of cancer. It is major surgery which involves the replacement of the recipient's diseased liver with a healthy organ from a donor. The removal of the diseased liver requires division of the major inflow and outflow structures from the liver, namely: the hepatic artery and portal vein (inflow), and bile duct and hepatic veins or IVC (outflow) (Figure 1A).

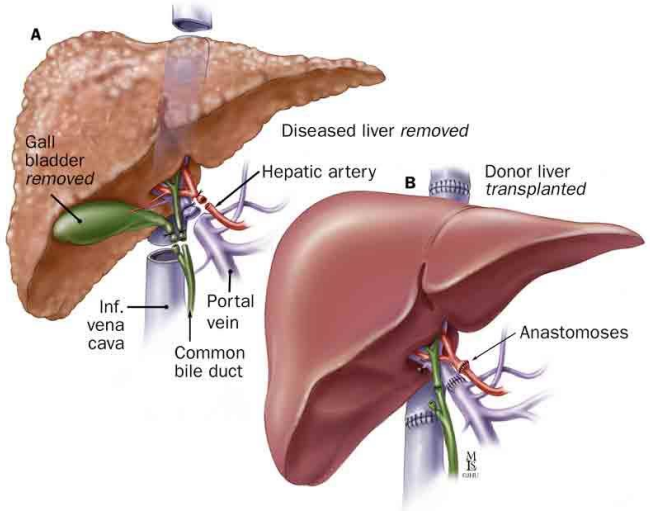


Figure 1: A - major structures that are divided during explant of a diseased liver. B - Common anastomoses performed during a liver transplant (Source: Wikipedia)

The donor liver must then be almost immediately implanted, requiring the anastomosis of the donor hepatic artery, portal vein, bile duct and vena cava to the divided ends of these structures in the recipient (Figure 1B). Beyond the immunological challenges, it is from these anastomoses that many of the complications of transplant surgery can arise. Each of the joins has the potential to leak, to occlude or to stricture.

1.1.4 The donor shortage

Over the last two decades, liver transplantation has become a victim of its own success: many more patients with a much wider range of conditions are being referred for transplantation such that the number of organs available from deceased donors cannot meet the demand. This organ shortage crisis is a global problem with the result that, in many transplant units, patients are more likely to die whilst waiting for an organ than in the first year after their transplant. According to NHSBT data from

2016/17, 1 in 8 patients was removed from the liver transplant waiting list due to death or becoming too unwell to be considered for surgery [2].

Great efforts have been made in recent years to increase the referral of organ donors: this has resulted in a 50% increase in the number of donors referred in the UK. Unfortunately this increase is largely due to additional donors declared dead by cardiovascular criteria ('donation after circulatory death', DCD) and other 'extended criteria' organ donors (older age, steatosis etc.). There has been a much smaller increase in the number of standard ('ideal') organ donors, declared dead by neurological criteria ('donation after brain death', DBD).

1.1.5 Types of Organ Donors

Organ donors can be broadly divided into two categories – living and deceased, with deceased donors divided into those declared dead according to neurological criteria (Deceased after Brainstem Death, DBD) and those declared dead by cardiovascular criteria (Deceased after Circulatory Death, DCD). DCD donors are further subdivided according to the Maastricht classification [3] (Figure 2).

In the UK, Western Europe and North and South America the majority of transplanted livers are derived from deceased donors. This is in contrast to much of South and East Asia where living donors are most commonly used.

1.1.6 Donation after circulatory death (DCD)

For many years (following the establishment of brain death criteria) in most countries, deceased donor organs were almost exclusively sourced from DBD donors. In such cases the donor remains on ventilatory support and cardiopulmonary function is maintained until the donor has been transferred to the operating room, preliminary dissection has been performed, cannulae placed and the organs are ready for cooling. This enables organs to be removed with minimal interruption to oxygenation before cooling and preservation. In contrast, in the case of DCD donors, death is certified after cardiac arrest using cardiovascular parameters; this causes an inevitable period of oxygen deprivation between cardiac arrest and cold preservation of the organs.

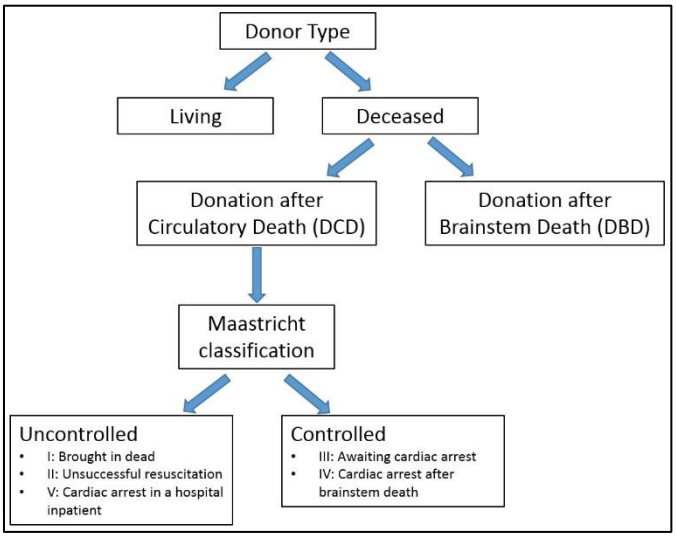


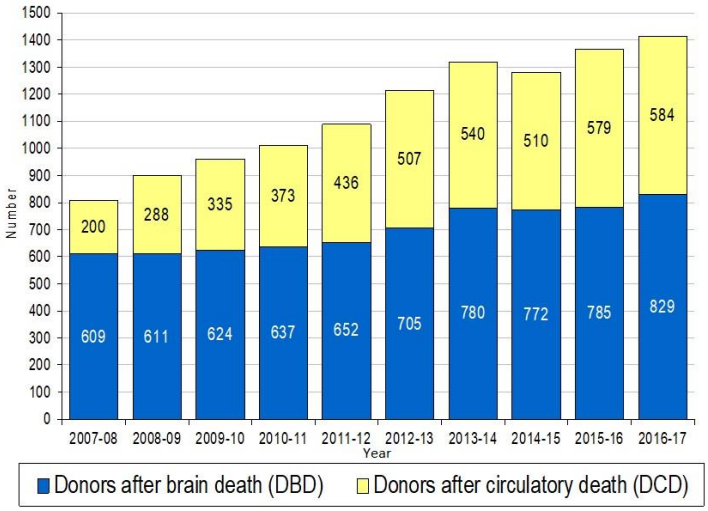
Figure 2: Categories of different donor types

DCD donors have been classified [3] as 'uncontrolled', in which death is not predicted (typically after failed resuscitation in an emergency room) and 'controlled' in which death is anticipated (typically under circumstances where life-support is withdrawn from a patient in whom continued treatment has been deemed to be futile). In the latter situation, following the confirmation of asystole, there follows a non-touch period which varies from 2 to 5 minutes according to local code of practice. Despite this, because death is anticipated and the transplant team can be mobilised in advance, the period of 'warm ischaemia' sustained by controlled DCD donor organs is usually much shorter than that of uncontrolled donors.

Livers from this DCD donors have been transplanted in substantial numbers in recent years, although very selectively, and with higher rates of primary non-function and other post-operative complications than DBD livers, as well as worse long-term graft survival [4-6]. This increase in complications results in an increased cost incurred with DCD transplantation [7]. In contrast, livers from uncontrolled DCD donors are not generally used for transplantation because of a high rate of primary non-function in the experience of those centres that have attempted this [8]. It has been estimated, however, that effective use of these potential donors would add greatly to the number of organ donors [9].

1.1.7 Increasing the Organ Donor Pool

As in many countries, the UK has seen a significant increase (more than 70%) in the number of deceased organ donors over the past decade. However, most of this increase is either from DCD donors (Graph 1) or those considered as 'extended criteria' donors (ECD) due to older age, steatosis or multiple co-morbidities, with the result that the livers from these donors are considered 'high risk' for transplantation. There has been a much smaller increase in the number of standard DBD organ donors.

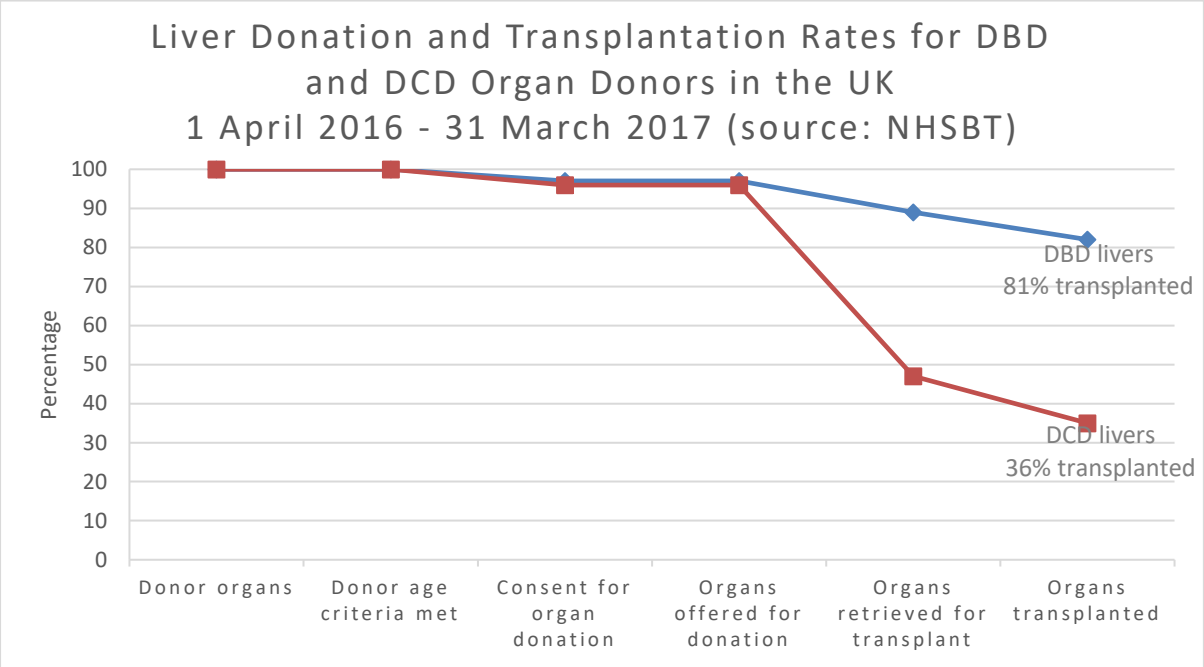


Graph 1: UK Deceased Donor Types from 2007 – 2017 (source: NHSBT)

Much emphasis is now placed on optimising the condition of those organs that are available, to enable a greater number of higher risk organs to be transplanted safely. The use of a higher risk organ does constitute a greater risk to the recipient, with an increased probability that the organ will never function and require immediate replacement (primary non-function, PNF), that it will function poorly and place the patient at risk of other complications (early allograft dysfunction; EAD) or that it will lead to later complications including multiple strictures of the biliary tree (ischaemic cholangiopathy; IC).

The serious effects of the organ shortage, with many patients dying on the waiting list, has led to increased interest in using donor livers which were previously thought unsuitable for transplantation. The use of these ECD and DCD donor livers for transplantation is now seen as essential if liver transplant units are to address the demand.

The safety and utility of DCD liver transplantation (and DBD to a lesser extent) would be greatly improved by a reliable test to assess viability in order to predict the outcome after transplantation.



Graph 2: Liver utilisation by donor type

Even after careful pre-retrieval donor selection, up to 45% of retrieved livers from DCD donors are discarded due to doubts about viability [10, 11]. In the UK in 2016/17 81% of all eligible i.e. those that fulfil nationally agreed criteria DBD livers were transplanted. For DCD donors this figure falls to 36% [12] (Graph 2). An effective means of pre-transplant viability assessment would not only allow greater use of higher risk donors but also minimise the risk of primary non-function by identifying and excluding non-viable organs before subjecting a patient to the risk of surgery.

1.1.8 Limitations of Current Preservation Techniques

Organs retrieved for transplantation undergo injury at several consecutive stages: 1) warm ischaemia prior to preservation, 2) cold preservation injury, 3) ischaemic rewarming during surgical implantation and 4) reperfusion injury. These consecutive events lead to a cumulative cellular injury that may not be compatible with recovery after transplantation.

Whilst many aspects of the transplant process have improved significantly in recent decades, one area that has remained unchanged is the organ preservation process. For the liver this involves flushing the

organ with several litres of cold preservation solution via the hepatic artery and portal vein and applying topical frozen saline slush *in situ* in the donor. Additional *ex-situ* perfusion may be performed to further cool the organ before it is placed in more cold preservation solution and packed in an ice box for transport. This means of preservation is known as Static Cold Storage (SCS) and is designed to rapidly cool an organ with the intention of halting all cellular processes using a solution designed to minimise cellular damage. In this way a good quality liver may remain viable for up to 12 hours. However, for most DCD and ECD organs, SCS is only suitable for up to 8 hours preservation.

Although cold preservation slows metabolism by 1.5- to 2-fold for every 10°C drop in temperature, considerable metabolic activity still occurs at 1°C [13]. This leads to ATP depletion and accumulation of succinate and other metabolites which lead to the generation of reactive oxygen species [14] that are the basis of ischaemia-reperfusion injury, when the organ is re-exposed to oxygenated blood at the time of transplantation. This deterioration is compounded by any injury that has occurred during the organ donation and retrieval process and poses significant limitations on the duration for which a liver can be preserved by SCS. Furthermore, once a liver has been placed on ice it remains inanimate with access prohibited making viability assessment impossible. Once cooled, the cessation of normal cellular activity also makes functional assessment impossible.

These shortcomings are particularly problematic in the higher-risk donor organs that form an increasing proportion of today's liver transplant practice. The very severe ischaemia-reperfusion-related morbidity that characterises transplantation of such organs is now a major limitation in meeting the demand for life-saving transplants.

An ideal means of organ preservation would possess the following characteristics:

- Reverse cellular injury that has occurred during donation and retrieval
- Prevent further deterioration of the organ
- Provide a means of viability assessment
- Avoid the accumulation of metabolic breakdown products

- Increase preservation times

It is hypothesised that normothermic machine perfusion (NMP) may possess these attributes and so offer a solution to the preservation problem.

1.2 Machine perfusion

1.2.1 Background

Early in the history of organ preservation and transplantation, pioneers in the field investigated machine perfusion. In the first half of the twentieth century Carrel, for example, perfused organs with normothermic, oxygenated serum and demonstrated gross viability for several days [15]. A number of early successful clinical liver transplants carried out by Dr Thomas Starzl, used machine perfusion with diluted blood under cold hyperbaric conditions [16]. This technique never gained popularity, partly due to its complexity and logistic challenges but also the subsequent introduction of effective cold flush preservation solutions [17]. In current clinical practice the principle forms of machine perfusion being considered are:

- Persufflation (PSF)
- Hypothermic machine perfusion (HMP)
- Subnormothermic machine perfusion (SnMP)
- Normothermic machine perfusion (NMP)

These may be administered in the following ways:

- For the full duration of transport and storage from retrieval in the donor until the organ is ready to be transplanted (preservation)
- At the transplant centre following a period (usually several hours) of SCS (post-SCS NMP)
- For a short period of time immediately prior to implantation (pre-implantation)

HMP and NMP are the techniques with greatest interest, with extensive debate regarding superiority and timing of intervention (preservation vs reconditioning). The relative merits of these arguments are discussed below with a primary focus on NMP.

1.2.2 Hypothermic machine perfusion (HMP)

The concept of ex-vivo liver HMP was first presented in 1967 with the demonstration of 24 hours of hypothermic perfusion in canines [18]. But with the advent of simple SCS preservation solutions this technology fell out of favour. It was not until the emergence of renal HMP in the 1980s to combat the high rates of delayed graft function (DGF) in ECD kidney transplants that there was renewed interest in liver HMP.

The mechanism of benefit of HMP is not fully understood, but probably relates to slowing cellular metabolism, the removal of metabolic products as well as the delivery of oxygen. The liver is perfused through the portal vein (PV) with a continuous, low-pressure (3-5mmHg) flow at a low temperature (4-10^{o}C) using an acellular, colloid-containing preservation solution. It is not clear whether portal perfusion alone is sufficient – maintaining the peribiliary vascular plexus appears to be vital for the prevention of ischaemic cholangiopathy but this plexus is principally supplied by the hepatic artery (HA). As a result some authors [19] have advocated dual perfusion (PV and HA) although perfusion of the intra- and extrahepatic biliary tree has been demonstrated via PV perfusion alone [20].

Experimental evidence shows that livers can be preserved by low-pressure oxygenated HMP (aka. hypothermic oxygenated perfusion; HOPE) using either continuous or preimplantation perfusion [21]. Oxygenated HMP reduces ischemia–reperfusion injury and protects against biliary injury in preclinical models; however, no evidence yet shows that HMP can extend liver preservation times [22, 23].

The feasibility and safety of HMP in humans was first demonstrated by Guarrera et al in 2010. They reported the outcomes from the transplantation of 20 DBD livers preserved by preimplantation HMP compared with matched SCS controls [24]. Preimplantation HMP provided safe preservation in this pilot study, with low EAD rates (5% vs. 25% of SCS livers). More recently the same group reported on

transplanting “orphan” livers (refused for transplantation by numerous centres) after preimplantation HMP and achieving reduced EAD rates, fewer biliary complications and shorter hospital stay compared with matched SCS controls [22].

For DCD organs the preliminary clinical experience was reported by Clavien’s group in Zurich. Their case series of eight DCD livers transplanted after preimplantation HMP showed no evidence of ischemic cholangiopathy at 8 months after transplant despite the marginal nature of the donors [25]. Most recently, the same group have published the findings from 50 in-house DCD livers that were treated with two hours of oxygenated HMP prior to transplantation. The outcomes were contrasted with those of 50 matched SCS DCD livers from a different transplant unit. The pumped livers showed improved graft survival at one year with reduced rates of non-anastomotic strictures (HMP 4/50 versus 11/50 SCS; p=0.09) [26]. However, questions remain about the comparability of the control group which experienced longer cold ischaemia times and concerningly high rates of graft loss at 1 year (32%).

Viability assessment during HMP is largely unexplored. There are no physiological or synthetic parameters that can act as an indicator of function, although there is a suggestion that perfusate transaminases might correlate with graft injury [24, 27, 28].

At present, two randomised controlled trials comparing SCS with dual vessel (NCT02584283) and portal vein only (NCT01317342) oxygenated HMP respectively are being conducted. Data from these will be useful in determining the role of HMP as a tool to improve outcomes after transplantation.

1.2.3 Normothermic Machine Perfusion: Pre-Clinical Evidence

Normothermic machine perfusion (NMP) is a technique that preserves a liver by perfusing it with oxygenated blood, medications and nutrients at normal body temperature in an attempt to simulate *in vivo* conditions. It is hypothesised that using this more physiological approach to organ preservation will have the following benefits:

- Reverse the damage to the organ that occurs during the organ donation process
- Provide a means to assess organ viability
- Increase preservation times

One of the earliest *ex vivo* normothermic liver perfusion systems was used by Ikeda et al. [29] as a means of comparing reperfusion of porcine livers at normothermic and sub-normothermic (32^{o}C) conditions. This technology was not pursued further until Shon et al described a series of porcine NMP liver transplants in 2001 . Shortly after this Friend et al. described similar perfusions using a simpler circuit incorporating a single pump [30]. This circuit (as described by Butler et al [31]) would form the basis for a surge of interest in this field.

Initial experiments compared NMP with SCS using a porcine DBD model. The livers were retrieved and subject to either 24 hours SCS or 24 hours NMP [32] following which they were reperfused with whole blood on the NMP circuit, as a surrogate for transplantation. NMP was shown to be superior in terms of haemodynamic, biochemical and histological parameters.

The next investigations [33] introduced a 60 minute agonal, warm ischaemic phase prior to organ retrieval, simulating a DCD model. The organs were then preserved for 24 hours using either SCS or NMP following which reperfusion on the NMP circuit was again used as a surrogate for transplant. All of the SCS livers were non-viable when reperfused with evidence of massive necrosis, no bile production and poor biochemical function. This was in contrast to the NMP livers where synthetic function, substrate utilisation and haemodynamic parameters all recovered well.

These early studies showed convincingly the benefits of NMP over SCS when applied to organ preservation, but did not address the question of whether a period of cold storage prior to NMP was harmful. This is important as it has significant logistical implications when applied to a clinical model of liver transplantation – does NMP need to be commenced at the time of retrieval or can it wait until the organ has reached the transplanting centre, thus experiencing a period of cold storage?

In a series of studies using the same previously described porcine DCD model the authors introduced initially 4 hours SCS prior to 20 hours NMP [34], and then 1 hour SCS prior to 23 hours NMP [35]. In both sets of experiments the effects of cold storage were compared to 24 hours NMP as a control. Samples were collected during NMP and the livers were not reperfused. The results showed that when livers were exposed to even a short 1 hour period of SCS prior to NMP they sustained significant hepatocellular damage and endothelial cell dysfunction although synthetic function was preserved. After 4 hours SCS there was also a statistically significant difference in synthetic function, bile production, transaminase levels and metabolic activity. These results suggest that even a short period of cold ischaemia prior to NMP has deleterious effects on the liver.

To confirm these results in a preclinical model of organ transplantation, a series of porcine liver transplants was performed [36]. Pig livers were cold-preserved or warm-preserved for either 5 hours or 20 hours, and then transplanted. As a model of DBD and DCD clinical scenarios, organs were cold-perfused *in situ* either at the time of cessation of circulation (as in a DBD organ donation) or after 40 and 60 minutes of warm ischaemia (simulating DCD organ donation). The two preservation times were selected because 5 hours is comfortably within, and 20 hours substantially beyond, the limit of the conventional cold preservation technology in pigs (in which a generally accepted limit for survival is 12 hours). The 40 and 60 minute periods of warm ischaemia are considerably longer than would be acceptable in current clinical practice where a 30 minute limit is widely used [37]. Indeed, success at

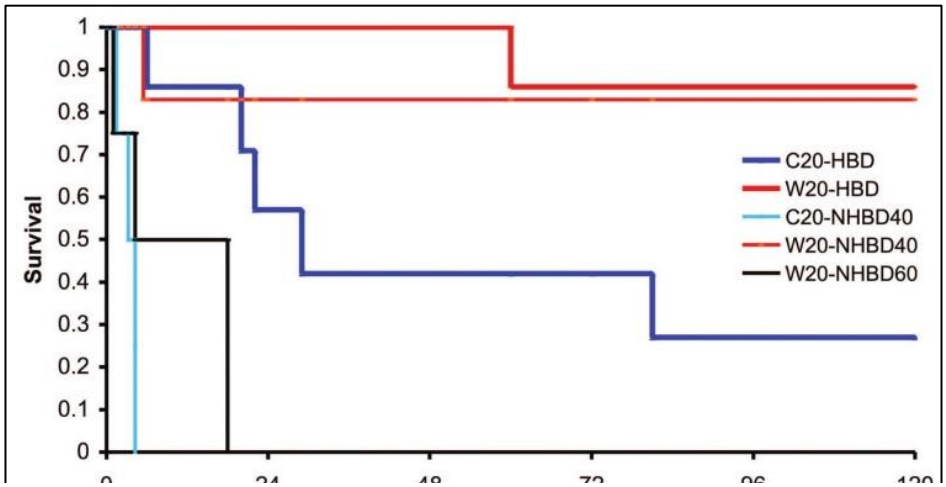


Figure 3: Recipient survival for different experimental groups preserved for 20 hours

40 minutes would raise the realistic prospect of transplantation of donor livers from uncontrolled DCD donors. Outcomes are illustrated in Figure 3.

There was no difference between the two groups at 5 hours of preservation. After 20 hours of preservation, for both DBD and DCD organs, there were significant advantages consistently with NMP compared to SCS with regards to postoperative enzyme release and animal survival. Notably, when comparing the DBD and DCD 20 hour warm-preserved groups (40 minute warm ischaemia), there was no difference in survival (86% versus 83%) or postoperative transaminase levels in recipients. At 60 minutes of warm ischaemia and 20 hours normothermic preservation there were no survivors.

1.2.4 Normothermic Machine Perfusion: Clinical Evidence

The first clinical series of normothermic machine perfused liver transplants to be published was a Phase I study in the United Kingdom which showed that prolonged, continuous NMP throughout preservation using the OrganOx *metra* was feasible and safe [38]. A total of 20 consecutive livers were perfused from 16 DBD and 4 DCD donors, all of which were successfully transplanted. The first 10 donors were well within current liver transplant criteria. As the study progressed increasingly marginal organs were perfused including: three very elderly donors (age 77 – 85), four DCD donors, one liver with severe steatosis, two livers with pre-retrieval transaminase over 1000 IU/L. Outcomes were compared with those from 40 historic matched controls, the results of which are reported in Table 1.

Outcome	**Control**	**NMP**	**p-value**
Primary end-point: 30 day graft survival, n (%)	39 (97.5)	20 (100)	1.000
Secondary end-points			
PNF, n (%)	0 (0)	0 (0)	1.000
EAD, n (%)	9 (22.5)	3 (15)	0.734
Peak AST first 7 days (IU/L) Median (range)	902 (218-8786)	417 (84-4681)	0.034
30 day mortality, n (%)	1 (2.5)	0 (0)	1.000
3 month survival, n (%)	39 (97.5)	20 (100)	1.000

Table 1: Outcomes from 20 patient pilot study of NMP

This was primarily a safety study and so was not powered, or intended, to demonstrate differences in outcomes when compared with the control group. Indeed results were similar between the two groups except for a significant difference in the peak aspartate transaminase (AST) level measured in the first seven days post-operatively, which was approximately half the value in the NMP group compared to control. This study was used as the basis for designing the subsequent randomised controlled trial which is central to this thesis.

Despite animal studies demonstrating the harmful effects of cold storage prior to NMP [34, 35], in clinical practice NMP has been used for organ reconditioning as well as preservation. This is partly due to the logistical challenges posed by NMP preservation, but also due to limitations of other types of technology that are being used. The first such case was published in early 2016 and described the resuscitation of an out-of-criteria DCD liver using NMP [39]. This case was later part of a case series of six discarded livers which, after being declined by all UK transplant centres, were reconditioned, five of which went on to be successfully transplanted [40].

One further case report has described the use of preimplantation NMP to assess and recondition a liver for transplant [41]. Again, this was an out-of-criteria (150 minutes WIT) DCD liver which, after 5 hours SCS, was reconditioned by a brief 132 minute period of NMP. During this time the biochemical and metabolic parameters were such that the liver was determined to be viable. It went on to be transplanted successfully.

As is apparent from the above descriptions, whilst there is extensive experimental evidence describing the potential benefits of NMP, the clinical evidence is limited at best. Hence the need for a randomised controlled trial to definitively determine whether NMP can justify the optimism of its proponents. Table 2 below lists the clinical studies using normothermic machine perfusion of the liver that have been published to date.

Study	Year	Group	Study design	No of livers	Percentage DBD/DCD	Primary outcome
Ravikumar et al [38]	2016	Oxford	Phase 1 pilot with retrospective matched-controls	20	80% DBD 20% DCD	30 day graft & patient survival
Selzner et al [42]	2016	Toronto	Phase 1 pilot with retrospective matched-controls	10	80% DBD 20% DCD	90 day graft & patient survival
Bral et al [43]	2016	Edmonton	Phase 1 pilot with retrospective matched-controls	9	66.7% DBD 33.3% DCD	30 day graft survival
Mergental et al [40]	2016	Birmingham	Case series	5	20% DBD 80% DCD	Markers that predict viability
Watson et al [44]	2017	Cambridge	Case series	12	25% DBD 75% DCD	Markers that predict viability
Watson et al [45]	2018	Cambridge	Case series	22	27.3% 72.7% DCD	Perfusate & bile markers that predict outcome

Table 2: Publications reporting transplanted NMP livers

1.2.5 Organ Viability Assessment:

Once a liver has been placed on ice it remains largely inanimate which, together with prohibited access, makes viability assessment impossible. It is widely acknowledged that a large number of organs are needlessly discarded each year due to uncertainty about whether or not they will function once transplanted. A central feature of much of the experimental and some of the clinical NMP work has been that livers which went on to be transplanted successfully could be predicted during NMP based on certain perfusion-related and biochemical parameters.

As early as 2001 it was hypothesised that NMP may allow an 'assessment of the quality of liver function' prior to transplantation [30]. Some of the earliest experimental work to suggest the possibility of using NMP to predict organ quality came from a series of five normothermic pig liver perfusions which were compared to five SCS livers [32]. After preservation for 24hrs both groups of

livers were then tested on the normothermic circuit as a surrogate for transplantation. Not only did the NMP livers perform better after reperfusion, but it was observed that it was possible to measure a range of biochemical, metabolic and synthetic parameters during NMP which it was hypothesised may reflect organ function and quality.

Later experimental work [35, 46] appeared to confirm that various measures of synthetic and biochemical liver function could be used to assess viability during NMP. In the previously described study from Brockmann et al [36] it was noted that, for NMP livers, it was possible to predict organ viability using parameters measured during perfusion. The transplanted NMP livers were categorised according to those which were transplanted successfully and those which developed liver failure. Important differences were observed in flow-dynamic, biochemical, synthetic and metabolic functional parameters (Table 3).

	Successful (n=13)	**Unsuccessful (n=4)**	***P***
Bile output (ml/h)	11.4 ± 1.4	2.8 ± 1.0	0.003
Base excess (mEq/L)	3.3 ± 1.7	-11.6 ± 3.0	0.02
AST (IU/L)	964 ± 302	3198 ± 677	0.01
ALT (IU/L)	62 ± 10	223 ± 75	0.015
HA (ng/ml)	108	6078	0.006
Portal pressure (mmHg)	2.5 ± 1	11.7 ± 2.0	0.006
Portal venous resistance	1.6 ± 0.9	35.4 ± 17.3	0.006

Table 3: Parameters predictive of post-transplant viability. Values presented are mean ± sd. HA - hyaluronic acid.

Much of the clinical work looking at the assessment of organ viability has focused on the use of discarded human livers with surrogate outcomes, such as histological findings, used to confirm viability. A series of 4 such livers, all from DCD donors, were perfused by the Groningen group [47] with a mean cold ischaemic time (CIT) of 415 minutes prior to NMP. All livers functioned well ex-vivo, in particular showing good bile production, stable transaminase levels, gradual normalisation over several hours of lactate and glucose, and satisfactory flows through the hepatic artery and portal vein. Histology from all organs showed no significant change after 6 hours NMP compared to baseline with all livers appearing viable. A clear limitation of this, and most other clinical studies, has been a lack of negative controls without which it is very difficult to draw reliable conclusions.

Despite this caveat, the Birmingham group [40] have established a clinical protocol that is being applied to determine whether a discarded liver is transplantable (Table 4). The criteria being used is somewhat arbitrary, based largely on findings from previous animal studies and recent case series and so have not been validated either experimentally or clinically. Initially, five discarded human livers were perfused and met this criteria, all of which went on to be transplanted successfully.

Within 3 hours of commencing NMP			
Required	Lactate < 2.5mmo/L	OR	Bile production
And 2 of the following 3 criteria			
Perfusate pH>7.3	Stable HA flow > 150ml/min & PV flow > 500ml/min	Homogenous graft perfusion with soft parenchymal consistency	

Table 4: Birmingham criteria for assessing organ viability

These criteria were subsequently refined (to remove the requirement for bile production) and applied to the 'viability testing and transplantation of marginal livers (VITTAL)' clinical study [48]. Here, 31 livers that had been declined by all UK transplant centres, and met other criteria to confirm their high risk nature, were assessed using normothermic perfusion. Of these, 22 met the required threshold for viability testing and so went on to be transplanted. 90 day graft survival was 100% although longer term outcomes are still awaited. Regardless, these preliminary findings would support the supposition that: i) many organs that would function after transplant are needlessly discarded, and ii) the Birmingham viability criteria are able to discriminate viable from non-viable livers.

Similar work by Watson et al [45] reported the findings from 47 perfusions of marginal and discarded livers, 22 of which went on to be transplanted. No specific criteria were applied to determine an organ's usability although it is notable that no correlation was observed between organ viability and bile production or lactate clearance, both of which are important elements of the Birmingham criteria. Three of the transplanted livers developed ischaemic cholangiopathy, all of which produced non-alkalotic bile with pH < 7.4. Further histological assessment of the bile ducts from 11 perfused livers determined that bile pH > 7.5 could be used to discriminate between those with significant (>50%)

circumferential stromal necrosis of septal bile ducts and those without. Bile pH was therefore suggested as a simple and useful tool to identify those livers at risk of developing ischaemic cholangiopathy.

The same group have now published their own criteria to determine the usability of livers after NMP assessment [45]:

• Maximum bile pH > 7.5

• Bile glucose concentration ≤3 mmol/L or ≥10 mmol less than perfusate glucose

• Able to maintain perfusate pH > 7.2 without >30 mmol bicarbonate supplementation

• Falling glucose beyond 2 h or perfusate glucose under 10 mmol/L which, on challenge with 2.5 g glucose, does subsequently fall

• Peak lactate fall ≥4.4 mmol/L/kg/h

• ALT <6000 IU/L at 2 h

To date these criteria have not been more widely applied in a formalised manner with the aim of determining the transplantability of an organ.

1.2.6 Mechanism of Action of NMP

NMP maintains the liver ex vivo in a fully functioning state, providing it with oxygen and nutrients at 37^{o}C. Several NMP circuits have been described in detail [30, 49-51]; these function on the principle of physiological preservation which maintains cellular metabolism throughout the preservation period. Principal components include the following: blood reservoir; pumps (some circuits comprise two pumps, for portal venous and hepatic arterial flow); an oxygenator; and a heat exchanger.

NMP's superiority over SCS in terms of synthetic and metabolic liver function and post-transplant survival has been shown in large animal models described above through evidence of ex-vivo bile

production, reduction of markers of cell injury, and post-transplant survival. However, the precise mechanisms underpinning the beneficial effects of NMP have not yet been fully elucidated. It is likely that perfusion helps maintain a healthy endothelium and replenish adenosine triphosphate (ATP). The importance of increasing hepatic ATP in human liver transplantation has previously been shown, with a direct correlation between high hepatic ATP content and good posttransplant outcomes [52]. NMP's role in ATP regeneration has been confirmed in murine experiments where rapid ATP recovery following initiation of NMP has been demonstrated [53, 54] as well as mitochondrial ATP-ase activity [55]. More recently, human studies have demonstrated histological evidence of glycogen repletion during NMP [40]. Glycogen is essential to maintain hepatocellular integrity and function by supplying glucose for ATP generation. Once glycogen is consumed, ATP depletion ensues, leading to irreversible cell injury and necrosis. In order to further exploit and maximize NMP's potential, a sound understanding of the injury and repair pathways affected during preservation will be important. This may be aided by a multiplatform proteomic approach in combination with computational biology.

1.3 Bile Production

Normothermic machine perfusion maintains the liver in a functioning physiological state. This offers the opportunity to measure various biochemical, metabolic, flow-dynamic and synthetic parameters during preservation which may provide an insight into the functional status of the liver which, in turn, may be an indication of its suitability for transplant. The rate of bile production in particular has long been thought to reflect the quality of the organ during NMP.

A key function of the liver is the production of bile, a fluid produced continuously by the liver which aids with the digestion of lipids in the small intestine. It is comprised of 97% water, 0.7% bile salts, 0.2% bilirubin, 0.51% fats (cholesterol, fatty acids, lecithin) and 200 meq/L inorganic salts [56]. Although bile salts make up a very small proportion of the total volume of bile, they are crucial to its production.

Bile production occurs through two distinct mechanisms – bile salt-dependent flow (BSDF) and bile salt-independent flow (BSIF). Bile that is produced by hepatocytes and secreted into canaliculi is almost entirely bile salt-dependent and contributes to approximately 75% of the total bile produced in humans [57]. BSIF is the result of direct secretion from cholangiocytes in response to external stimuli such as hormones e.g. secretin.

1.3.1 Bile Salt Production

The bile salts found in bile originate from two different sources:

1. Recycled from the entero-hepatic circulation. This is an extremely efficient process which recycles approximately 90% of the total bile salt pool with the remainder excreted in the faeces.
2. Synthesised de novo from cholesterol though a cascade of 16 enzyme-mediated reactions in sinusoidal hepatocytes.

For efficient recycling and transport of bile salts from the sinusoids into the canaliculi, as well as for controlling this process, hepatocytes are equipped with an elaborate array of transporters and regulatory mechanisms.

1.3.1.1 The enterohepatic circulation

The enterohepatic circulation describes the flow of bile salts through the liver, biliary tree, intestine and then back, via the portal circulation, to the liver. Approximately 95% of intestinal bile salts are recovered by active transport, mainly in the ileum, through the action of the apical sodium-dependent bile acid transporter. This co-transports two sodium ions together with one molecule of bile acid into the intestinal epithelial cells [58]. Once they reach the portal circulation the bile salts are usually albumin-bound and, on reaching the liver, are efficiently removed by transport proteins located at the sinusoidal membrane of hepatocytes (see Figure 5 below).

During fasting, the concentrations of primary bile salts (cholic acid and chenodeoxycholic acid) in sinusoidal blood are approximately 10 to 20 µmol/L. In the post-prandial state these levels can rise as high as 50 µmol/L [59].

1.3.2 Incorporation of Entero-hepatic Bile Salts in Bile

The initial uptake of bile salts from the sinusoids is predominantly through a sodium-dependent process mediated by the sodium-taurocholate co-transporting polypeptide (NTCP) [60]. Sodium-independent uptake is via organic anion transporting polypeptides (OATP). These two families of transporters are located in the basolateral sinusoidal membrane of the hepatocyte in direct contact with the sinusoidal fluid.

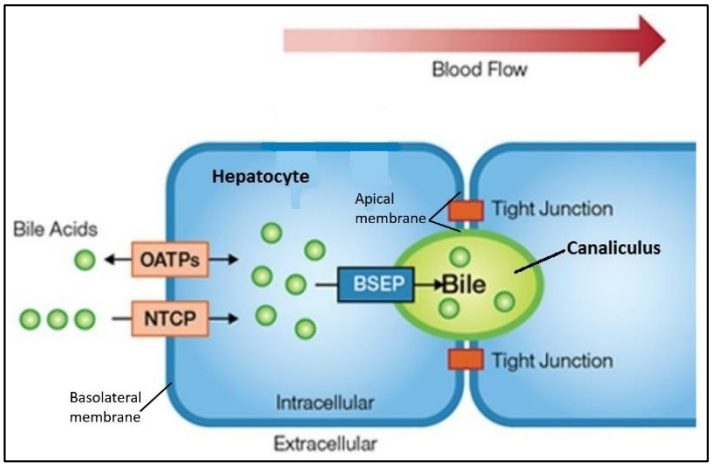


Figure 4: Movement of bile salts through the hepatocyte (Source: Ref [50])

The intracellular transport of bile salts is poorly understood but results in their movement from the basolateral hepatocyte membrane to the apical membrane adjoining the canaliculi (Figure 4). Export of bile salts into the canaliculi occurs against a steep concentration gradient and so is an ATP-dependent process mediated by the bile salt export pump (BSEP) [60].

The secretion of bile salts into canaliculi generates an osmotic gradient which draws in water and so generates the bile salt-dependent component of bile flow. In addition to this, bile salts stimulate biliary lipid secretion. The combination of biliary phospholipids and bile salts form mixed micelles which enables the solubilisation in bile of cholesterol and other lipophilic compounds [61].

1.3.3 De novo synthesis of bile salts

The de novo synthesis of bile salts from cholesterol occurs through two main biosynthetic pathways known as the 'classical' and 'alternative' pathways. These are a complex series of enzyme mediated reactions across almost all major cellular structures which will not be described in detail here but are illustrated in Figure 5. They result in the hydroxylation, reduction, and oxidation of cholesterol to

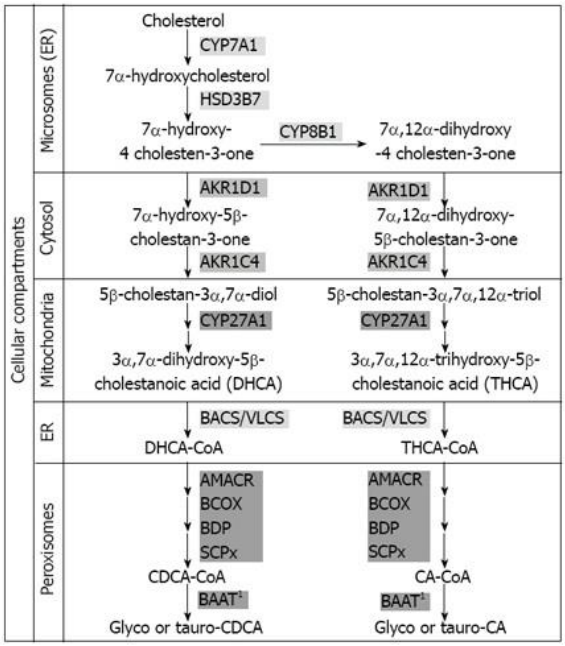


Figure 5: The classical pathway of bile acid synthesis. Enzymes are shown in grey boxes. (Source: Ref [50])

produce the two primary bile salts found in humans – cholic acid (CA) and chenodeoxycholic acid (CDCA), both of which are insoluble in water. As a final step, the primary bile salts are conjugated by the addition of either a taurine or glycine group to produce either:

- glycocholic acid
- glycochenodeoxycholic acid
- taurocholic acid
- taurochenodeoxycholic acid

Taurine and glycine are both water soluble and so confer these properties to part of the bile salt. The other end is hydrophobic, so producing the overall amphipathic properties of bile salts. The resulting micelles that form enable the incorporation of insoluble molecules in bile.

1.3.4 Exogenous administration of bile salts

Early in the development of NMP it was observed that, after approximately 10 hours perfusion of porcine livers, bile production ceased [62]. This was accompanied by a progressive fall in measurable bile salt levels in the perfusate. Histology from these organs showed evidence of cholestasis and damage to the biliary canaliculi. It was hypothesised that the absence of the entero-hepatic recirculation of bile salts during NMP leads to the progressive depletion of bile salts in the circuit. The ensuing bile salt deficiency prevents the generation of the bile salt-dependent component of bile which constitutes over 75% of bile that the liver produces. To test this, during subsequent perfusions, the perfusate was supplemented with bovine sodium taurocholate (NaTC) to replace the bile salts that were being lost.

Following this change it was immediately apparent that, rather than declining as the perfusion progressed, bile production was sustained for the duration of NMP (Figure 7). The bovine sodium taurocholate was shown to be readily taken up by porcine livers and secreted into the bile that was

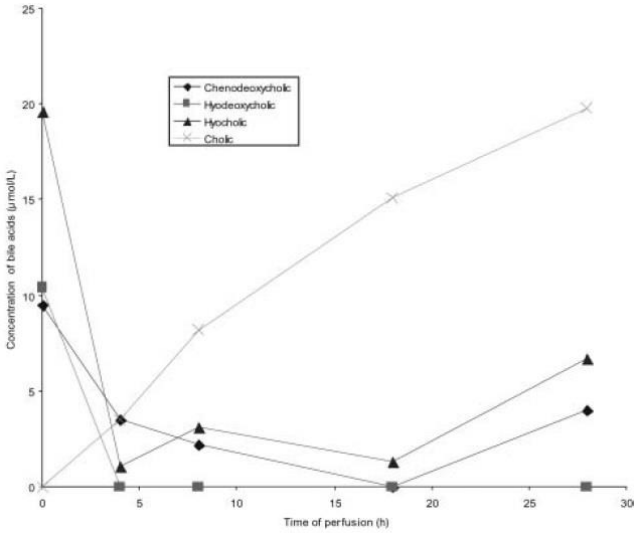


Figure 6: Bile acid concentrations in bile during a supplemented liver perfusion

produced, accompanied by a decline in measured levels of native bile salts in the bile. Finally, there was also a visible improvement in the histology (Image 1) from these supplemented livers, with no evidence of cholestasis or damage to the biliary canaliculi.

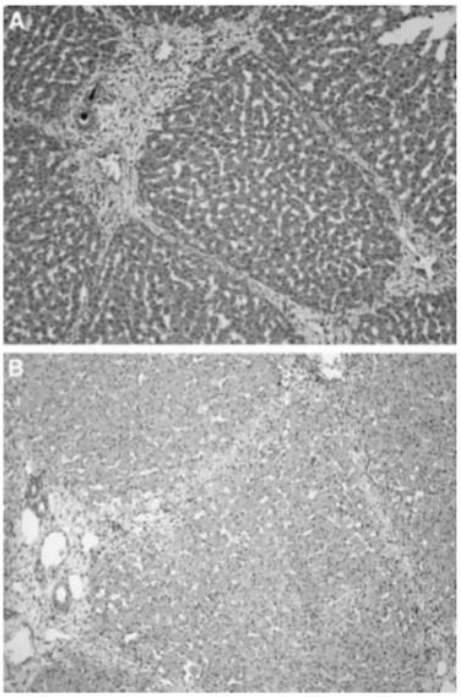


Image 1: H&E stained slides from porcine liver after 40 hours of unsupplemented (A) and supplemented (B) normothermic perfusion. Inspissated bile is clearly visible within the canaliculi of the unsupplemented perfusion in contrast to the perfusion receiving bile salts.

This study has been used as the justification for bile salt supplementation during NMP throughout the clinical development of this technology. The rate of addition of bile salts during the porcine studies was 7ml/min of a 2% NaTC solution. For clinical perfusions this has been arbitrarily reduced to 1ml/min of a 2.5% NaTC solution. This is despite it being unknown whether the human liver can utilise bile salts of non-human origin and what is the optimum dose required to achieve sufficient bile flow and prevent cholestatic injury.

1.4 Bile Production during Normothermic Liver Perfusion

The first group to suggest a relationship between bile production and organ quality was Imber et al [32]. They compared five porcine NMP livers with five SCS livers, and used 24 hours of NMP with whole blood based perfusate as a surrogate for transplantation. Livers preserved by NMP showed superior bile production for the duration of their reperfusion, compared to SCS.

Several years later the same group used a porcine model of DBD and DCD liver transplant to investigate the effects of donor type and duration of warm ischaemia on outcome after

transplantation [36]. They reported a significant difference in the bile production during NMP between livers that were transplanted successfully (n=13) and those that failed (n=4) after transplant (11.4ml/hr vs 2.8ml/hr; p=0.003).

In clinical practice it has been more difficult to investigate this relationship due to the small number of human NMP transplants that have been performed. Sutton et al perfused 12 discarded human livers at 37^{o}C [63] and divided them into two groups – those with good bile production (>30g in six hours; n=6) and those with poor bile production (<20g in six hours; n=6). When compared with the poor bile production livers, those with good bile production showed better lactate clearance, lower transaminase and alkaline phosphatase levels, higher bile bilirubin levels and less hepatic necrosis on histology.

In contrast to these findings, Watson et al [45] performed 47 normothermic liver perfusions after SCS on a range of livers, some of which were intended for transplantation and others which were not. Of these, 22 resulted in transplants, two of which had not originally been intended for use. For these perfusions the perfusate was not supplemented with bile salts although the duration of most did not extend beyond eight hours. No relationship was seen between bile production and organ viability or cholangiopathy. Of note, three transplanted livers developed cholangiopathy, all of which showed bile pH < 7.4. In livers that were ultimately not transplanted, bile pH < 7.4 was associated with significant cholangiocyte necrosis. None of these findings were seen in livers with bile pH > 7.5 leading to the suggestion that bile pH can be used to predict cholangiopathy development.

Despite the lack of validated clinical evidence for a relationship between bile production during NMP and organ viability, the above evidence was sufficient to justify the inclusion of bile production in the Birmingham NMP liver viability criteria [40] (see Table 4). These criteria were applied to six discarded human livers that underwent normothermic reconditioning, five of which met the criteria and went on to be transplanted successfully.

As described above, it is widely accepted that bile production reflects organ quality but, to date, there is no validated clinical data to back-up this assertion. It is therefore an area that warrants further investigation.

1.5 Biliary Strictures after Liver Transplantation

A well-recognised complication after liver transplantation is the development of strictures in the biliary tree. These can be characterised according to their anatomical location as either: anastomotic strictures (AS) or non-anastomotic strictures (NAS).

1.5.1 Anastomotic Biliary Strictures

Anastomotic strictures occur at the choledochocholedochostomy or choledochojejunostomy join, depending on which sort of anastomosis has been performed, with reported rates of approximately 5 – 31% [64-66]. They are more common following a bile leak and in duct-to-duct anastomoses [64] and usually present with obstructive jaundice that is typically managed by endoscopic or percutaneous transhepatic insertion of a biliary stent [67].

Radiologically these strictures are characterised by >70% narrowing of the duct lumen diameter at the level of the biliary anastomosis. This is often, but not always, accompanied by dilation of the proximal bile duct.

1.5.2 Non-anastomotic Biliary Strictures

Non-anastomotic strictures (NAS) are diffuse biliary strictures that can occur throughout the biliary tree. They can lead to cholestasis, cholangitis and a progressive decline in liver function, culminating in organ failure requiring re-transplantation. The cause of NAS can be due to recurrent biliary disease (primary sclerosing cholangitis, primary biliary cirrhosis), which is usually difficult to prevent. However, in the majority of cases the development of these strictures is believed to be a result of ischaemic damage that occurs during the organ donation, procurement, preservation or transplant process in the absence of hepatic artery thrombosis, a condition known as ischaemic cholangiopathy (IC). It is

strongly associated with DCD liver transplantation with a 2014 meta-analysis reporting rates of 16% [68] in this group of livers compared to 3% in DBD transplants. This study also highlighted the contribution of prolonged DCD warm ischaemia time (WIT) in the development of IC. Unfortunately, heterogeneity in the WIT definition limited the number of studies that could be used for this part of the analysis. Of note, the reported rates of IC refer only to strictures that are clinically apparent, usually requiring intervention. The incidence of radiologically apparent strictures in otherwise asymptomatic individuals is unknown.

Whilst the mechanism behind the development of IC is not fully understood, there is evidence that the following aspects of the transplant process impact upon the risk of its development [69]:

- DCD donor type
- functional warm ischaemia time (fWIT) – the time from onset of organ ischaemia (systolic blood pressure < 50mmHg or oxygen saturations < 70%) to the start of aortic perfusion.
- cold ischaemia time – the time from the start of aortic perfusion in the donor to organ reperfusion in the recipient
- ischaemia-reperfusion injury

The strong association between DCD donor type and IC has been a major factor preventing the widespread utilisation of these livers.

1.5.3 Characterisation of Biliary Strictures

Strictures of the biliary tree can be diagnosed using either ultrasound, CT-scan, magnetic resonance cholangio-pancreatography (MRCP), endoscopic retrograde cholangio-pancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC). A direct cholangiogram (ERCP or PTC) is the gold standard [70] but with recent advances in magnetic resonance imaging technology, MRCP can achieve comparable levels of diagnostic accuracy [71, 72].

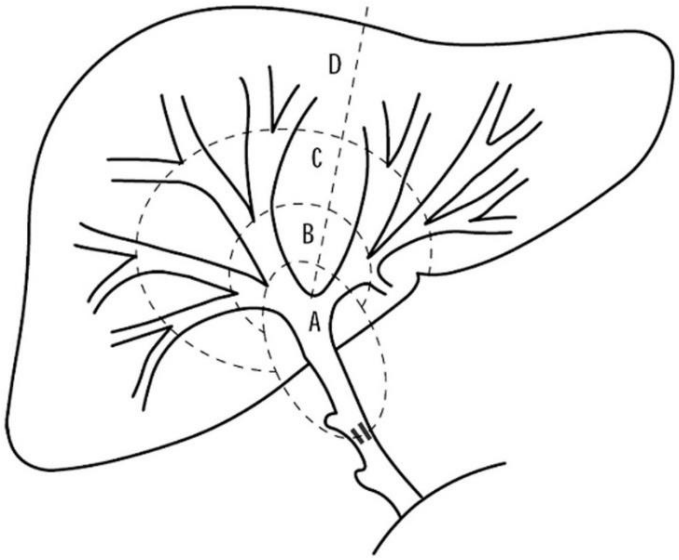


Figure 7: Schematic representation of the anatomical zones of the biliary stricture location as described by Buis et al. A - extrahepatic bile duct; B - 1st to 2nd order branches; C - 2nd to 3rd order branches; D - liver periphery

Biliary strictures are characterised radiologically by the presence of dilation of the proximal bile ducts up to a focal segment of narrowing. There have been limited attempts to devise a classification system for these. The most notable is from Buis et al [73], who attempted to incorporate the number of strictures, degree of narrowing, anatomical location and other radiological features to grade the severity of disease (Figure 8). Anatomical location localised lesions according to the order of bile duct involved and which lobe was affected.

A similar system is employed in the Leiden Biliary Stricture Classification [71]. Here strictures are again categorized into four regions (Figure 9): A) within 1cm of the biliary anastomosis; B) affecting anywhere from the donor common bile duct to 2cm above the common hepatic duct bifurcation; C) affecting the left or right hepatic ducts; D) affecting the second order ducts or beyond.

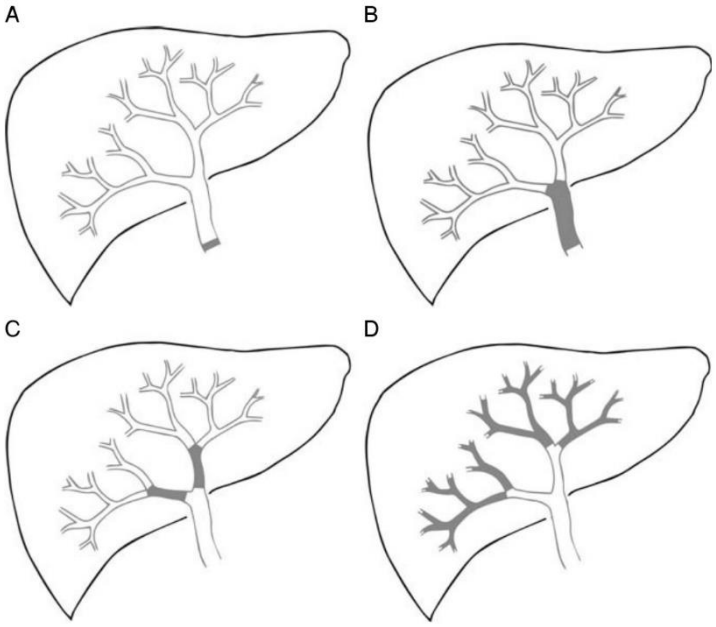


Figure 8: The Leiden Biliary Stricture Classification

Whilst these systems produced a useful descriptive system for reporting strictures, the findings did not correlate with the severity of symptoms or biochemical derangement and so were of limited clinical relevance. As a result there remains no accepted standard nomenclature for the reporting of AS or NAS.

This lack of correlation with clinical findings is the major limitation in the radiological diagnosis of NAS. As noted above, the rate of biliary strictures in asymptomatic individuals following liver transplantation is unknown. Those rates reported in the literature relate only to individuals with clinical manifestation of NAC, usually in the form of deranged liver function or cholangitis.

2 Aims, Objectives and Hypotheses

2.1 Aims:

The primary purpose of this work is to demonstrate the benefits of using normothermic machine perfusion as a means of liver preservation when compared with the current standard method, static cold storage.

There are three secondary aims:

1. To demonstrate that parameters measured during normothermic machine perfusion can be used to predict organ quality and viability and so be used to guide organ utilisation.
2. To demonstate that the human liver can utilise bovine bile salts.
3. To demonstrate that routine use of magnetic resonance cholangio-pancreatography (MRCP) imaging at 6 months after liver transplantation is useful in the diagnosis of non-anastomotic biliary strictures.

2.2 Objectives:

The following objectives have been set in order to fulfil the aims:

1. Conduct a randomised controlled trial to compare normothermic machine perfusion with static cold storage in human liver transplantation.
2. To collect an analyse machine perfusion data and perfusate biochemistry from transplanted NMP livers to ascertain if there is a relationship between these parameters and post-operative outcome.
3. To perform bile salt analysis of the perfusate and bile from livers during NMP.
4. To arrange for all participants transplanted as part of the randomised controlled trial to undergo a protocol MRCP after 6 months. These will then be assessed by radiologists blinded to means of organ preservation. Findings will be compared with clinical outcomes.

2.3 Hypotheses:

1. Normothermic machine perfusion of the liver improves outcomes after transplantation when compared with conventional static cold storage.
2. Parameters measured during normothermic machine perfusion of the liver correlate with outcomes after transplantation
3. Exogenously administered bovine bile salts can be utilised by human livers during normothermic machine perfusion
4. Routine use of magnetic resonance cholangio-pancreatography useful in the diagnosis of biliary strictures in asymptomatic individuals following liver transplant.

3 Methods

3.1 Clinical Trial

3.1.1 Consortium for Organ Preservation in Europe

The Consortium for Organ Preservation in Europe (COPE) is a multinational, European collaboration between Universities, transplant centres, SMEs and the European Society of Organ Transplantation (ESOT). It is the official organ preservation taskforce for ESOT with the stated aim to advance and develop organ preservation technologies by performing clinical and translational studies with on-going experimental refinement. By testing the quality and safety, increasing the efficiency and refining preservation strategies it was established to bring transplant technologies from the bench to the bedside.

The consortium was established by Professor Rutger Ploeg and funded by a European Commission Seventh Framework Programme grant (No 305934) which was secured in October 2012. The Consortium has been responsible for running three multi-centre, international, clinical trials (including the present study), two multi-centre, experimental programmes and the establishment of a biobank containing samples collected as part of the three clinical trials.

3.1.2 Study Design

This investigator-initiated, multinational, open-label, randomised controlled trial was sponsored by the University of Oxford and funded through COPE. The protocol was developed by consensus between the Chief Investigator (Professor Peter Friend), Clinical Research Fellows, trial site Principal Investigators (PI), the University of Oxford Clinical Trial Research Group, Surgical Intervention Trials Unit and the Centre for Statistics in Medicine and complied with all aspects of the CONSORT guidelines for clinical trials [74]. The seven trial sites included four UK transplant centres (Addenbrooke's Hospital, Cambridge; King's College Hospital, London; Queen Elizabeth Hospital, Birmingham; Royal Free Hospital, London) and University Hospital, Essen, Germany; University Hospitals, Leuven, Belgium; and Hospital Clinic, Barcelona, Spain. The trial protocol was registered prior to commencing

recruitment (ISRCTN 39731134). Approval for the study was obtained from national research ethics committees and medical device regulatory bodies in each trial region.

No significant changes were made to the trial design after the start of recruitment. The flow of patients through the trial is depicted below.

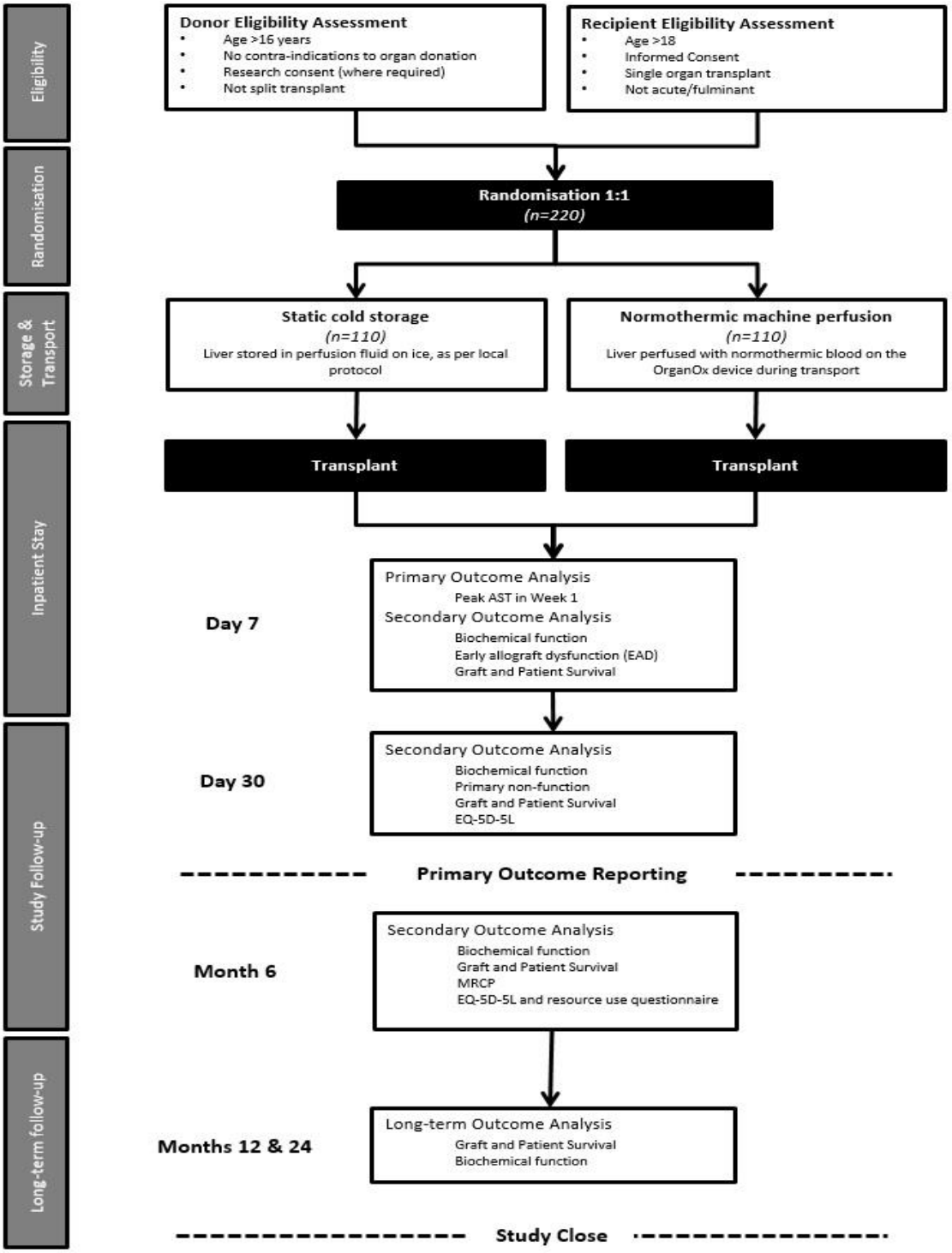


Figure 9: Flow of participants through the trial

3.1.3 Ethics and Regulatory Approval

3.1.3.1 UK

To perform a clinical trial involving a non-CE marked medical device in the UK it is necessary to obtain approval from a National Research Ethics Committee (NREC) specialising in device trials and from the Medicines and Healthcare products Regulatory Agency (MHRA). Having achieved this, it is then necessary to obtain Research and Development (R&D) approval at each individual trial site. The National Institute for Health Research (NIHR) can provide additional support in the form of research nurses at each NHS trial site if it agrees to adopt the study on to its Clinical Research Network (CRN) Portfolio of studies.

Following several months of discussions the trial protocol was agreed by all interested parties and approved by the trial sponsor in January 2014. Additional documents to have been approved were:

- Patient information sheet
- Letter to patients on the waiting list
- Letter to GP for patients enrolled in the trial
- Evidence of trial funding
- Letter of support from the trial sponsor

These can all be found in appendix 1.

In the UK, all documents were submitted simultaneously via an online Integrated Research Application System (IRAS).

A Letter of No Objection (CI/2014/0007) was issued by the MHRA on 26th February 2014

Ethics approval (14/LO/0182) was granted by the London Dulwich research ethics committee on 11th April 2014.

3.1.3.2 Belgium

Before seeking research ethics approval in Belgium, it is first necessary to obtain approval from the competent authority (R&D equivalent) at the study site. In Leuven, this process was coordinated by the local PI, with approval obtained in July 2014. Ethics approval followed in November 2014 with the trial going live on 1st December 2014.

3.1.3.3 Spain

Due to specific legal requirements in Spain, the University of Oxford had never previously been able to successfully establish a multi-national clinical trial involving a Spanish trial site. In particular, most Spanish regulatory authorities require individual Spanish trial sites and investigators to be separately named in the clinical trials insurance policy. As trial sponsor, the University of Oxford had in place a comprehensive multi-national clinical trials insurance policy but was not able to specifically name individuals within this. After extensive legal negotiations between the University of Oxford and Hospital Clinic Barcelona trial site, it was eventually agreed that the existing clinical trials insurance policy was sufficient without specific individuals being named. This process meant that, whilst research ethics committee approval was obtained in Spain on 30th June 2014, formal approval from the sponsor to start the trial was not granted until 14th November 2014. This process was largely coordinated by the local PI in Barcelona.

3.1.3.4 Germany

During 2012 and 2013 the German transplant community was engulfed by a scandal involving manipulation of the organ transplant waiting list and which culminated in the suspension and imprisonment of several transplant surgeons [75]. The effect of this was a drop in levels of organ donation and much greater scrutiny around transplant-related clinical research.

On this background, attempting to start this trial in Germany proved challenging. The Deutsche Stiftung Organtransplantation (DSO; German Organ Transplantation Foundation) required assurances about patient safety and demanded approval for the study from additional German transplant centres

not involved in the trial but which could potentially receive a machine perfused liver if the original intended consented recipient proved unable to have a transplant. The ensuing delays this caused meant that final study approval in Germany was not obtained until 20th November 2015. This process was largely coordinated by the local PI in Essen.

The time taken to obtain R&D approval at each study site is shown in Figure 11.

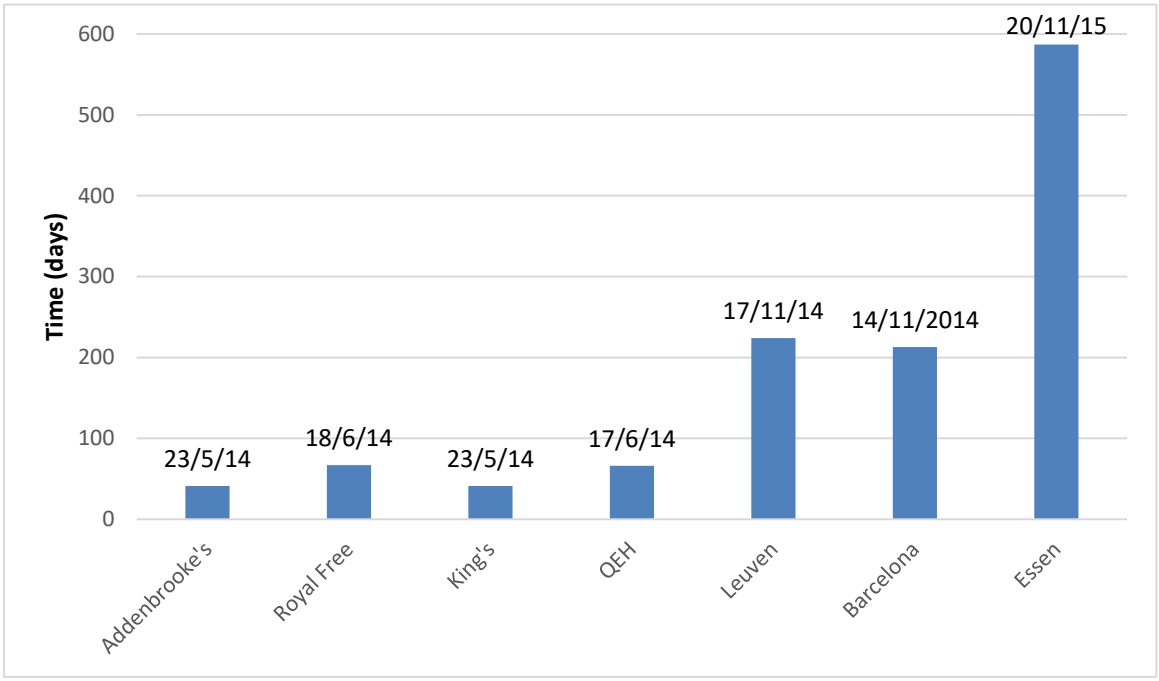


Figure 10: Time (days) taken to obtain R&D approval at each trial site

3.1.4 Data Monitoring Committee

Prior to commencing recruitment a Data Monitoring Committee (DMC) was established comprised of four independent clinicians with relevant expertise and a statistical expert. The DMC met periodically to review accruing data with the stated aim of safeguarding the interests of the trial participants, potential participants and future patients and assessing the safety of the interventions. The DMC was to advise the study chief investigator if, in its view, the study should be terminated due to major clinical disadvantages in one of the study arms. No interim analysis of trial data was performed.

3.1.5 Inclusion and Exclusion Criteria

Inclusion criteria for donors, livers and recipients were deliberately broad to include the full spectrum of clinical scenarios encountered in standard liver transplant practice.

3.1.5.1 Donor eligibility criteria

Livers from deceased organ donors were eligbible for enrolment provided they fulfilled the following criteria:

- Donor age over 16 years
- DBD or Maastricht category III circulatory death (DCD) donors
- Participating surgeon willing to consider transplanting the liver whether assigned to SCS of NMP
- No consent from donor family for research (where applicable). This was not a requirement in the UK or Spain but was a necessity in Germany and Belgium.

3.1.5.2 Donor exclusion criteria

- Age under 16 years
- Liver intended to be split
- Prisoners

3.1.5.3 Recipient eligibility criteria

Liver transplant recipients were eligible for enrolment provided they fulfilled the following criteria:

- Aged over 18 years
- Active on the liver transplant waiting list at a participating trial site
- Listed to receive a liver-only transplant

3.1.5.4 Recipient exclusion criteria

- Recipients with acute/fulminant liver failure were not eligible due to their poor prognosis regardless of organ quality i.e. the outcome was more likely to be related to the disease than the characteristics of the donor organ.
- Unable to provide informed consent

3.1.6 Consent

Following review by the Liver Advisory Group at NHSBT it was agreed that, in the UK, donor families were not required to provide specific consent to be included in this study (because involvement in the trial did not affect the destination of the liver). In Belgium, Spain and Germany research consent was required.

All eligible potential transplant recipients in each centre were provided with verbal and written information about the trial. It was a requirement of the NREC that participants should have at least 24 hours to consider whether to participate in the trial. In practice this meant that consent was obtained whilst the patient was on the waiting list and then affirmed on the day of transplant.

All researchers involved in the consent process were required to have demonstrated evidence of Good Clinical Practice (GCP) accreditation. This is an international ethical and scientific quality standard for the conduct of clinical trials with clear guidelines relating to obtaining informed consent in the contextof a clinical trial.

3.1.7 Randomisation

Once an eligible donor organ had been allocated to a consented eligible recipient, randomisation was performed using an online randomisation tool. Livers were assigned to NMP or SCS with 1:1 allocation ratio as per a computer generated randomisation schedule using variable block randomisation stratified by transplant centre and donor type.

3.1.8 Static Cold Storage group

In this arm of the study the organ retrieval, preservation, transport and transplant process followed normal standard practice at each centre. Following the routine retrieval procedure, the liver was placed in ice-cold perfusion solution (according to local protocols) on the back-table, followed by storage in cold perfusion solution within an icebox. The organ was transported to the recipient centre, and removed from storage prior to implantation for standard back-table preparation. The duration of

cold storage was dictated by logistics and local policy. The trial protocol did not stipulate the type of organ preservation solution to be used or the duration of preservation.

3.1.9 Normothermic Machine Perfusion group

The OrganOx *metra* is a normothermic liver perfusion device for clinical use, the experimental precursor to which was first described by Butler et al [31] in 2002 and the essential circuit design of which has remained largely unaltered (Figure 12).

The circuit incorporates a centrifugal pump, membrane oxygenator and heat exchanger. Arterial perfusion passes directly from the centrifugal pump, via the membrane oxygenator, into the hepatic artery. A second outlet from the oxygenator passes to a reservoir. Adjustments in the degree of occlusion of this outlet by a variable pinch valve are used to alter flow to the reservoir, and hence the pressure in the hepatic artery. Pump speed and pinch valve occlusion settings are automated to

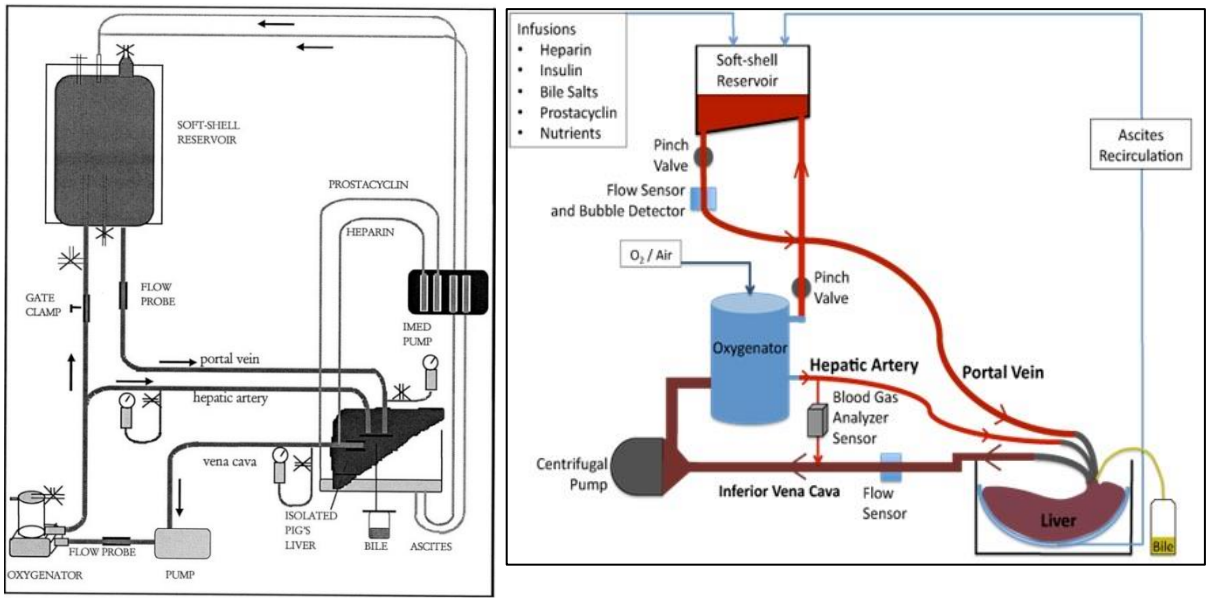


Figure 11: NMP circuit described by Butler et al (left) and the circuit currently used in the OrganOx metra *(right)*

achieve a hepatic artery pressure of 75mmHg. The portal vein is perfused via a soft-shell reservoir using gravitational force.

Image 2: Primed OrganOx metra

Following randomisation to NMP, a device and accompanying clinician were transported to the donor hospital. The device was set-up whilst the retrieval procedure was on-going. A sterile disposable set was mounted on to the device chassis and primed with 500ml Gelofusine (B. Braun Ltd) mixed with three units of donor-matched packed red cells (Image 2). The following additional substrates are added to the perfusate either as a bolus prior to starting NMP or via device mounted syringe drivers and pumps:

3.1.9.1 Bolus

- Cefuroxime – 750mg vial reconstituted with 10ml of 0.9% normal saline
- Heparin – 10000u (2ml at 5000u/ml)
- Calcium gluconate – 10ml of 10% concentration

3.1.9.2 Infusions

- Heparin – 25,000u in 30ml 0.9% normal saline
- Epoprostenol sodium (Flolan) – 10ml of glycine buffer is added to the 0.5mg vial of Flolan. Using a microbial filter, 5ml is drawn out of the vial into a 30ml syringe. A further 25ml 0.9% normal saline is added to achieve a total volume of 30mls.

- Bile salts (bovine sodium taurocholate) – 30ml 0.9% normal saline is injected into the vial containing 5.6g sodium taurocholate. The mixture is agitated and then 30ml is drawn into a syringe via a microbial filter.
- Insulin (actrapid 100u/ml) – 200units is drawn up and mixed with an additional 28mls 0.9% normal saline to achieve a total volume of 30mls.

3.1.9.3 Other

- Sodium bicarbonate ($NaCO_3$, 8.4%) – added to the circuit following device priming and prior to connection of the liver to the device to correct the perfusate pH, aiming for pH 7.3. Additional boluses are titrated according to the pH following connection of the liver.
- Fat free parenteral nutrition (Nutriflex Special, B. Braun Ltd) – infusion starts automatically when a perfusate glucose value of below 10mmol/L is entered.

3.1.10 Rationale for use of NMP Additives

It is important to note that, with the exception of bile salts, the evidence is limited for the inclusion of each of the additives described above. Most were used in a trial and error fashion on the basis of 'common sense' during the early development of the NMP [31]. Once a combination of medications and infusions was stumbled upon it was left largely unchanged until now. Questions therefore remain about the necessity of using heparin in the absence of any platelets or significant clotting factor levels. Similarly, some groups have questioned the need for insulin, observing similar rise and falls in perfusate glucose levels regardless of whether insulin is added.

The effectiveness of epoprostenol is also unclear. It has a short half-life and should be kept out of light which causes degredation of the molecule. As part of the OrganOx *metra*™ circuit, epoprostenol is kept in a clear syringe which is exposed to normal light. Therefore, even if the addition of epoprostenol is helpful, it is likely degraded to an ineffective form within a short period of setting up the device.

Fat free parentral nutrition is used to discourage the deposition of lipid droplets in the liver parenchyma. It is well described that steatotic livers have poorer outcomes after transplantation, something thought to be related to the impact of cold storage on the lipid droplets in the hepatocytes [76]. Whether steatosis is also a problem for liver preserved using NMP is unknown.

3.1.11 Liver Backbench Preparation

Following explant of the donor organ, a liver back-table procedure was performed with removal of the diaphragm, superfluous tissue from the inferior vena cava, and skeletonisation of the inflow and outflow vessels [77]. Very careful attention was paid to haemostasis, in particular with reference to the phrenic veins.

Once complete, the organ was cannulated in preparation for NMP. This involved the following steps:

1. The supra-hepatic IVC was closed using either a linear vascular stapler (Image 3) or a suture.

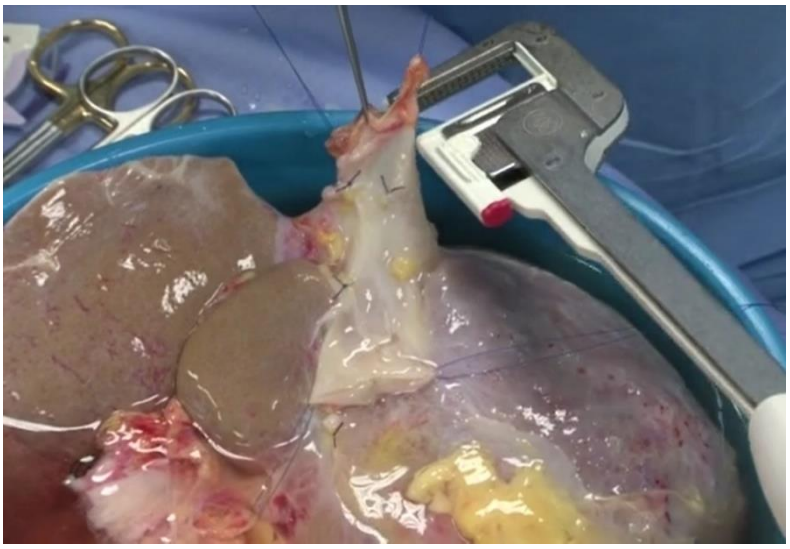


Image 3: Stapling of the supra-hepatic IVC

2. The infra-hepatic IVC was cannulated using a wire-reinforced 28Fr cannula (Sorin Ltd) (Image 4). This was secured in place by a purse-string suture with water-tight reinforcement using a nylon tape snugger.

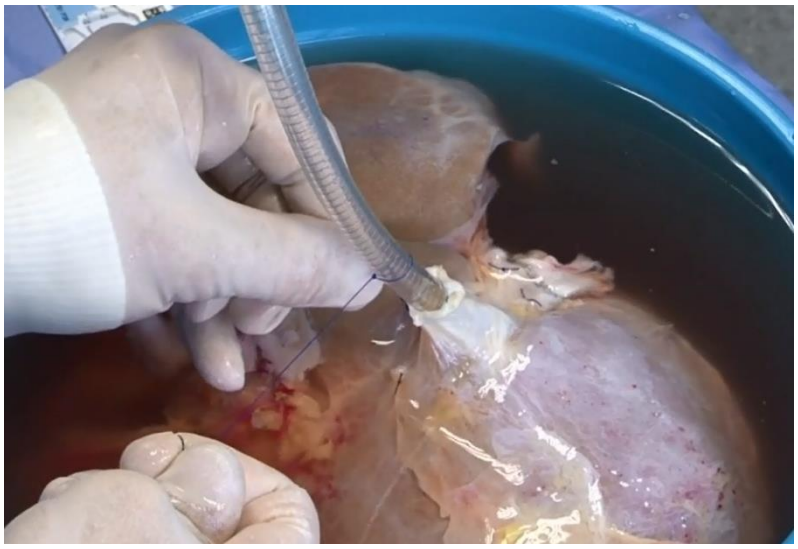


Image 4: Cannulation of the infra-hepatic IVC

3. The hepatic artery was cannulated using a wire-reinforced 10Fr cannula (Medtronic Ltd) secured in place using a 2-0 vicryl ligature (Image 5). Aberrant arterial anatomy was encountered in approximately 30% of cases. During the early part of the trial it was ensured that the necessary expertise was available at the donor hospital to perform arterial reconstructions of these aberrant vessels on to the main hepatic artery. This enabled perfusion via a single vessel either by:

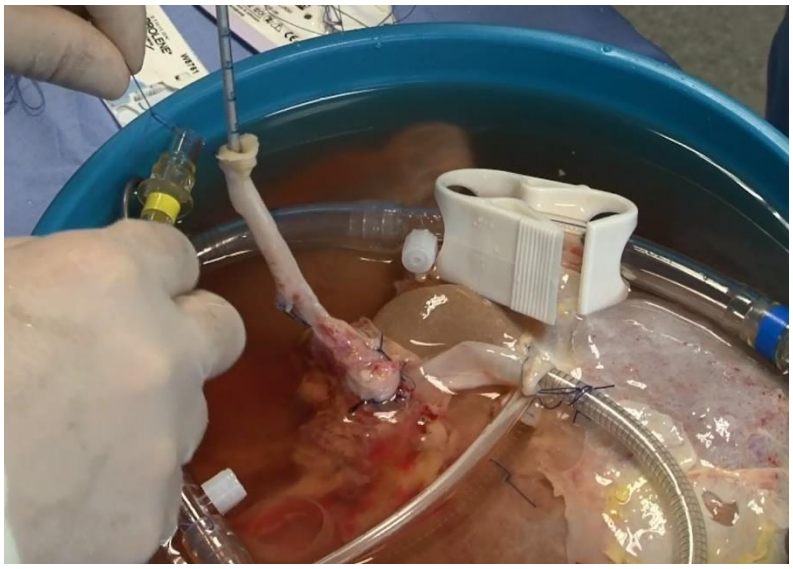


Image 5: Cannulation of the hepatic artery

Image 6a: Reconstruction of an aberrant right hepatic artery on to the gastro-duodenal artery. Also shown is an aberrant left hepatic artery arising from the left gastric artery

   a. anastomosis of the aberrant right hepatic artery to the gastro-duodenal artery (Image 6a) or;

b. the use of the common iliac artery as an extension graft to enable safe cannulation when an aberrant left hepatic artery was arising from the left gastric artery too close to the origin of the common hepatic artery (Image 6b).

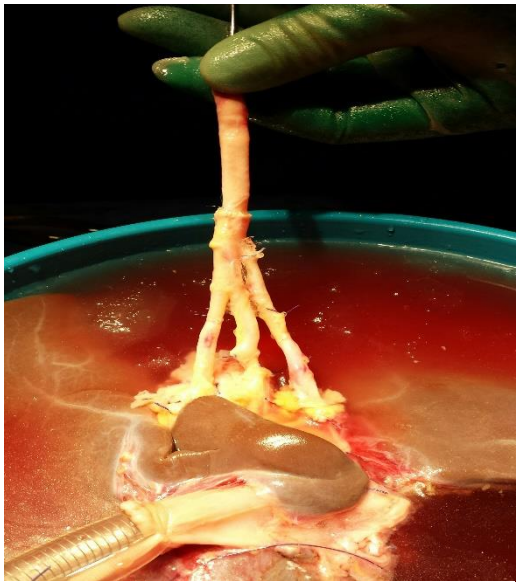


Image 6b: Iliac vessel extension graft used to enable safe cannulatiuon due to aberrant arteries arising close to the origin of the common hepatic artery

If the required expertise to perform an artertial reconstruction was not available then perfusion through two arteries was performed by either:

a. Cannulation of the aorta to perfuse through the coeliac artery and SMA (Image 7a).

b. Use of a y-piece adaptor to connect two arterial cannulae for dual hepatic artery perfusion (Image 7b).

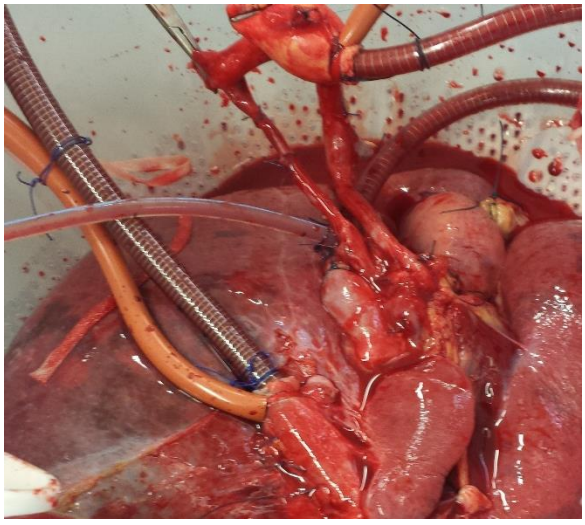


Image7a: Aortic cannulation

Image7b: Dual arterial perfusion

4. The portal vein was cannulated using a 20Fr wire reinforced cannula (Sorin Ltd) secured in place using a 2-0 vicryl ligature (Image 8).

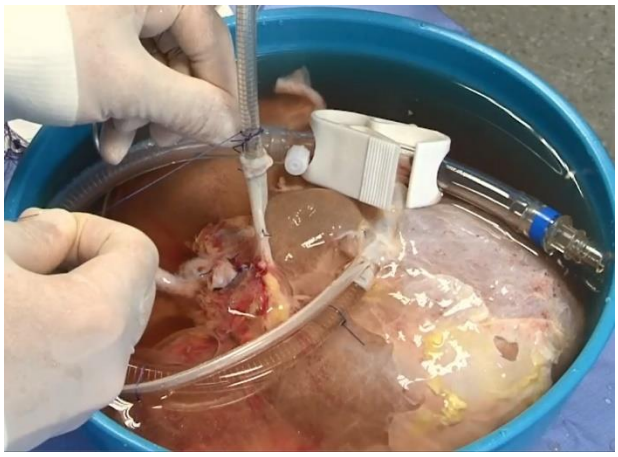


Image 8: Portal vein cannulation

5. The bile duct was cannulated using an appropriately sized silicon tube secured in place using a 2-0 vicryl ligature (Image 9).

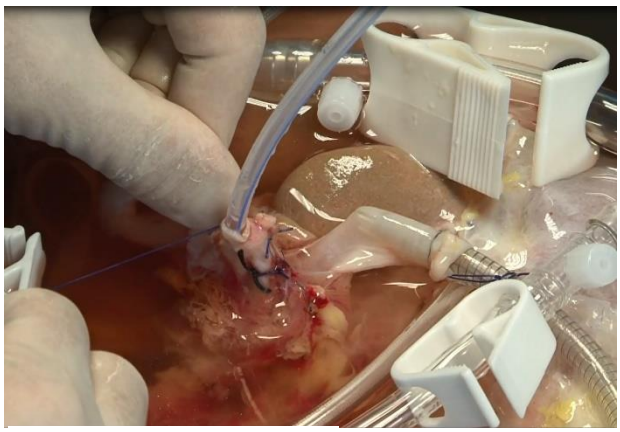


Image 9: Bile duct cannulation

Once all cannulae had been sited, they were flushed with at least 500ml of Gelofusine, taking care to avoid entraining air into the vasculature. This process was used to test for any fluid leakage from the cannulae or vessels, removed any trapped air from inside the organ, and filled the organ with fluid to contribute to the overall perfusate

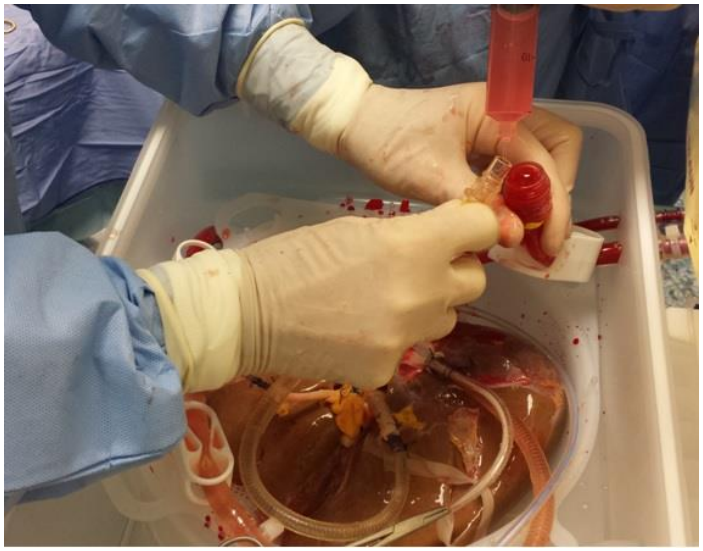


Image 10: Connecting the cannulated liver to the device

volume. The liver was then transferred into the OrganOx *metra* liver bowl where the cannulae were connected to the machine circuit (Image 10) and the perfusion commenced (Image 11).

The organ then remained connected to the device throughout the duration of transport and storage until implantation. The minimum protocol-stipulated NMP duration was 4 hours, the time needed for ATP repletion in animal (DCD) studies [42]. The maximum allowed NMP duration was 24 hours in line with the experience in the phase-I study and the regulatory approval for the device.

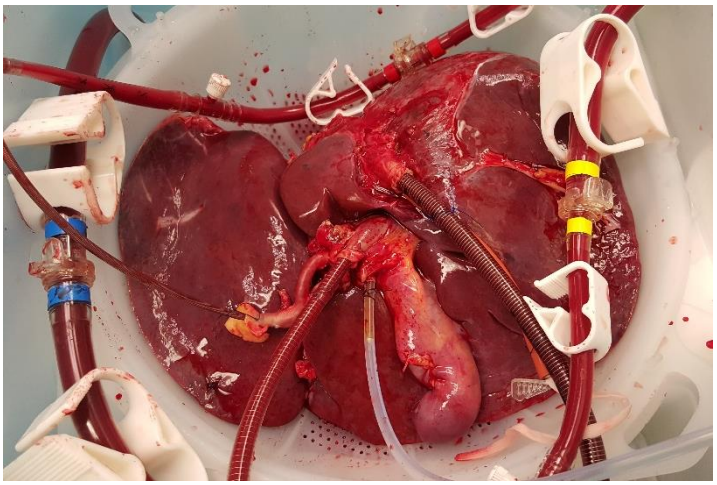


Image 11: Liver during normothermic perfusion

3.1.12 The Development of Dual Perfusion Technique

As described above, aberrant hepatic artery anatomy was the most frequently encountered challenge that had to be overcome during liver cannulation, occurring in approximately 30% of cases. During the early NMP cases, a transplant consultant from the recipient centre would always attend the retrieval meaning that the appropriate skill set was available to perform an arterial reconstruction should it be necessary. However, this arrangement was difficult to sustain.

The first occasion on which aberrant anatomy was discovered in the absence of the required expertise for reconstruction was during the retrieval from a 54 year old DBD donor. Early in the operation the retrieval team identified a replaced right hepatic artery arising from the superior mesenteric artery (SMA). Fortunately, as this was identified early, it was possible to request for the aotic tube to be retrieved intact with the coeliac axis and SMA in place. It was therefore possible to cannulate the distal aorta whilst oversewing the proximal end (Image 7a). This technique was effective for perfusing through the coeliac axis and SMA simultaneously via a single cannula but was extremely problematic due to bleeing from the proximal aorta suture line.

The alternative solution of using a Y-connector piece for dual cannulation had been discussed during trial management meetings. Whilst it was logistically feasible it had never been tried clinically or experimentally. The first clinical case occurred in August 2015. The donor was a 43 year old DCD who had been severely acidotic for 24 hours prior to organ retrieval. The liver had only been accepted by one centre nationally and was considered marginal. At retrieval the liver was noted to be steatotic and, due to the more rapid nature of a DCD retrieval, the aberrant right hepatic artery arising from the SMA was not noticed until the aortic patch had already been divided. On hearing that this already marginal liver was also steatotic, the receiveing surgeon at the transplanting centre was inclined not to use it. However, I proposed that we use NMP to assess the liver's behaviour on the machine. He agreed.

I therefore cannabilised another unused NMP circuit to obtain the Y-piece (present in the liver box as part of the priming circuit) and an additional aortic cannula. The single end of the Y piece was connected to the main inlet for arterial perfusion. The double end was connected to two arterial cannulae. These were secured into the lumens of the two arteries (common hepatic artery and SMA). On commencing perfusion it was immediately apparent that this strategy was successful. There was good flow through both vessels with minimal bleeding. This liver went on to be transplanted successfully.

This technique was used on six further occasions in the study. It was also notable that livers in which dual perfusion was used achieved higher mean arterial flows than those with single vessel perfusion. This is unlikely to be related to any factors inherent in these livers with aberrant anatomy – they should still have a similar cross-sectional area of arterial vasculature inside the liver parenchyma and so have similar arterial resistance. The observed differences in flow most likely represents the greater cross-sectional area of using two cannulae rather than one, suggesting that cannula diameter may be a limiting factor in hepatic arterial flow during NMP. It was not possible to investigate this hypothesis further during the study but could form the basis of future work.

3.1.13 Study End Points

3.1.13.1 Selection of a Primary End Point

Evidence is lacking regarding the best endpoint to use in an efficacy study in liver transplantation. The most important outcome is probably graft survival at one year. But with many units achieving one year graft survival in excess of 90%, powering a study to achieve a measurable improvement in this outcome would require very large numbers of particpants.

There are a number of early biochemical outcome measures that have been shown to correlate with long term graft and patient survival. These include initial poor function[78] (IPF), early allograft dysfunction[79] (EAD), and early changes in transaminase levels [80-82]. IPF and EAD are both binary measures based on the degree of abnormality of certain biochemical and functional parameters exceeding defined thresholds in the first week post-transplant. Meanwhile, serum peak-AST is a continuous variable defined as the peak measured rise in AST levels in the first week post-transplant. When selecting a primary outcome for the present trial it was agreed that it would be ideal to use one year graft survival. However, the large numbers of particpants required to power such study meant that an appropriate surrogate endpoint was sought. The two strongest options were EAD and peak-AST. The use of continuous variables as outcome measures are considered to be more sensitive than binary measures which, as a consequence of transforming continuous data into a binary response, inevitably results in a loss of power to detect differences[83]. For this reason, peak-AST was selected as the primary outcome for this trial.

3.1.13.2 Primary End Point

The primary endpoint was defined as the difference in peak serum aspartate transaminase (AST) level within seven days post-transplant between the two treatment arms. In contrast to alanine transaminase (ALT) which is found primarily in hepatocytes, AST is also found in heart, skeletal muscle, kidneys and red blood cells. Despite this, the magnitude of AST rise following transplantation is regarded as a reliable reflection of the degree of injury a liver has sustained and is consistently found

to correlate more strongly than ALT with the degree of hepatocellular injury that a liver has sustained

.

A number of studies have demonstrated a relationship between peak AST in the early post-transplant period and patient survival, graft survival, early graft dysfunction and primary non-function following liver transplantation [80, 81, 84] (Figure 12). Peak AST is also significantly elevated in liver allografts with histological evidence of moderate to severe perfusion injury [85, 86].

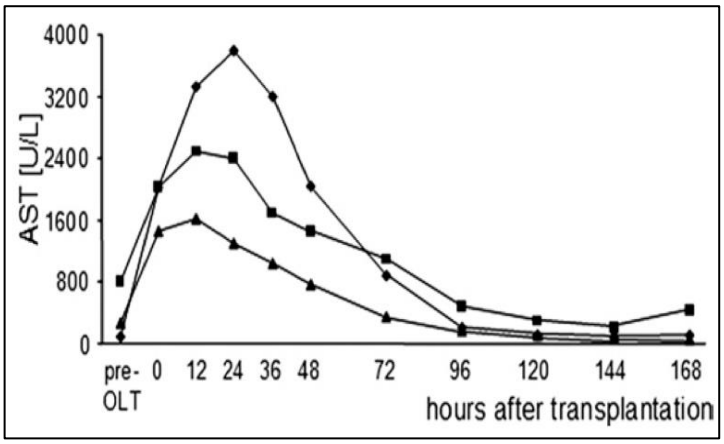


Figure 12:Peak AST in those who died (◆), lost graft (■) or survived with a functioning graft (▲) within 90 days post-transplant. (Eisenbach 2009)

The first post-transplant AST value was measured 12 to 24 hours post-reperfusion. This was stipulated primarily to mitigate concern that NMP may have a 'wash out' effect on AST that could result in artificially low measurements in the very early post-operative period.

3.1.13.3 Power Calculation

Data from 416 liver transplant recipients from University Hospital Essen demonstrated the geometric mean of peak AST to be 608.59 IU/L.

Following discussions between the Trial Management Committee and liver transplant clinicians at the participating trial sites, a clinically significant effect was defined as a 33% difference in peak serum aspartate transaminase level (AST) within 7 days post-transplant between the two treatment arms:

$$\frac{Peak\ Serum\ (SCS) - Peak\ Serum\ AST(NMP)}{Peak\ Serum\ AST(SCS)} \geq 0.33$$

This 33% difference was chosen arbitrarily and was felt to reflect the minimum transaminase reduction required to translate into a meaningful change in clinical outcome. There was little evidence on which to base this assertion; indeed there is little evidence to support the use of any outcome measure in an efficacy study in liver transplantation.

This study was powered to detect this clinically significant 33% reduction (to 401.67 IU/L) with 90% power at a 5% significance level, requiring 220 transplants (110 per arm). 2011/12 NHSBT data suggested that 12% of livers retrieved would not be transplanted. Assuming losses of 15%, randomisation of 260 livers into the trial was therefore calculated to be required to achieve adequate power.

Results are reported as an intention-to-treat analysis. Livers intended for a randomised recipient that were not retrieved for any reason were withdrawn from the trial and not included in later analysis.

Pre-specified subgroup analyses were performed to determine the treatment effect on the primary end point and some secondary end-points according to donor type (DCD versus DBD). A p-value of 0.05 or less was considered to indicate statistical significance. Analyses were conducted with the use of Stata software package.

No interim analyses of study end points were carried out. At regular intervals, confidential safety analyses were performed by an independent Data Monitoring Committee, which compared the reported rates of adverse events between the two trial groups.

3.1.13.4 Secondary End Points

The full range of secondary end points that were measured as part of the trial are described in detail below:

Objective	Outcome Measures
To compare graft and patient survival between NMP and SCS livers.	1. Primary non-function: defined according to the United Network for Organ Sharing (UNOS) criteria as irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes. 2. Graft survival at 30 days and 6, 12 and 24 months following transplantation. 3. Patient survival at 30 days and 6, 12 and 24 months following transplantation.
To compare biochemical liver function between NMP and SCS livers.	1. Daily serum bilirubin, GGT, AST and INR at days 1-7 following transplantation. 2. Daily serum lactate at days 1-7 whilst in high level (ITU/HDU) care 3. Serum bilirubin, GGT, AST and INR at day 30 and months 6, 12 and 24 following transplantation. 4. Early allograft dysfunction (EAD) [79]; defined by any one of: a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant b. INR >1.6 on day 7 post-transplant. c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant The Olthoff definition of EAD was chosen as it is the most widely used internationally and so the most appropriate choice in the context of a multi-national trial.
To compare the physiological response to reperfusion between NMP and SCS livers	1. Post-reperfusion syndrome, defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than one minute during the first five minutes after reperfusion. This will be assessed in the context of vasopressor use [87, 88] 2. Length of stay in Level 2 or 3 (HDU/ITU) care 3. Length of hospital stay 4. Need for renal replacement therapy (haemodialysis, haemofiltration, haemodiafiltration) 5. Estimated Glomerular Filtration Rate (eGFR)

To compare evidence of reperfusion injury between NMP and SCS livers.	Histological evidence of reperfusion injury in post-reperfusion biopsies (taken immediately prior to abdominal closure). These will be compared to baseline pre-reperfusion biopsies (on removal of the liver from SCS/NMP) and graded using standard histological criteria [85, 89]
To compare evidence of ischaemic cholangiopathy between NMP and SCS livers.	Evidence of non-anastomotic biliary stricturing on magnetic resonance cholangiography (MRCP) at 6 (range 5-7) months post-transplant.
To assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation.	1. Perfusion parameters (logged automatically by the device): a. Arterial and caval pressures (in mmHg) b. Arterial, portal and caval flow rates (in mmHg) c. pO_2, pCO_2 and pH d. Blood temperature (^oC), Glucose (mmol/L) and bile production (ml/h) 2. Perfusate ALT and AST at 15 minutes, 1 hour and the end of NMP 3. Perfusate IL6, TNF, vWF at 15 minutes, 1 hour and the end of NMP 4. In addition to these pre-specified outcomes, additional biological samples will be taken for the COPE WP7 bioresource at specified timepoints as detailed in Appendix 2.
To assess the feasibility and safety of NMP as a method of organ storage and transportation.	1. Organ discard rate 2. Perfusate culture. At the end of preservation a sample will be taken for microbiological culture (cold preservation or warm perfusate). 3. Adverse event rates and severity, graded according to the Clavien-Dindo classification [90] as described in Appendix 4. a. Recipient infection b. Biopsy proven acute rejection c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks) d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis) e. Reoperation rate f. Technical complications/device failures

To assess the health economic implications of normothermic liver perfusion.	Limited health economic analysis utilising: 1. Logistical costs, measured using national unit costs where available. 2. Healthcare resource use; measured by a combination of hospital episode records and a patient-completed resource use log. 3. Quality of life by delivery of the EQ-5D-5L questionnaire at baseline, day 30 and month 6 post-transplant.

Table 5: Secondary outcomes measured in the COPE trial

3.1.14 Baseline Assessments

3.1.14.1 Donor Demographics

The following donor demographic data was recorded for all randomised livers:

- Age
- Sex
- Height
- Weight
- BMI
- Race (White, Afro-Caribbean, other)
- Cause of death (CVA, hypoxia, trauma, other)
- Type of donor (DBD, DCD)
- UK Donor Risk Index (UK-DRI) – calculated
- Eurotransplant Donor Risk Index (ET-DRI) [91]– calculated
- Peak serum AST
- Peak serum ALT
- Peak serum sodium
- Length of ITU stay
- BMI

3.1.14.2 Donor Risk Index (DRI)

The DRI was first described by Feng et al [92] to quantify the effect of different donor characteristics on the outcome following liver transplantation. They applied a cox regression model to UNOS data from 1998-2002 to identify seven donor characteristics that independently predicted significantly increased risk of graft failure. These were combined to produce the following calculation:

Donor risk index = exp[(0.154 if 40≤ age <50) + (0.274 if 50≤ age <60) + (0.424 if 60≤ age <70) + (0.501 if 70 ≤ age) + (0.079 if COD = anoxia) + (0.145 if COD = CVA) + (0.184 if COD = other) + (0.176 if race = African American) + (0.126 if race = other) + (0.411 if DCD)+(0.422 if partial/split)+(0.066 ((170–height)/10))+(0.105 if regional share)+(0.244 if national share)+(0.010×cold time)]

Equation 1: Donor risk index equation. Key: COD - cause of death; CVA - cerebrovascular accident

Table 6 below expands the concept described by this equation to illustrate the relative change in risk with the inclusion of each additional risk factor.

Donor profile	N (%)	Range of calculated DRI	95% CI for adjusted 1 year survival estimates
None			
Under 40	6814 (34.0%)	Ref.	84.8–86.4
40 – 49	1174 (5.9%)	1.17	82.9–86.9
50 – 59	653 (3.3%)	1.32	79.0–84.8
60 – 69	299 (1.5%)	1.53	77.7–85.9
70+	140 (0.7%)	1.65	61.3–76.3
COD – CVA/other or black			
Under 40	2683 (13.4%)	1.16–1.20	81.8–84.6
40 – 49	2128 (10.6%)	1.35–1.40	79.8–83.1
50 – 59	2293 (11.5%)	1.52–1.58	77.2–80.5
60 – 69	1445 (7.2%)	1.77–1.84	73.4–77.8
70+	655 (3.3%)	1.91–1.99	72.4–78.7
(COD – CVA + Race – black) or (COD – CVA + Race – black)			
Under 40	276 (1.4%)	1.38–1.43	71.7–81.3
40 – 49	365(1.8%)	1.61–1.67	73.7–82.0
50 – 59	290 (1.5%)	1.81–1.89	73.7–82.8

60 – 69	132 (0.7%)	2.11–2.19	63.4–78.4
70+	58 (<0.5%)	2.27–2.37	71.6–91.0
DCD or split liver			
Under 40	344 (1.7%)	1.51–1.52	75.4–83.8
40 – 49	34 (<0.5%)	1.76–1.78	45.6–78.0
50 – 59	17 (<0.5%)	1.98–2.01	73.0–100.0
60 – 69	6 (<0.5%)	2.30–2.33	33.3–100.0
70+	3(<0.5%)	2.49–2.52	23.9–100.0
(DCD or split liver) + at least one other factor			
Under 40	129 (0.6%)	1.74–3.30	64.9–80.5
40 – 49	51 (<0.5%)	2.03–3.85	60.7–84.7
50 – 59	20 (<0.5%)	2.29–4.34	49.0–91.5
60 – 69	14 (<0.5%)	2.66–5.04	46.4–97.0
70+	0 (0.0%)	2.88–5.45	N/A

Table 6: Calculated donor risk index and 1-year graft survival with 95% confidence interval estimates for specified donor profiles. Key: DRI – donor risk index; CI – confidence interval; COD – cause of death; CVA – cerebrovascular accident. Ref [92]

This model, developed by Feng et al., was subsequently adjusted and validated in the Eurotransplant population [91]:

ET-DRI = exp[0.960((0.154 if 40≤age<50)+(0.274 if 50≤age<60)+(0.424 if 60≤age<70)+(0.501 if 70≤age)+(0.079 if COD = anoxia)+(0.145× if COD = CVA)+(0.184 if COD = other)+(0.411 if DCD)+(0.422 if partial/split)+(0.105 if regional share)+(0.244 if national share))+(0.010×(cold ischemia time−8h)) +0.06((latest lab GGt (U/L)-50)/100)+(0.180 if rescue offer)]

Equation 2: Eurotransplant Donor Risk Index equation. Key: COD = cause of death; CVA - cerebrovascular accident

The ET-DRI has been validated in the Eurotransplant population but not in the UK. Therefore a similar cox regression model was applied to NHSBT registry data to develop a UK-DRI validated in the UK transplant population :

UKDRI = exp[1.0620 + 0.0111(age) + (0.7383 if DCD) + (0.5084 if split liver) – 0.0082(height) + (0.4253 if diabetes) + (0.1313 if smoker) + 0.0082*(bilirubin) – 0.0101(albumin)]

Equation 3: United Kingdom Donor Risk Index (UK-DRI)

The ET-DRI was applied to all trial livers whilst the UK-DRI was applied only to those enrolled in the UK. This was partly because it had only been validated in the UK population, but also due to difficulties in collecting the necessary data from non-UK sites.

3.1.14.3 Recipient Demographics

The following recipient demographic data was recorded for all randomised livers that went on to be transplanted:

- Age
- Sex
- Indication for transplant
- MELD score (calculated from INR, creatinine, bilirubin)
- BMI
- eGFR

A baseline assessment of quality of life using the EQ-5D-5L was performed at the time of initial consent to the study.

3.1.15 Recording of operative and perfusion parameters

The following data was collected during the retrieval, preservation and implant process:

3.1.15.1 Organ retrieval data

The times recorded for DBD donors (SCS group) were:

- Cessation of donor circulation (cross clamp)
- Start of cold perfusion (should be the same unless technical problem)
- Liver removal and placement on ice
- Degree of steatosis (graded mild, moderate, severe) – surgeon's assessment
- Quality of *in-situ* perfusion (graded poor, moderate, good)
- Perfusion solution used for aortic perfusion
- Perfusion solution used for organ transport

Additional times recorded for DCD donors (SCS group) were:

- Withdrawal of support
- Onset of functional warm ischaemia (SBP < 50 mmHg or O_2 saturation < 70%)

In addition, the following parameters were recorded for all machine perfused livers:

- Initiation of normothermic machine preservation
- Cessation of normothermic machine preservation (cold flush)
- Volume of bile produced

3.1.15.2 Operative parameters

These include:

- Total operative time: defined as time from knife-to-skin to skin closure.
- Anastomotic time (secondary warm ischaemia): defined as time between removal of organ from ice (SCS) or perfusion device (NMP) to organ reperfusion (portal or arterial)
- Presence of post-reperfusion syndrome (defined as a decrease in mean arterial pressure (MAP) of more than 30% from the pre-implantation baseline value for more than one minute during the first five minutes after reperfusion
- Use of vasopressors prior to and after reperfusion
- Intraoperative transfusion of blood products (measured in units).
- The use of veno-venous bypass or porto-caval shunt
- Type of caval anastomosis (standard end-end, piggyback (end-side or side-side)

3.1.16 Post-operative Data

The post-operative care of trial participants was conducted according to normal local protocols.

3.1.16.1 Outcome assessment

The following biochemical outcomes were recorded:

- Daily serum samples for the first 7 days post-transplant:

    - Serum bilirubin (measured in µmol/l)
    - Serum gamma-glutamyl transferase (GGT; measured in IU/L)
    - Serum aspartate transaminase (AST; measured in IU/L)
    - Serum alanine aminotransferase (ALT; measured in IU/L)
    - Serum creatinine (Cr; measured in µmol/L)
    - International normalised ratio (INR)
- Daily serum lactate (measured in mmol/L) whilst admitted on ITU/HDU.

As previously described, the first measurements were collected at 12 to 24 hours post-transplant. For subsequent measurements, in the event that more than one measurement was taken in a 24 hour period, the measurement taken closest to the specified time-point was used.

Other outcomes that were recorded include:

- Length of stay in level 2 or 3 care (ITU/HDU) (days)
- Total length of hospital stay (days)
- Requirement for renal replacement therapy (haemodialysis (HD), haemodiafiltration (HDF), haemofiltration (HF))
- Estimated Glomerular Filtration Rate (eGFR)
- Graft and patient survival at day 10 post-transplant
- Primary non-function, defined as irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes (UNOS definition).

3.1.17 Day 30 Follow Up

Where possible, this visit coincided with a routine outpatient appointment. If the recipient was an inpatient, assessment was made in hospital where appropriate.

3.1.17.1 Outcome assessment

The following biochemical outcomes were recorded at day 30 post-transplant:

- Serum bilirubin (measured in µmol/l)
- Serum gamma-glutamyl transferase (GGT; measured in IU/L)
- Serum aspartate transaminase (AST; measured in IU/L)
- International normalised ratio (INR)

Other outcomes that were recorded include:

- Graft and patient survival at day 30 post-transplant
- Requirement for renal replacement therapy (HD, HF, HDF) at any time
- Estimated Glomerular Filtration Rate (eGFR)
- Healthcare resource use (by means of a patient-completed log, hospital admissions data and medical records)
- Quality of life (measured by means of the EQ-5D-5L questionnaire).

3.1.18 Longer Term Follow-up

Additional follow-up data was collected at six months, 12 months and 24 months. This included graft and patient survival as well as biochemical data. At the six month follow-up appointment, quality of life information was recorded and an MRCP was performed looking for evidence of ischaemic cholangiopathy.

The information collected at 24 months is not included in this thesis.

3.1.19 Sample Collection

Part of the purpose of the COPE project was to establish biobank containing samples collected from the three COPE clinical trials. The samples that were collected from the trial described in this thesis are detailed in Appendix 2.

The samples were all stored in the trial biobank. An application system was developed where-by interested parties could submit a study proposal for a project using these samples. All of the basic science work described in this thesis has used these samples.

3.1.20 Trial Logistics

This trial involved the use of a relatively complex, non-CE marked (at the start of the trial) medical device being used in clinical practice during what is a fast paced and complex process (organ retrieval and transplant). This inevitably entailed challenges related to training, transport and clinical use of the device across different transplant centers in different countries. A non-exhaustive description of these challenges is detailed below.

3.1.20.1 Device transport

The OrganOx *metra* has an estimated battery life of 2.5 hours. The journey time between donor hospital and transplant centre is often in excess of this time and therefore battery power alone cannot be relied upon when transporting a liver on the machine. It was therefore necessary to ensure that all ambulances which were used for device transport were equipped with a 12V DC to 240V AC power inverter. Prior to starting the trial an agreement was reached with Amvale Medical Transport Ltd to make this adaptation to several of their vehicles (Image 12). Similar arrangements were made in Leuven, Belgium and Essen, Germany. In Barcelona it was not possible to make these arrangements. Instead a back-up power supply was sourced and taken with the team for NMP retrievals.

Image 12: DC to AC power inverter fitted in Amvale ambulance

3.1.20.2 SCS liver data and sample collection

Collecting the required biopsy, perfusate, bile, blood and urine samples from each NMP liver did not require additional personnel as there was always someone present with the liver during NMP. Collecting these samples from SCS livers was more challenging. It was not feasible to rely on individuals at each transplant centre to do this – transplants often occur during anti-social hours so it was not predictable who would be available. It was therefore deemed necessary to employ a group of Transplant Technicians (TTs) to attend each SCS transplant where they could ensure all of the required samples and data were collected.

A job description was drafted, approved by the University of Oxford and then advertised within Oxford University Medical School and to all Medical Sciences post-graduate students. Applicants were short-listed and interviewed.

Successful applicants then attended a one day training course. This included details of the three COPE clinical trials, training on the trial database and instructions on sample collection. Between them the TTs organised an on-call rota. If a liver was randomised to SCS the appropriate on call TT was contacted and attended the transplant operation. I remained in regular contact with them during this process to deal with any problems as and when these arose.

3.1.20.3 Device training

In conjunction with the device manufacturer, OrganOx Ltd, a NMP training program was developed. This consisted of educational literature, a training presentation, training videos and hands-on device set-up and organ cannulation using discarded human livers or pig livers. This training system was implemented in Leuven, Essen and Barcelona with varying success.

Essen were confident using the device autonomously from their first perfusion and did so successfully for the duration of their involvement in the trial.

Although comprehensive training was provided, the Leuven transplant team preferred that personnel from Oxford should be in attendance for most of their NMP livers: this was feasible due to the excellent transport links between the UK and Belgium, and was provided.

The Barcelona transplant team had extensive previous experience in the use of other perfusion systems, although user-related problems resulted in the first two NMP livers not being transplanted. This issue was resolved by an extended period of further training using discarded human livers before successfully recommencing trial recruitment.

3.1.21 Sources of bias

A challenge inherent to any open-label trial is the potential for the introduction of bias, and this study was no exception. Many potential sources of bias were anticipated in advance of commencing recruitment and so every effort was made to minimise and mitigate their effects.

3.1.21.1 Consent

As described above, evidence of GCP accreditation was required from all researchers before they were able to participate in the trial consent process. It was also a requirement that patients had at least 24 hours to consider whether they wished to be involved in the study which, in practice, meant that they had to be consented prior to the day of their transplant.

In practice, there were instances where this process was not adhered to. In particular, there were instances where, on arrival at the transplant centre with the liver undergoing normothermic perfusion it then became apparent that the original intended recipient was not fit for surgery and so an alternative recipient had to be found at short notice. On these occasions there may not have been an appropriate recipient that was already consented for inclusion in the trial. As a consequence, these recipients were approached at the time of their arrival in the transplant centre on the day of their transplant. They were then having to consider not only whether to proceed with the transplant operation itself, for which they may have been waiting months or even years, but also whether to do

so in the context of a clinical trial using a device which was still in development and undergoing assessment. The validity of consent obtained in these circumstances is questionable and, indeed, could constitute coercion for enrollment in the trial as the patient is not truly in a situation where they can consider the alternatives to participation in the study. To my knowledge this occurred on two occasions. The clinican involved was advised regarding the inappropriateness of seeking consent in this manner and a letter was sent by the chief investigator to to all site PIs reminding them of their obligations under the code of Good Clinical Practice. No further action beyond this was taken.

3.1.21.2 Liver enrollment

The study protocol stipulated that a liver should only be enrolled if it would be considered potentially suitable for transplant regardless of the arm to which it was assigned. Early in the trial, or possibly, even prior to commencing recruitment, some investigators expressed a preconception that NMP would improve the quality of livers during preservation and so potentially mean that livers which would not usually be suitable for transplant could then be made usable. There was a concern that this belief could alter surgeon behavior to consider enrolling livers that would fall outside the acceptance criteria in the hope that these would be assigned to the NMP arm. This would either translate into an artificially high discard rate in the SCS arm or worse outcomes in the NMP arm if it resulted in poorer quality livers being transplanted.

To mitigate this risk, all investigators were provided with clear guidance from the outset as to the eligibility requirement for donor livers. At periodic intervals during the trial investigators were sent a reminder via email explicitly stating the importance of maintaining equipoise throughout trial recruitment to guarantee the academic integrity of the results. This was reinforced at the annual trial principal investigators' meeting.

3.1.21.3 Liver Assessment

During liver NMP it is possible to perform continuous real-time monitoring of flow-dynamic, biochemical and metabolic organ parameters. There is evidence to suggest that some of these

measures can be used to predict organ quality although this has not been validated. The use of these parameters to determine the usability of an organ was not prohibited in this study as this is inherently part of the NMP technology. However, investigators were given no instuctions that perfusion-derived parameters should be used to determine whether or not to transplant any given liver. In the future, should the application of this data to clinical practice result in differences in organ utilization between the two trial arms it would be seen as an intrinsic effect of NMP.

3.1.21.4 Preservation Time

The trial did not stipulate that NMP and SCS organs had to be treated similarly with regards to preservation duration. The only requirement was that the duration of NMP was between 4 – 24 hours. The time of transplant was to be determined by operating department logistics. Some investigators expressed a preconception that NMP could safely extend the duration of liver preservation. It was unknown if this was the case and so could translate into worse outcomes in the NMP arm should this be translated to clinical practice.

To prevent this scenario from arising all investigators were provided with clear guidance from the outset emphasizing that the timing of organ implantation should only be determined by local logistical arrangements and not be impacted by mode of preservation. At periodic intervals during the trial investigators were sent a reminder via email explicitly stating the importance of maintaining equipoise throughout trial recruitment to ensure the integrity of the results. This was reinforced at the annual trial principal investigators meeting.

The emergency nature of transplant surgery, combined with the well-established practice of minimising cold ischaemia times due the detrimental impact of prolonged ischaemia on graft and patient survival, meant that the stipulated minimum NMP time of 4 hours could sometimes also be a problem. Many surgeons will start the recipient operation in advance of an organ's arrival in order to minimise preservation time. For an NMP liver this could mean a surgeon wishing to remove the liver from the device before the stipulated 4 hours. Therefore, during each NMP liver transplant this

protocol stipulation was re-emphasised. The statistical analysis plan also made it clear that any NMP livers that received less than 4 hours of normothermic preservation would still be included in the intention-to-treat (ITT) analysis but excluded from the per-protocol (PP) analysis.

3.1.21.5 Surgeon Behaviour

Perhaps the most difficult aspect to control within a clinical trial are the individual preferences of clinicians, that have evolved often over the course of many years. The introduction of a new technology that requires alterations in surgeon behaviour can inevitably be difficult. For this reason the protocol was designed to be flexible in as many respects as possible, in order to require as few alterations to normal practice as possible. This included: ensuring that the final decision regarding whether to use an organ was at the discretion of the transplanting surgeon; allowing surgeons to follow their preferred practice during the organ retrieval; allowing surgeons to follow their preferred practice for flushing the liver prior to implantation.

3.2 Statistical Analysis

A Statistical Analysis Plan was written prior to completion of study enrolment to provide a detailed description of the intended methods of statistical analysis. This can be found in Appendix 3.

3.2.1 Primary Outcome

The distribution of peak serum AST between the two arms will be assessed by a Normal plot for each treatment group. If this appears to be normally distributed the difference in peak serum AST between the two groups will be compared by using ANOVA with adjustment for stratification factors: participating (recipient) centre and donor type (DBD and DCD).

If peak serum AST has departure from Normality the first approach will be transformation. If the data cannot transform to Normal distribution, the difference in peak serum AST between arms will be analysed using Mann-Whitney U test. The difference will be presented along with 95% confidence

intervals (CI) for the difference in medians. If a non-parametric analysis needs to be performed there will be no adjustment for stratification factors or other covariates.

The analysis comparing the difference in peak serum AST between the two groups using ANOVA will be repeated with additional adjustment for important prognostic factors.

Interim analyses of primary and secondary endpoints could only be carried out if requested by the DMC. This scenario did not arise during the course of the trial.

3.2.1.1 Pre-specified subgroup analysis

Subgroup analyses will be performed for donor type (DCD vs. DBD), donor risk index (ET-DRI) and MELD Score. Value intervals for ET-DRI will be 'low', 'medium' and 'high' based on the 33rd percentiles. Value intervals for MELD Score will be <9, 10-19, 20-29, 30-39, ≥40 based on the UNOS standard reference ranges [93].

The study is not powered to detect differences in the subgroups and should only be regarded as hypothesis-generating. Interaction methods will be used to look for consistency of treatment effect across the different subgroups and reported using forest plots.

3.2.2 Analysis to address secondary aims

The secondary aims will determine the effect of NMP compared to SCS on graft and patient survival, biochemical liver function, physiological response to reperfusion, reperfusion injury and ischaemic cholangiopathy. They also will assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation and to assess the feasibility, safety and health economic implications of NMP as a method of organ storage and transportation.

Binary outcomes will be reported in terms of proportions along with 95% confidence intervals and will be assessed using chi-squared test. Logistic regression will be performed to adjust for potential confounders and the treatment difference will be reported using odds ratios with relevant 95% confidence intervals. Continuous outcomes will be reported as mean with standard deviation (SD) or

as median and interquartile range (IQR), and compared using the T-test if normally distributed, or by the Mann-Whitney U. The treatment difference will be reported in terms of difference in means together with 95% confidence intervals. Time-to-event outcomes will be analysed using survival analysis methods, including Kaplan-Meier and Cox proportional hazards regression model with calculation of hazard ratios. The proportional hazards (PH) assumption will be tested and if non-proportionality is present appropriate methodology will be used such as stratified Cox (SC) model and extended Cox model containing time-dependent variables. Median survival times will be reported together with 95% confidence intervals.

Outcomes will be reported with 95% confidence intervals and p-values to 3 decimal places. A p-value of less than 0.05 will be regarded as statistically significant.

Primary timepoint for secondary outcomes analysis is at 6 months with long-term follow-up at 2 years.

3.2.2.1 Primary Non-function

Primary Non-Function (PNF) is defined as irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes (UNOS definition).

Presence of PNF will be compared across the two randomised groups by performing unadjusted and adjusted analysis as described above to take account of the stratification factors and important prognostic factors such as peak AST, DRI and MELD Score.

3.2.2.2 Graft and patient survival

Graft survival is defined as time (in days) from transplant to graft failure. Patients who die with a functioning graft will be censored at their date of death. Patients who are still alive with a functioning graft at the end of the study will be censored at their last known date alive with functioning graft.

Patient overall survival is defined as time (in days) from transplant to patient death. Patients still alive will be censored at their last known alive date. Graft survival and patient overall survival will be

compared across the two groups using the methods described above, reporting at 6 months and at long-term follow-up time points. Cox proportional hazards regression model will be performed both in a univariate and multivariate framework adjusting for stratification factors and known or suspected prognostic factors such as donor and recipients demographics (BMI, DRI, indication for transplant, MELD Score).

3.2.2.3 Biochemical liver function

Liver function will be explored by assessing biochemical components of the blood serum. These are a number of serum biochemical tests: Biliburin, Gamma glutamyl transpeptidase (GGT), Aspartate transaminase (AST), International normalised ratio (INR), creatinine and lactate dehydrogenase. All of these tests are interpreted using reference ranges.

There are circumstances where the INR is medically corrected. Although this is expected to be very uncommon, cases of INR being medically corrected will be recorded and this value used instead of the ordinary one. Proportion of INR medically corrected will also be reported overall and by treatment arm.

As Bilirubin, GGT, AST, INR and creatinine are measured daily at days 1-7 following transplantation and serum lactate daily at days 1-7 whilst in ITU/HDU care, they will be assessed using a number of different methods: their average value as well as area under the curve (AUC) will be calculated and compared across the two treatment arms.

Bilirubin, GGT, AST, INR and creatinine will also be measured at day 30 and month 6, 12 and 24 following transplantation. A mixed model for repeated measurement will be used at 24 months to compare each of these measures across the two groups.

3.2.2.4 Early allograft dysfunction

Early allograft dysfunction (EAD) has been defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function [79]:

a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant

b. INR >1.6 on day 7 post-transplant.

c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant

In case of missing data in any of the three variables required to define the EAD, this will be considered as present if any of the available values satisfies the specified criteria. In case of Bilirubin and/or INR missing due to the patient being discharged before Day 7 post-transplant, this will be considered as absence of EAD as the recipient would not be discharged earlier if the graft was not functioning. Subjects with missing data due to patient death or graft failure will be excluded from this analysis as expected to be few in numbers in the first 7 days post-transplant.

Presence of EAD will be compared across the two groups using the methods described above to adjust for potential confounders such as DRI and MELD Score.

3.2.2.5 Physiological response to reperfusion

Post-reperfusion syndrome is defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than a minute during the first five minutes after reperfusion:

$$\frac{MAP_{baseline} - MAP_{5mins}}{MAP_{baseline}} > 0.30 \text{ mmHg } for > 1min$$

Need for renal replacement therapy relates to the requirement of treatments for renal failure such as haemodialysis, haemofiltration and haemodiafiltration.

Differences in proportions of both post-reperfusion syndrome, need for renal replacement therapy and post-reperfusion lactate levels between groups will be assessed by performing unadjusted analysis as described above. Duration of renal replacement therapy at Day 7 will also be compared between the two groups using appropriate tests for continuous outcomes as described above.

Length of hospital stay and length of stay in HDU/ITU care will be summarised as median together with interquartile range and will be compared between the two groups using Kruskal-Wallis test.

3.2.2.6 Anastomotic and non-anastomotic strictures on MRCP

The protocol stipulated that all study participants should undergo magnetic resonance cholangiopancreatography (MRCP) with T2-weighted turbo-spin echo sequences at 6 months (range 5 – 7 months) post-transplant unless contraindicated. Three independent consultant radiologists were appointed, all of whom were experienced in the assessment of liver transplant radiology and MRCP image analysis. They were Dr Arvind Pallan (AP; Queen Elizabeth Hospital, Birmingham), Dr Sara Upponi (SU; Addenbrooke's Hospital, Cambridge) and Prof John Karani (JK; King's College Hospital, London). All scans were reviewed by two of the radiologists (AP and SU), blinded to the method of organ preservation, with disparities adjudicated by the third radiologist (JK).

Evidence of ischaemic cholangiopathy was taken as the presence of extra-anastomotic biliary structuring in the absence of hepatic artery thrombosis [72]. Due to the lack of any existing grading system for biliary strictures, an initial pilot series of 10 scans was reviewed by all three radiologists to determine the level of concordance between them in their interpretation of scans.

The findings will be reported as follows: i) normal biliary tree; ii) anastomotic stricture (>70% of luminal diameter); iii) unequivocal evidence of non-anastomotic stricture anywhere in the biliary tree; iv) both anastomotic and non-anastomotic biliary strictures.

Proportions of MRCP evidence of ischaemic cholangiopathy will be compared across treatment arms by performing unadjusted analysis.

3.2.3 Safety Analysis

Safety outcomes to be reported will be:

- Organ discard rate (number of livers not suitable for transplantation)
- Adverse event:

- Recipient infection (defined as a positive microbiological culture result)
- Biopsy-proven acute rejection episodes
- Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks)
- Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
- Reoperation rate
- Technical complications/device failures

Their severity will be graded according to the Clavien-Dindo classification [90] (see Appendix 4). Adverse events with grading ≥ IIIb will be considered Serious Adverse Events (SAE). Rates of adverse events and organ discards will be compared between NMP and SCS groups by examination of 95% confidence intervals for the difference in incidence. The number of patients with any serious adverse events will be compared by assessment of the difference in incidence with 95% confidence interval. The number of serious adverse events per patient will be reported.

3.3 Analysis of Machine Perfusion Parameters

During NMP continuous displays of pressures, flows, metabolic (pH) and synthetic (bile production) liver function were available to the operator. In addition, lactate measurements were carried out using external blood gas analysis. The trial protocol did not stipulate the manner in which these parameters should be interpreted.

Once trial recruitment was complete, an ad hoc analysis was performed in which NMP organs were categorised according to those which, following transplant, displayed minimal preservation injury (MPI; peak AST <250 IU/litre) and those with severe preservation injury (SPI; peak AST >1000 IU/litre). The AST thresholds used to define the MPI and SPI groups were arbitrary based on the trial results. To maximise the likelihood of identifying organ characteristics that could be used to discriminate between a ‘good’ and ‘bad’ liver it was necessary to investigate those livers at the extremes of

outcomes in this study. In clinical practice it is widely accepted that an AST < 250 IU/L following liver transplant is very low, although an AST > 1000 IU/L would not be considered high. This was not the case for NMP livers though where post-transplant transaminsaes of over 1000 IU/L were uncommon. The groups were assessed for comparability with regards to the following donor and recipient characteristics:

- Donor details – type (DBD/DCD), age, sex, ET-DRI
- Recipient details – age, sex, MELD
- Duration of NMP

Biochemical analysis of the perfusate was performed and compared between groups, measuring the following:

- Liver function tests – bilirubin, gamma-glutamyltransferase (GGT), alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase
- Additional biochemistry – C-reactive protein, urea, lactic acid
- Haemolysis index – this reflects the degree of haemolysed red cells measurable in the perfusate.

The following synthetic function parameters were compared between groups:

- Bile production

The following clinical outcome measures were compared between groups:

- Post-reperfusion syndrome
- HDU/ITU and hospital stay

A D'Agostino–Pearson normality test was performed to assess data distribution. Parametric data were analysed using an unpaired Student's t-test and non-parametric data were analysed using a Mann–Whitney U-test.

3.4 Bile salt analysis

From 13 consecutive NMP livers with preservation times greater than 12 hours, four hourly perfusate and bile samples were collected.

Bile was collected directly via aspiration of the silicone tube draining the bile duct (Image 13). Bile samples were collected in cryovials which were snap frozen in dry ice before later being transferred to storage at -70°C.

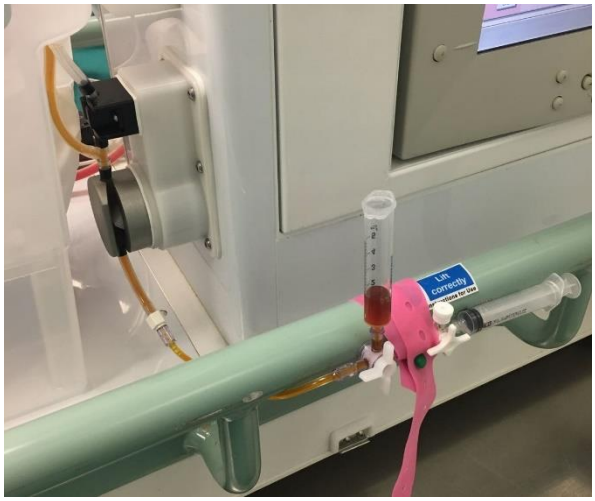


Image 13: Arrangement of biliary drainage for collection of bile samples

Perfusate samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. These were allowed to stand for 30-60 minutes after which they were centrifuged at 1500 RCF/G for 15 minutes. The supernatant was then transferred to a cryovial which was snap frozen in dry ice before later being transferred to storage at -70°C.

Analysis of bile samples was performed using electrospray ionisation-mass spectrometry (ESI-MS) to determine the concentration of endogenous produced and exogenous administered bile salts present in the bile. Results were analysed to determine the pattern of endogenous bile salt depletion and establish if bovine bile salt supplementation is necessary or effective.

For perfusate samples, ESI-MS was used to determine the concentration of endogenous produced and exogenous administered bile salts present in the perfusate. Perfusate bile salt levels were compared to established accepted physiological bile salt normal ranges [59] to determine if sufficient exogenous

bile salts are being provided to the NMP circuit. Results were analysed to determine the pattern of endogenous bile salt depletion and establish if bovine bile salt supplementation is necessary or effective.

Analysis of bile salt levels in the perfusate and bile of NMP levels was performed by personnel in the Sheffield Children's Hospital Department of Clinical Chemistry following standardised electrospray-ionisation mass spectrometry procedures. The process is described in detail in Appendix 5.

3.4.1 Bile salts results analysis

The change in abundance of each bile salt over time in the perfusate and bile was plotted in a scatter graph. The Pearson correlation coefficient was calculated to assess for a relationship between this abundance and time for each bile salt. Differences between the concentrations of each bile salt at different time points was assess using a Student t-test.

Change in the concentration of bile salts was also assessed in the context of hourly bile production with the Pearson correlation coefficient used to assess for a relationship.

4 Results

4.1 Clinical Trial

4.1.1 Personal Involvement

Prior to commencing enrolment for this study it was envisaged that we would recruit a team of several researchers between whom an on call rota would be devised in order to provide 24/7 cover for the UK trial sites. In practise this did not materialise and so, for the duration of the study, I was on call to perform randomisations and attend the NMP liver retrievals and transplants. At various intervals during the trial there were temporary arrangements in place to provide help in the form of an additional researcher or perfusionist. These people were: Dr Rubens Macedo-Arantes, Ms Annemarie Weissenbacher and Mr Chris Morris who provided cover on an ad hoc basis. For the first six months that the study was open I also had to attend all SCS transplants in order to collect data and samples as the transplant technicians had not yet been recruited.

As a consequence of the arrangements described above, I attended in excess of 100 retrieval procedures and over 90 liver transplant operations during the course of the trial. This included attending many DCD donors that did not proceed, perfusing livers that went on to be discarded or transplanted into non-consented recipients, attending several NMP livers in Leuven and also attending approximately a dozen SCS liver transplants. Through necessity, I also became proficient at performing the liver backbench procedure, something I carried out independently on over 50 occasions. I also remotely supervised all Transplant Technicians that attended the SCS liver transplants.

4.1.2 Study Participants

During the trial design, it was originally envisaged that 260 donor livers would need to be randomised to achieve the 220 transplanted livers required to power the primary endpoint. In practice it required 335 randomisations to reach this target. The reasons for this are described below.

For logistical reasons randomisation had to occur before the retrieval team reached the donor hospital with the result that more donor livers than anticipated were found to be not retrievable and so not suitable for inclusion in the trial. Only livers that were actually retrieved from the donor were included in the statistical analysis. Thus, these non-retrieved livers were excluded.

Transplant centres almost always accept a liver for a specific recipient. In the context of this trial, the recipient had to be someone that had already consented to being part of the trial. For reasons of clinical priority, the designated transplant recipient can change at very short notice, and this occurred more frequently than had been anticipated during the trial design. If the new recipient had not provided prior consent to be part of the trial then the liver was not able to be used in the statistical analysis and so was excluded from the study.

37 DCD donors did not proceed to asystole. These were excluded from the study

Between 26th June 2014 and 8th March 2016, 335 livers were randomised; 170 were allocated to NMP and 164 to the control arm. One randomisation occurred in error before R&D approval was in place; that liver was excluded. Sixty-four livers (33 in the NMP and 31 in the SCS arm) were excluded after randomisation and 48 livers (16 in the NMP and 32 in the SCS arm) discarded before transplantation, leaving 222 successfully transplanted livers. Of these, two livers (1 NMP, 1 SCS) did not have any AST values available and so could not be included in the primary outcome analysis. The flow of livers through the trial is depicted in the CONSORT diagram on the following page (Figure 17).

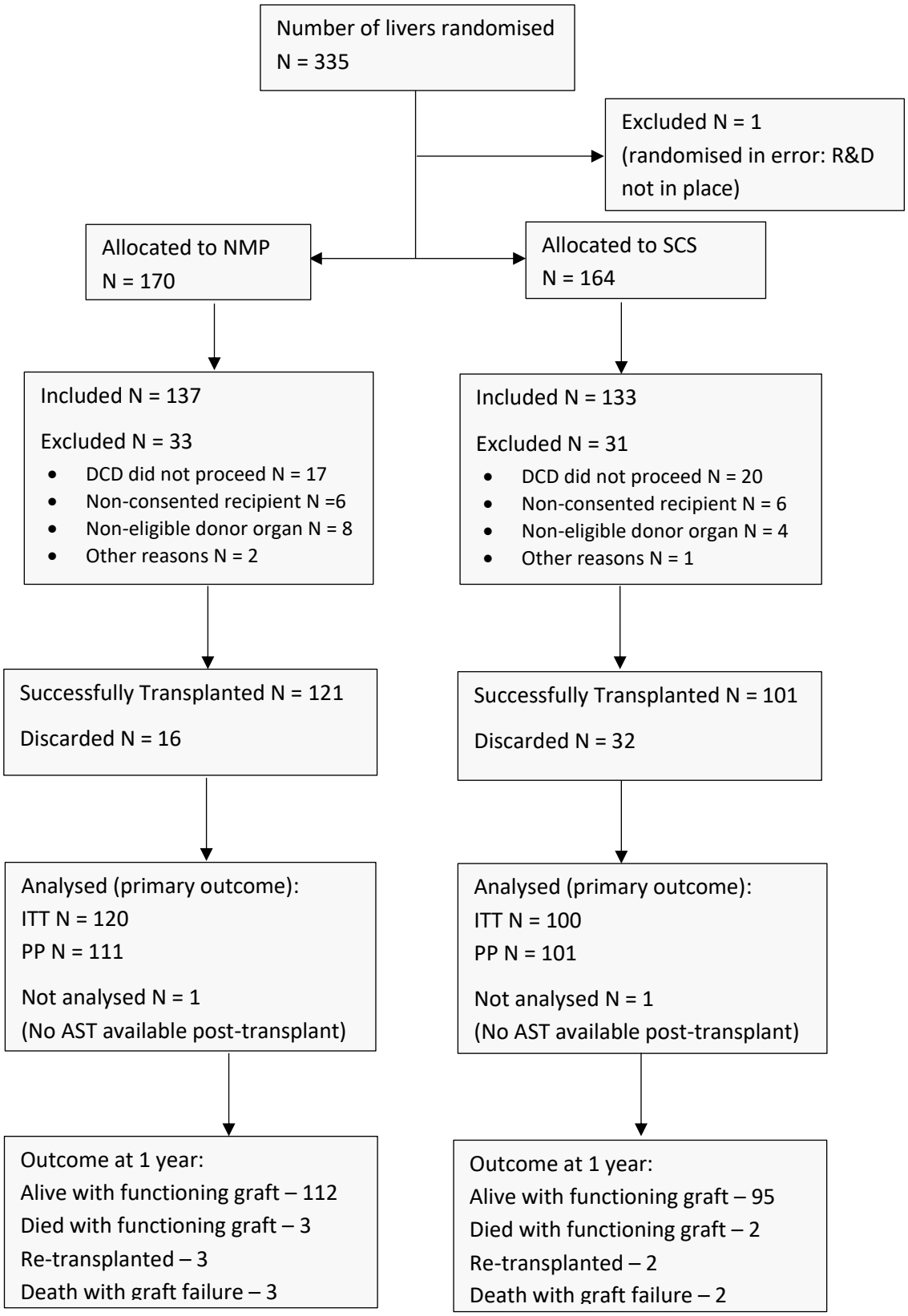


Figure 13: CONSORT diagram depicting flow of participants through the trial

4.1.3 Baseline Characteristics

Baseline characteristics are reported with different totals according to whether they refer to donors or recipients. In accordance with CONSORT guidelines, significance testing of baseline donor characteristics was not performed. However, this was an open label study and so it is feasible that the preservation and recipient characteristics, which are generated after randomisation, could have been affected by preservation method. Therefore preservation and recipient baseline characteristics were compared to check the groups remained balanced using Mann-Whitney non-parametric test for continuous variables and Chi-square test for categorical variables. Tables 7 and 8 show that the livers were similar in the two groups in terms of both donor and recipients characteristics.

Stratification factors *(all randomised livers)*	**NMP (N = 170)**	**SCS (N = 164)**
Donor type*		
DBD	107 (62.9%)	104 (63.4%)
DCD	63 (37.1%)	60 (36.6%)
Donor demographics *(retrieved livers)*	**NMP (N = 137)**	**SCS (N = 133)**
Gender*		
Female	54 (39.4%)	57 (42.9%)
Male	81 (59.1%)	76 (57.1%)
(missing)	*2 (1.5%)*	*0 (0.0%)*
Age^	56 (45, 67) (16, 84)	56 (47, 66) (20, 86)
Ethnicity*		
African-Caribbean	3 (2.2%)	1 (0.8%)
Caucasian	131 (95.6%)	128 (96.2%)
Other	1 (0.7%)	4 (3.0%)
(missing)	*2 (1.5%)*	*0 (0.0%)*
Cause of death		
CVA	74 (54.0%)	74 (55.6%)
Hypoxia	30 (21.9%)	32 (24.1%)
Trauma	17 (12.4%)	16 (12.0%)
Other	14 (10.2%)	11 (8.3%)
(missing)	*2 (1.5%)*	*0 (0.0%)*
BMI^	26.26 (23.66, 30.52) (16.42, 46.65)	27.01 (23.74, 30.56) (17.24, 49.96)
(missing)	*2 (1.5%)*	*0 (0.0%)*
UK-Donor risk index^	1.53 (1.19, 2.63) (0.78, 6.35)	1.49 (1.22, 2.44) (0.77, 4.58)
(missing)	*41 (29.9%)*	*53 (39.8%)*
ET-Donor risk index^	1.72 (1.47, 2.09) (0.98, 4.31)	1.72 (1.50, 2.10) (1.06, 3.49)
(missing)	*16 (11.7%)*	*19 (14.3%)*

*Table 7: Donor baseline characteristics. ^Median, IQR and full range reported. *Frequency and column % reported*

Preservation Details *(transplanted livers)*	**NMP (N = 121)**	**SCS (N = 101)**	
Functional warm ischaemia time[$] (minutes)[^ ‡] (Applies to DCD livers; N = 55, 34 NMP, 21 SCS)	21 (17, 25) (9, 93)	16 (10, 20) (2, 32)	
Cold ischaemia time prior to NMP (minutes)[^] (N = 120)	126 (106.5, 143) (49, 218)	-	
Machine perfusion time (minutes)[^] (N = 120)	547.5 (372.5, 710.5) (85, 1388)	-	
Total Preservation time from cross clamp in donor to organ reperfusion in recipient (minutes)[^]	714 (542, 876) (258, 1527)	465 (375, 575) (223, 967)	
Steatosis assessed pre-preservation (clinical diagnosis, not histological)*[#]			
None	36 (29.8%)	44 (43.6%)	
Mild	55 (45.5%)	45 (44.6%)	
Moderate	18 (14.9%)	10 (9.9%)	
Severe	11 (9.1%)	2 (2.0%)	
(missing)	*1 (0.8%)*		
Quality of perfusion*			
Good	106 (87.6%)	83 (82.2%)	
Moderate	11 (9.1%)	7 (6.9%)	
Poor	3 (2.5%)	1 (1.0%)	
(missing)	*1 (0.8%)*	*10 (9.9%)*	
Recipient demographics	**NMP (N = 121)**	**SCS (N = 101)**	**p-value**
Gender*			0.717
Female	35 (28.9%)	27 (26.7%)	
Male	86 (71.1%)	74 (73.3%)	
Donor type*			0.209
DBD	87 (71.9%)	80 (79.2 %)	
DCD	34 (28.1%)	21 (20.8%)	
Age^	55 (48, 62) (20, 72)	55 (48,62) (22, 70)	0.713
Cause of Liver Failure*			0.782
Alcoholic	36 (29.8%)	29 (28.7%)	
Auto-Immune Hepatitis	2 (1.7%)	5 (5.0%)	
Drug Induced	0 (0.0%)	0 (0.0%)	
Hepatitis B	3 (2.5%)	2 (2.0%)	
Hepatitis C	4 (3.3%)	4 (4.0%)	
Hepatocellular Carcinoma on background of Cirrhosis	15 (12.4%)	16 (15.8%)	
Hepatocellular Carcinoma without Cirrhosis	4 (3.3%)	2 (2.0%)	
Metabolic	1 (0.8%)	0 (0.0%)	
Non-Alcoholic Fatty Liver Disease	2 (1.7%)	3 (3.0%)	
Non-Alcoholic Steato-Hepatitis	9 (7.4%)	8 (7.9%)	
Other Cancers	1 (0.8%)	0 (0.0%)	
Primary Biliary Cirrhosis	10 (8.3%)	3 (3.0%)	
Primary Sclerosis Cholangitis	18 (14.9%)	13 (12.9%)	
Other	16 (13.2%)	16 (15.8%)	
BMI^	26.18 (23.12, 32.39) (18.02, 50.99)	26.94 (24.36, 30.42) (18.91, 42.95)	0.626
(missing)	*0 (0.0%)*	*1 (1.0%)*	
Retransplant*	12 (9.9%)	8 (7.9%)	0.605

MELD score^ (calculated at time of transplant)	13 (10, 18) (6, 35)	14 (9, 18) (6, 29)	0.970
UK	13 (10, 17) (6, 33)	14 (9, 18) (6, 28)	
Essen, Germany	17 (14, 19) (13, 23)	15.5 (14, 17) (14, 17)	
Barcelona, Spain	16 (8, 26) (8, 35)	14 (9, 16) (8, 29)	
Leuven, Belgium	19 (13.5, 25) (13, 26)	16 (16, 20) (9, 27)	
eGFR^	87.36 (69.61, 107.66) (33.45, 156.43)	92.22 (69.72, 104.24) (30.19, 155.04)	0.928
(missing)	*4 (3.3%)*	*3 (3.0%)*	
ET-Donor risk index^	1.70 (1.47, 2.07) (0.98, 4.31)	1.71 (1.50, 2.01) (1.06 3.49)	0.610
(missing)	*13 (10.7%)*	*13 (12.9%)*	

*Effect reported is: geometric mean ratio [% reduction] for Peak AST; odds ratio for EAD; difference in proportions (%) for Post Reperfusion Syndrome and Need for RRT; not reported for outcomes where medians are reported and for survivals.

$ Functional warm ischaemia applies to DCD donors and is measured from the onset of functional warm ischaemia (systolic BP < 50mmHg or O_2 saturations < 70%) to cross clamp

#Measurement of the degree of steatosis was based on clinical assessment by the retrieval surgeon

^Test not performed due to few events and no events in one arm.

‡Median and IQR reported, non-parametric test used.

Table 8: Preservation and recipient characteristics

4.1.4 Numbers Analysed

The primary outcome was planned as an intention-to-treat analysis with livers remaining in their assigned randomisation arm for analysis regardless of which method of preservation was used. Of the 222 transplanted livers, two (one NMP, one SCS) did not have any AST values available and so could not be used for primary outcome reporting. The remaining 220 livers consisted of 120 NMP and 100 SCS. This discrepancy was the result of a significant difference in the number of discarded livers in each trial arm and reduced the study power from 90% to 89.7%.

A separate per-protocol analysis was also performed. Eight livers were considered protocol violators as they were perfused on the machine for less than 4 hours (the NMP duration stipulated in the protocol was 4-24 hours). One crossover (from NMP to SCS arm) occurred due to an accessory left hepatic artery arising from the aorta preventing effective cannulation. This left 214 livers for the per-protocol analyses (112 and 102 in the SCS and NMP arms, respectively) and 212 livers specifically for the per-protocol primary outcome analysis (111 and 101 in the SCS and NMP arms, respectively).

4.1.5 Exclusions

Livers were excluded after randomisation if they were later found to be ineligible for the trial. The reasons are listed in Table 9:

Reason	NMP	SCS	Total
DCD did not proceed	17	20	37
Non-consented recipient	6	6	12
Non-eligible donor organ	8	4	12
Other	2	1	3
Total	**33**	**31**	**64**

Table 9: Reasons for liver exclusion from the trial

Other reasons include:

- DCD donor arrested before retrieval team arrived (n=1)
- DCD donor became DBD donor therefore re-randomised (n=1)
- Randomised in error as NMP logistically not possible due to staff absence (n=1)

4.1.6 Discarded Livers

Discarded livers are those which were retrieved but were deemed by the transplanting surgeon to not be usable and so were discarded. These are reported below followed by the reasons for discards.

Discarded	NMP	SCS	Total
No	121 (88.3%)	101 (75.9%)	222 (82.2%)
Yes	16 (11.7%)	32 (24.1%)	48 (17.8%)
Total	137	133	270
Difference = -12.4% (95% C.I. -21.4%, -3.3%) p = 0.008			

Table 10: Discarded livers by treatment arm

Reason	NMP	SCS	Total
Steatosis	13	24	37
Prolonged warm ischaemia time	2	6	8
Poor NMP perfusion parameters	5	0	5
Device user error	4	0	4
Donor problem e.g. malignancy	2	2	4
Abnormal lesion	0	3	3
Poor in-situ perfusion	1	2	3
Fibrosis	1	1	2
Liver too large for recipient	1	1	2
Capsular damage	1	1	2
Device error	1	0	1
Injury to hepatic artery	0	1	1
Parenchymal damage	1	1	1

Table 11: Reasons for organ discard. Note that some livers were declined for more than one reason

The null hypothesis that there was no difference in discard rate between the two groups was tested and there was strong evidence that this could be rejected: difference in discard rates was -12.4% (95% C.I. -21.4% to -3.3%), p=0.008.

A detailed description for the causes of discard of NMP livers is available in Appendix 6.

4.2 Primary Outcome

4.2.1 Data completion

There was a very high data completeness overall of over 98%. Some AST values were missing in the first 7 days post-transplant particularly in centres that do not collect this routinely i.e. use ALT for standard biochemical liver function tests. The primary outcome was assessed provided at least two AST values were available (i.e. AST values available for at least two days post-transplant). No missing data were imputed. The number of AST values available by treatment arm is shown in Figure 20 below:

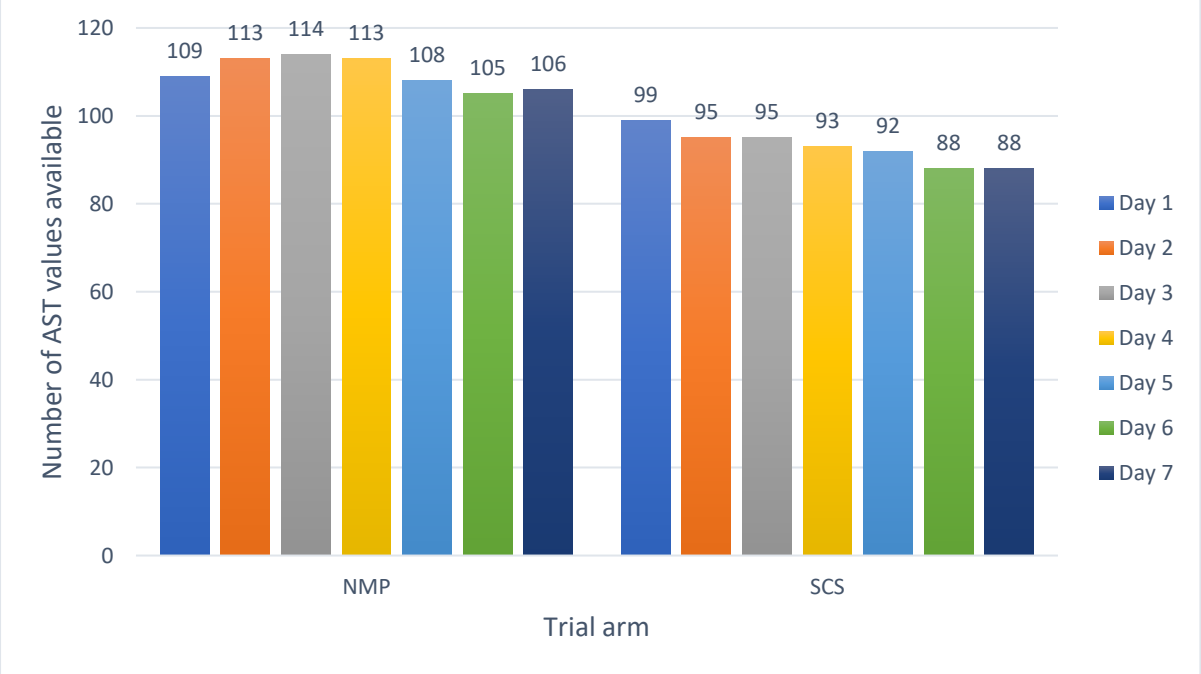


Figure 14: Number of trial participants for whom AST values were available on each of the first 7 post-operative days for each trial arm. Please not that in the NMP arm the denominator for each value is 121; In the SCS arm the denominator is 101.

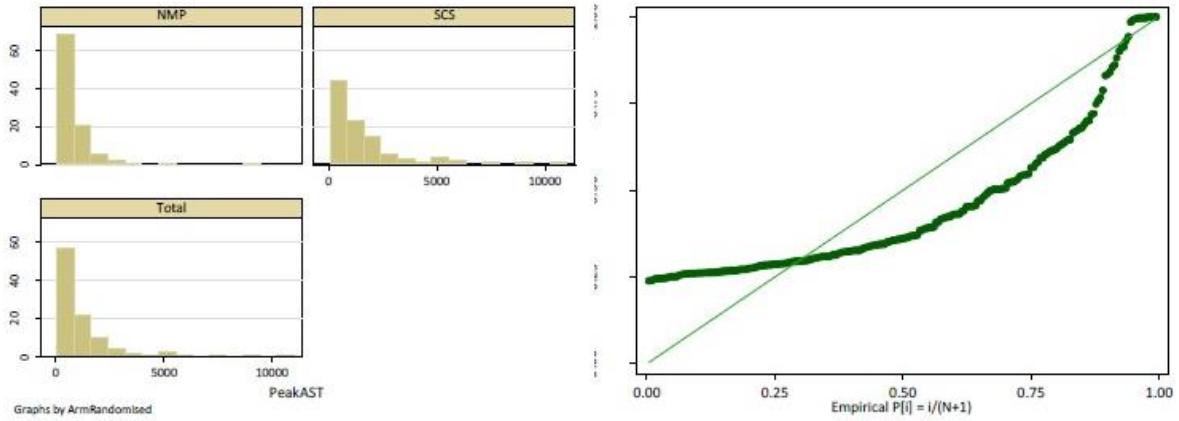
4.2.2 Assessment of AST distribution

As expected from previous reports [94], in all centres the majority of peak AST values occurred in the first 48 hours post-transplant (Table 12).

Day of Peak AST	NMP	SCS	Total
1	97 (80.8%)	93 (93.0%)	190 (86.4%)
2	13 (10.8%)	4 (4.0%)	17 (7.7%)
3	4 (3.3%)	1 (1.0%)	5 (2.3%)
4	0 (0.0%)	1 (1.0%)	1 (0.5%)
5	1 (0.8%)	1 (1.0%)	2 (0.9%)
6	2 (1.7%)	0 (0.0%)	2 (0.9%)
7	2 (1.7%)	0 (0.0%)	3 (1.4%)
Total	120	100	220

Table 12: Post-operative day of occurrence of peak AST in each trial arm

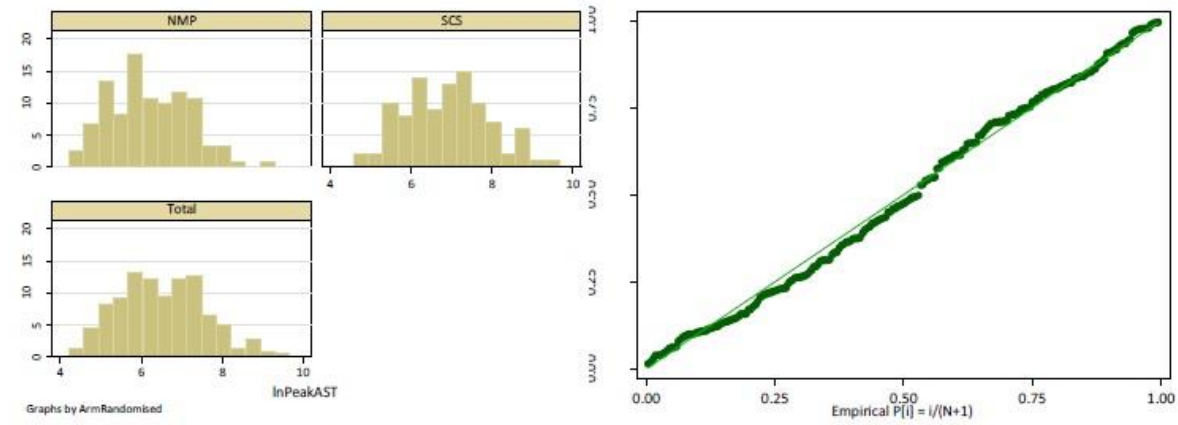
The D'Agostino-Pearson test was used to confirm that the peak AST values were not normally distributed. A log conversion of these values resulted in a normal distribution being achieved. For peak AST all statistical tests were applied to these values to assess for differences in the geometric mean.



Treatment group	Obs	W'	V'	z	p-value
NMP	120	0.57944	40.470	8.291	0.00000
SCS	100	0.70649	24.233	7.072	0.00000

Figure 15: Assessment for Normality of distribution of peak AST

The p-values of the test above (Figure 19) indicates there is enough evidence to reject the null hypothesis that the peak AST follows a Normal distribution.



Treatment group	Obs	W'	V'	z	p-value
NMP	120	0.98494	1.450	0.832	0.20274
SCS	100	0.98554	1.194	0.393	0.34718

Figure 16: Assessment for Normality of distribution of (ln) peak AST

The p-value of the test is now not statistically significant (Figure 20) suggesting the log-transformation of the Peak AST is normally distributed.

4.2.3 Primary outcome results

The natural logarithm of Peak AST was analysed using Analysis of Variance (ANOVA) adjusting for the following stratification factors: centre and donor type. Results from the ANOVA model are shown in the following table.

	NMP	**SCS**	**Difference / Mean ratio [% reduction]**
Mean ln Peak AST (95% C.I.)	6.183 (6.007, 6.359)	6.881 (6.679, 7.084)	-0.698 (-0.963, 0.433)
Geometric mean peak AST (95% C.I.)	484.5 (406.4, 577.6)	973.7 (795.2, 1192.3)	0.498 (0.382, 0.649) [50.2% (35.1%, 61.8%)]

Table 13: Primary outcome results from the adjusted analysis (ANOVA model)

These findings were confirmed in the unadjusted analysis from the t-test. The reduction in peak AST between the NMP and the SCS group was 50.2% (95% C.I. 35.1% to 61.9%, p<0.001).

4.2.4 Subgroup analysis by donor type, ET-DRI and MELD score

The subgroup analysis was performed by including the interaction between the treatment and donor type in the ANOVA model. This interaction proved to be statistically significant (p-value=0.012), suggesting that the treatment outcome effect was significantly affected by donor type.

The size of the treatment effect differs in the two donor type subgroups, but is statistically different for both groups being larger in the DCD livers: the reduction in the peak AST between the NMP and the SCS group was 40.2% (95% C.I. 19.3% to 55.7%, p=0.001) in DBD livers and 73.3% (95% C.I. 53.7% to 84.6%, p<0.001) in DCD livers.

Donor Type	Obs	NMP	SCS	Effect (geometric mean ratio)	[95% C.I.]	p-value
DBD	167	526.2 (427.3, 647.9)	880.2 (708.5, 1093.5)	0.598	[0.443, 0.807]	0.001
DCD	53	389.7 (278.0, 546.4)	1458.1 (944.7, 2250.5)	0.267	0.154, 0.463]	<0.001

Table 14: Treatment effect on Peak AST for donor type subgroups

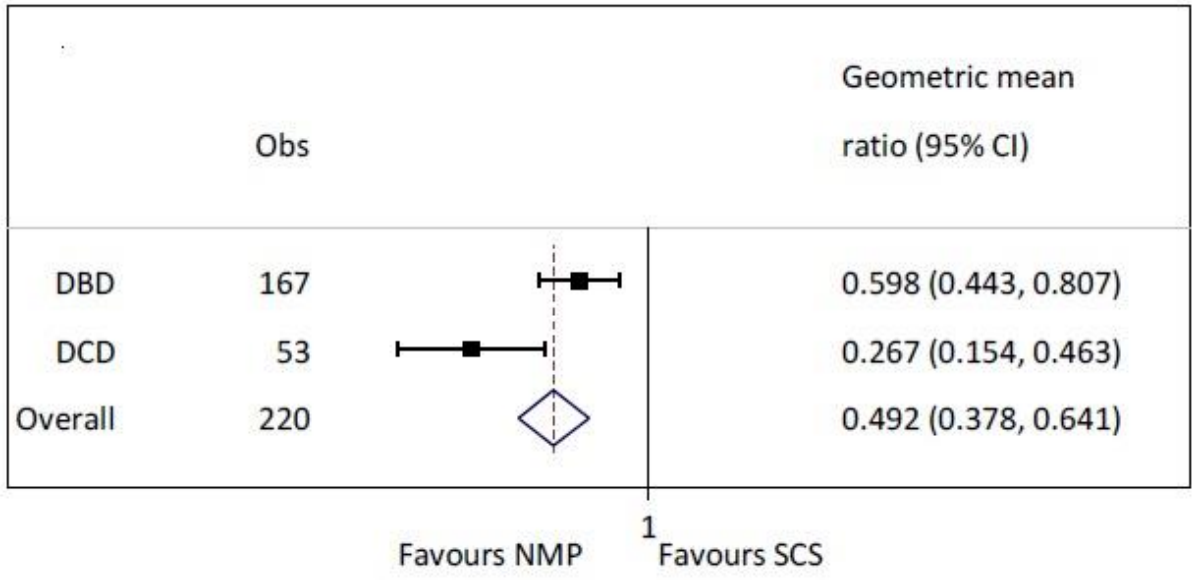


Figure 17:Forest plot for subgroup analysis of Peak AST by donor type

Subgroup analyses were also performed for the MELD Score and Eurotransplant Donor Risk Index (ET-DRI). When tested as interactions there were no statistically significant differences within these subgroups (Treatment*ET-DRI p=0.217; treatment*MELD score p=0.991).

4.2.5 Sensitivity analysis – per-protocol population

The primary analysis for the log peak AST was repeated in the per-protocol population (refer to Numbers Analysed section). In this analysis the livers were analysed according to the treatment actually received and to the centre where they were eventually transplanted rather than those that randomised them (note some livers were rejected for the original recipient but transplanted into other

consented recipients, at a different trial centre). The treatment effect remained significant in the per-protocol population and the reduction only changed slightly (reduction 49.3% (95% CI 33.7% to 61.2%)) as show in the table below.

	NMP	**SCS**	**Difference / Mean ratio [% reduction]**
Mean ln Peak AST (95% C.I.)	6.212 (6.028, 6.396)	6.891 (6.698, 7.084)	-0.679 (-0.947, -0.410)
Geometric mean peak AST (95% C.I.)	498.6 (414.8, 599.4)	982.9 (810.4, 1192.2)	0.507 (0.388, 0.663) [49.3% (33.7%, 61.2%)]

Table 15: Primary outcome results from the adjusted analysis (ANOVA model) – Per Protocol population

4.3 Preservation Outcomes

Important preservation outcomes are shown in Table 16 below:

Preservation Details *(transplanted livers)*	**NMP (N = 121)**	**SCS (N = 101)**	**p-value**
Functional warm ischaemia time[$] (minutes)[^ ‡] (Applies to DCD livers; N = 55, 34 NMP, 21 SCS)	21 (17, 25) (9, 93)	16 (10, 20) (2, 32)	0.003
Cold ischaemia time prior to NMP (minutes)[^] (N = 120)	126 (106.5, 143) (49, 218)	-	
Machine perfusion time (minutes)[^] (N = 120)	547.5 (372.5, 710.5) (85, 1388)	-	
Total Preservation time from cross clamp in donor to organ reperfusion in recipient (minutes)[^]	714 (542, 876) (258, 1527)	465 (375, 575) (223, 967)	0.0000
Steatosis assessed pre-preservation[*#]			0.019
None	36 (29.8%)	44 (43.6%)	
Mild	55 (45.5%)	45 (44.6%)	
Moderate	18 (14.9%)	10 (9.9%)	
Severe	11 (9.1%)	2 (2.0%)	
(missing)	*1 (0.8%)*		

Table 16: Preservation details

Functional warm ischaemia time applies only to DCD organs and was significantly longer for NMP livers compared with SCS (21 mins vs 16 mins; p=0.003).

In the NMP group, the cold ischaemia time prior to commencing machine perfusion was 126 mins. This was comprised of the liver explant time (approximately 45 mins) and the back-table preparation and cannulation of the organ.

Total preservation time was markedly longer in the NMP group (NMP 714 mins vs SCS 465 mins; p=0.000).

A larger proportion of the organs that went on to be transplanted in the NMP group were clinically assessed by the retrieval surgeon to be either moderately or severely steatotic (NMP 24% vs SCS 11.9%; p=0.019).

4.3.1 Post-reperfusion characteristics

Post-reperfusion syndrome is defined as a >30% fall in mean arterial pressure following reperfusion which lasts at least one minute and occurs within five minutes of reperfusion. Post-reperfusion haemodynamics were documented in 218 cases: post-reperfusion syndrome was more common in the SCS (32 out of 97) than the NMP group (15 out of 121), a statistically significant difference (–20.6%, 95% confidence interval –31.6 to –9.5%; *P* < 0.001).

Post-reperfusion syndrome	**NMP (n=121)**	**SCS (n=101)**
No	106 (87.6%)	65 (67.0%)
Yes	15 (12.4%)	32 (33.0%)
Total	121	97
Difference = -20.6% (95% C.I. -31.6%, -9.5%) p = 0.000		

Table 17: Incidence of post reperfusion syndrome in NMP and SCS liver transplants

The difference in the incidence of post-reperfusion syndrome was despite reduced requirements for vasopressors in NMP livers in the post-reperfusion period, shown in the table below:

	NMP (n=121)	**SCS (n=101)**
Pre-reperfusion vasopressor infusion *missing*	92 (76.0%) *2 (1.7%)*	82 (81.2%) *6 (5.9%)*
Post-reperfusion bolus *(missing)*	41 (33.9%) *4 (3.3%)*	60 (59.4%) *9 (8.9%)*
Post-reperfusion vasopressor infusion *(missing)*	65 (53.7%) *1 (0.8%)*	80 (79.2%) *9 (8.9%)*

Table 18: Post-reperfusion vasopressor usage

Post reperfusion lactate levels (in the transplant recipient) were compared between groups using non-parametric test due to the non-normal distribution of the variable. This comparison was significant (p=0.018). The collection of this data started after 81 livers had been randomised (in March 2015). As a result there are a large number of missing values.

	NMP (n=81)	SCS (n=51)	p-value
Median (IQR)	3.6 (2.6, 4.2)	4.1 (3.2, 5.0)	0.018

Table 19: Post-reperfusion lactate levels measured in the transplant recipient at 15-30mins following reperfusion

4.4 Biochemical outcomes

4.4.1 Early allograft dysfunction

The presence of EAD was more common in the SCS arm than in the NMP arm and the odds ratio is statistically significant (OR=0.263 (95% C.I. 0.126, 0.550); p<0.001) showing those in the NMP arm were approximately 74% less likely to develop EAD than those in the SCS group.

EAD	NMP	SCS	Total
No	107 (89.9%)	68 (70.1%)	175 (81%)
Yes	12 (10.1%)	29 (29.9%)	41 (19.0%)
Total	119	97	216
Difference = -19.8% (95% C.I. -30.4%, -9.2%)			p-value = 0.000
Odds Ratio = 0.263 (95% C.I. 0.126, 0.550)			p-value = 0.000

Table 20: Early allograft dysfunction by treatment group

An adjusted analysis was performed using logistic regression adjusting for Donor type, MELD Score and ET-DRI. The adjusted odds of EAD in the NMP arm are about 72% less than in the cold storage arm (OR=0.276 (95% C.I. 0.124, 0.611); p-value=0.002).

4.4.2 Biochemical liver function

Liver function was assessed by measurement of different biochemical tests in the first 7 days post-transplant. These were compared between treatment groups by means of area under the curve (AUC) and their average value over day 1-7.

There was a significant difference between groups only for the AUC of the Bilirubin (p=0.022) and the AST (p<0.001), as shown from the table below. Median and IQR are reported as well as the values available in each group.

Biochemical test (AUC)	NMP	SCS	p-value
Bilirubin ***n***	12.72 (7.10, 25.15) *119*	17.25 (9.25, 30.79) *101*	0.022
AST ***n***	854 (514.5, 1651) *112*	1649 (801, 2961.5) *99*	0.000
GGT ***n***	1615 (914.5, 2308) *93*	1785 (1016, 2605.5) *81*	0.260
INR ***n***	7.3 (6.65, 8.02) *118*	7.26 (6.66, 8.3) *100*	0.604
Creatinine ***n***	5.75 (3.94, 8.40) *119*	6.44 (4.47, 9.9) *101*	0.155

Table 21: Results for area under the curve (AUC) of biochemical tests by treatment group

The average value of different biochemical measurements over the 7 days was calculated for each patient irrespective of any missing value/measurement. The Mann-Whitney test was then used to compare them between treatment arms as the mean measurement was not normally distributed.

As above, the only statistically significant differences over the first seven days post-transplant were observed for the Bilirubin ($p=0.029$) and the AST ($p<0.001$). No significant differences were observed at 30 days and 6 months apart from Creatinine levels at 30 days ($p=0.019$). These results are shown in Appendix 7.

4.4.3 Length of HDU/ITU stay and hospital stay

The length of hospital stay and ITU/HDU stay were compared between groups using non-parametric test. There is not enough evidence to reject the hypothesis the length of stay is the same in the two groups ($p=0.926$ for length of hospital stay; $p=0.339$ for length of ITU/HDU stay).

	NMP (N=121)	SCS (N=101)	p-value
Length of hospital stay	15 (10, 24)	15 (11, 24)	0.926
Length of HDU/ITU stay	4 (2, 7)	4 (3, 7)	0.339

Table 22: Length of HDU/ITU stay and hospital stay. Median and (inter-quartile range)

4.5 Graft and patient survival

Ten patient deaths occurred during the 1 year follow-up showing a survival of 0.949 (95% C.I 0.890 to 0.977) in the NMP group and of 0.958 (95% C.I 0.902 to 0.982) in the SCS group.

Eight deaths occurred during the 6 month follow-up showing a survival of 0.958 (95% C.I 0.902 to 0.982) in the NMP group and of 0.970 (95% C.I 0.909 to 0.990) in the SCS group.

At the 30 days follow-up 3 deaths occurred, all in the NMP group showing a survival of 0.975% (95% C.I 0.925 to 0.992). Since no deaths occurred in the SCS group in the first 30 days post-transplant, the survival rate is shown as 1.000 and no 95% C.I. is calculated.

There were in total 10 graft failures, all occurring in the 6 months post-transplant. The graft survival at 6 months is 0.950 (95% C.I 0.893 to 0.977) in the NMP group and 0.960 (95% C.I 0.897 to 0.985) in the SCS group. No further graft failure occurred after the 6 months follow-up, showing graft 1 year survival rates similar to the 6 months ones.

At the 30 days follow-up 7 graft failures occurred, 5 in the NMP group and 2 in the SCS group showing a graft survival rate of 0.959 (95% C.I 0.904 to 0.983) and 0.980 (95% C.I 0.923 to 0.995), respectively.

There was no evidence of a significant difference in patient and graft survivals between the two treatment groups (log rank test p=0.901 and p=0.695, respectively)

Cox proportional hazards regression models were not performed due to the very few events observed for both survival outcomes.

Treatment group	Deaths at 30 days	Deaths at 6 months	Deaths at 1 year	Deaths overall
NMP	3	5	6	6
SCS	0	3	4	5
Total	3	8	10	11

Table 23: Recipient deaths by treatment group

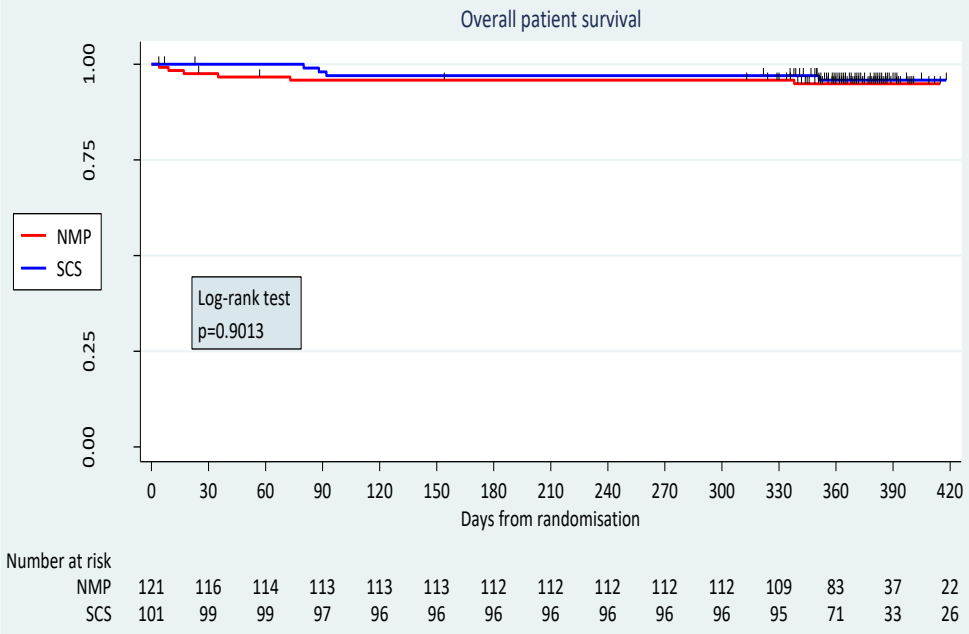


Figure 18: Kaplan-Meier plot for patient survival with log-rank test

Treatment group	Graft failures at 30 days	Graft failures at 1 year
NMP (n=121)	5	6
SCS (n=101)	2	4
Total	7	10

Table 24: Graft failure at 1 year by treatment group

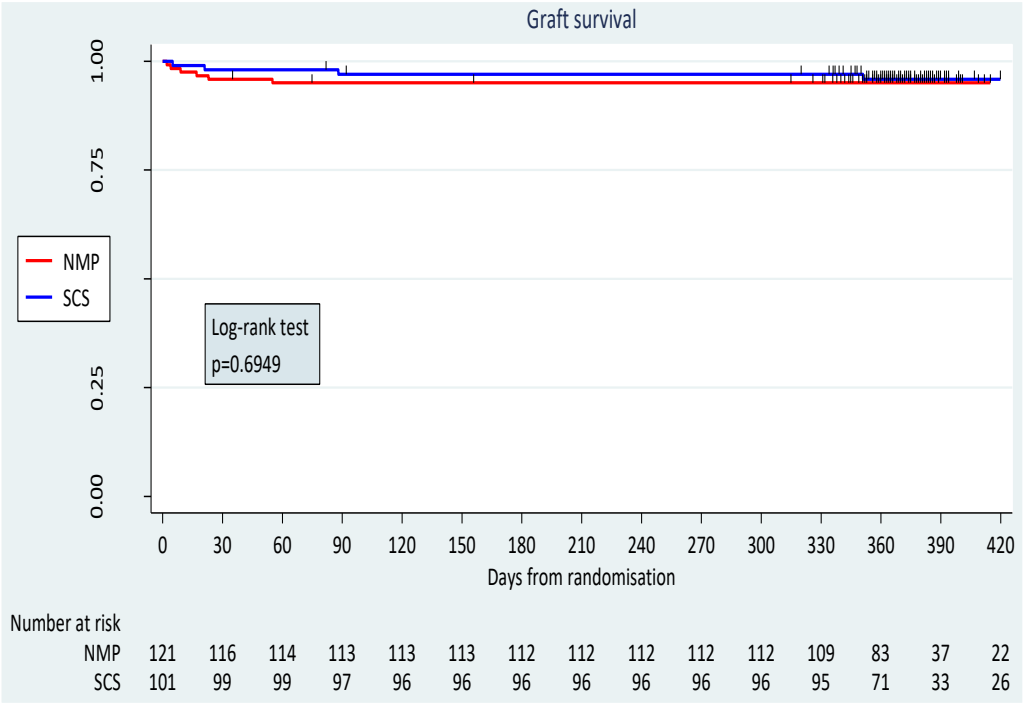


Figure 19: Kaplan-Meier plot for graft survival and log-rank test

4.6 Safety Outcomes

All reported adverse events were reviewed by two independent clinicians blinded to treatment group. After removal of duplicates, the seriousness of the events was reviewed to confirm the appropriate Clavien-Dindo grading had been allocated (see Appendix 4 for grading). Any event graded IIIb or above was considered a serious adverse event (SAE). No statistical tests were applied to these data.

The proportion of patients for whom adverse events were reported was similar in the two arms (55.4% NMP, 95% confidence interval 46.1–64.4% versus 57.4% SCS, 95% confidence interval, 47.2–67.2%) with a larger total number of events reported for SCS livers (128 NMP versus 164 SCS). Of these, a greater proportion of the serious adverse events (Clavien–Dindo grade ≥IIIb) were in the SCS than NMP arm (16.4% NMP (21 out of 128) versus 22% SCS (36 out of 164)). No statistical tests were applied to these data.

Patients with	**NMP (N=121)**	**SCS (N=101)**	**Total**
No events reported	54 (44.6%)	43 (42.6%)	97 (43.7%)
Adverse events (95% C.I.)	67 (55.4%) (46.1%, 64.4%)	58 (57.4%) (47.2%, 67.2%)	125 (56.3%)

Table 25: Number of patients for whom adverse events were reported by treatment group

Clavien-Dindo grading	**NMP**	**SCS**	**Total**
I	15 (11.7%)	30 (18.3%)	45 (15.4%)
II	64 (50.0%)	72 (43.9%)	136 (46.6%)
IIIa	28 (21.9%)	26 (15.9%)	54 (18.5%)
IIIb	8 (6.3%)	9 (5.5%)	17 (5.8%)
IVa	5 (3.9%)	15 (9.2%)	20 (6.9%)
IVb	3 (2.3%)	9 (5.5%)	12 (4.1%)
V	5 (3.9%)	3 (1.8%)	8 (2.7%)
Total	128	164	292

Table 26: Clavien-Dindo grading of adverse events by treatment group

Classification	NMP	SCS	Total
AE	107 (83.6%)	128 (78.1%)	235 (80.5%)
SAE	21 (16.4%)	36 (22.0%)	57 (19.5%)
Total	128	164	292

Table 27: Number of adverse events and serious adverse events reported by treatment group

Event Category	NMP	SCS	Total
Infection	25 (19.5%)	17 (10.4%)	42 (14.4%)
Chest	1	1	2
Blood	10	3	13
Biliary	6	0	6
Abdominal	2	3	5
Gastrointestinal	4	5	9
Other	2	5	7
Hepatic	44 (34.4%)	48 (29.3%)	92 (31.5%)
Bile leak	2	1	3
Biliary stricture (anastomotic)	9	11	20
Ischaemic cholangiopathy	1	3	4
Biliary other	1	0	1
Drainage of ascites	0	1	1
Hepatic artery aneurysm	0	1	1
Hepatic artery thrombosis	2	4	6
Hepatic artery stenosis	5	3	8
Hepatic artery other	0	2	2
Hepatic vein thrombosis	1	0	1
Portal vein thrombosis	2	0	2
Portal vein stenosis	2	0	2
Portal vein other	1	0	1
Graft dysfunction	3	2	5
Rejection	12	13	25
Other	3	7	10
Cardiovascular	5 (3.9%)	5 (3.1%)	10 (3.4%)
Congestive heart failure	1	0	1
Myocardial infarction	2	3	5
Other	2	2	4
Dermatologic	1 (0.8%)	0 (0.0%)	1 (0.3%)
Seroma	1	0	1
Gastrointestinal	5 (3.9%)	6 (3.7%)	11 (3.8%)
Colitis	0	1	1
Diarrhea	3	2	5
Other	2	3	5
Genitourinary	8 (6.3%)	17 (10.4%)	25 (8.6%)
Renal insufficiency	6	13	19
UTI	2	3	5
Other	0	1	1

Respiratory	4 (3.1%)	9 (5.5%)	13 (4.5%)
Cold/flu	0	1	1
Pneumonia	4	6	10
Shortness of breath	0	1	1
Other	0	1	1
Bleeding complications	9 (7.0%)	6 (3.7%)	15 (5.1%)
Bleeding – no transfusion required	0	2	2
Haemorrhage (Bleeding requiring transfusion)	3	0	3
Bleeding from hepatic artery	1	1	2
Bleeding from liver parenchyma	2	0	2
Other	3	3	6
Fluid Collection	7 (5.5%)	18 (11.0%)	25 (8.6%)
Abdominal	5	10	15
Pleural	2	7	9
Other	0	1	1
Device error	1 (0.8%)	-	1 (0.3%)
Device user error	2 (1.6%)	-	2 (0.7%)
Other systemic diseases	17 (13.3%)	38 (23.2%)	55 (18.8%)
Total	128	164	292

Table 28: Full breakdown of adverse events by treatment arm

4.7 Anastomotic and non-anastomotic biliary strictures on MRCP

An MRCP was performed on 155 (81 NMP, 74 SCS) of the 222 transplanted trial patients. There was consensus between AP and SU on the interpretation of 138 of these scans. The remaining 17 were re-reviewed by JK to reach a majority decision for all.

There was no significant difference in the rate of non-anastomotic strictures for DBD (NMP 7.4% (4 out of 54) versus SCS 5.4% (3 out of 55); *P* = 0.678) or DCD (NMP 11.1% (3 out of 27) versus SCS 26.3% (5 out of 19); *P* = 0.180) livers. Only one patient in each trial arm developed clinically relevant evidence of ischaemic cholangiopathy in the first year after transplant, both of whom were re-transplanted.

Similarly, there was no significant difference in the rate of anastomotic strictures for DBD (NMP 40.7% (22 out of 54) versus SCS 41.8% (23 out of 55); *P* = 0.909) or DCD (NMP 48.1% (13 out of 27) versus SCS 57.9% (11 out of 19); *P* = 0.515) livers.

Radiological Finding	NMP (n=27)	SCS (n=19)
No strictures	13	6
Anastomotic stricture	11	8
Non-anastomotic stricture	1	2
Both	2	3

Table 29: Number and type of strictures reported for DCD livers by treatment arm

Radiological Findings	NMP (n=54)	SCS (n=55)
No strictures	28	30
Anastomotic stricture	22	22
Non-anastomotic stricture	4	2
Both	0	1

Table 30: Number and type of strictures reported for DBD livers by treatment arm

4.8 Machine perfusion parameters indicative of organ quality

The following continuously monitored parameters (mean ± s.d.) by the third hour of NMP were measured for all livers that went on to be successfully transplanted. The measured haemodynamic parameters were: hepatic artery flow (280 ± 120 ml/min) and portal vein flow (1.11 ± 0.2 l/min). The measured metabolic parameters were: pH (7.31 ± 0.17) and lactate clearance from 9.99 ± 3.13 mmol/l at 15 min NMP to 0.93 ± 0.63 mmol/l by 4 h NMP. The measured synthetic parameter consisted of bile production (9.17 ± 11.16 ml/h). Notably, 18 transplanted NMP livers produced no/minimal bile during perfusion. All but one of these functioned after transplant. There was no correlation between bile production and post-transplant liver function or later development of non-anastomotic biliary strictures.

On the following pages are shown the mean hepatic artery and portal vein flows as well as mean pH and bile production for all transplanted NMP livers at one hour, two hours, three hours and the end of perfusion. The final scatter graph depicts the changes in lacatate levels with time for all transplanted NMP livers.

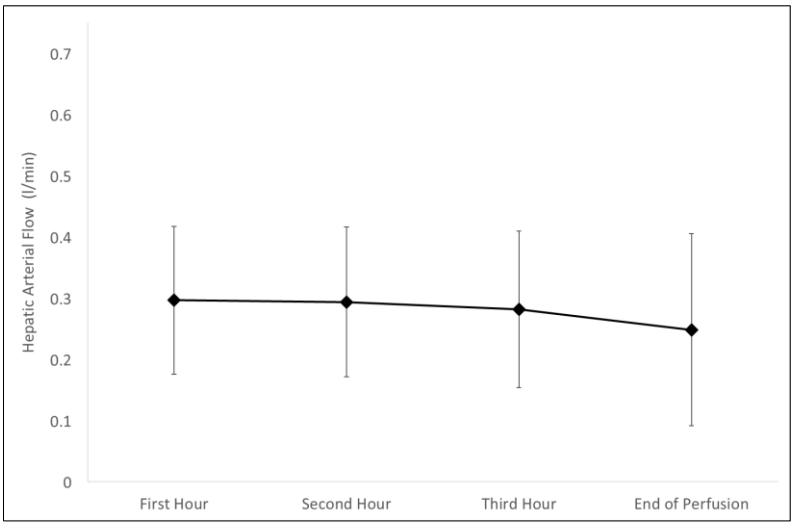


Figure 20: Hepatic artery flow during perfusion for all successfully transplanted NMP livers

Time Point	Mean flow (litres/min)	Standard Deviation
First hour	0.296	0.121
Second hour	0.294	0.122
Third hour	0.282	0.128
End of perfusion	0.248	0.157

Table 31: Hepatic artery flow during perfusion for all successfully transplanted NMP livers

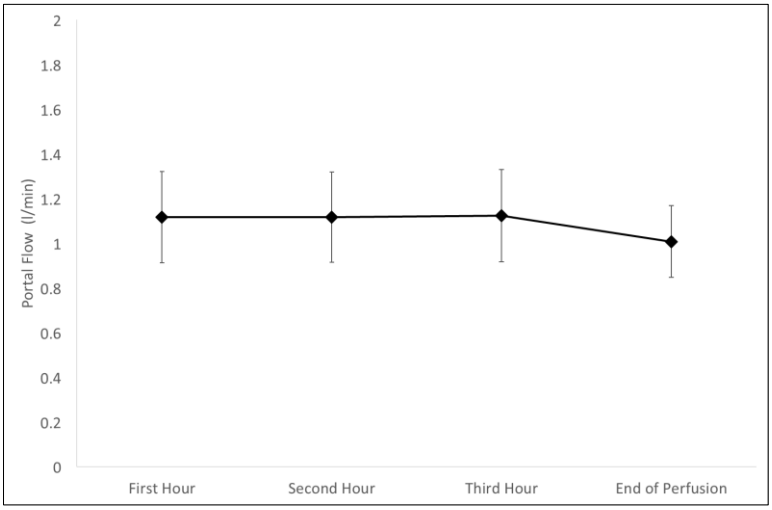


Figure 21: Portal vein flow during perfusion for all successfully transplanted NMP livers

Time Point	Mean flow (litres/min)	Standard Deviation
First hour	1.118	0.205
Second hour	1.118	0.202
Third hour	1.124	0.206
End of perfusion	1.008	0.160

Table 32: Portal vein flow during perfusion for all successfully transplanted NMP livers

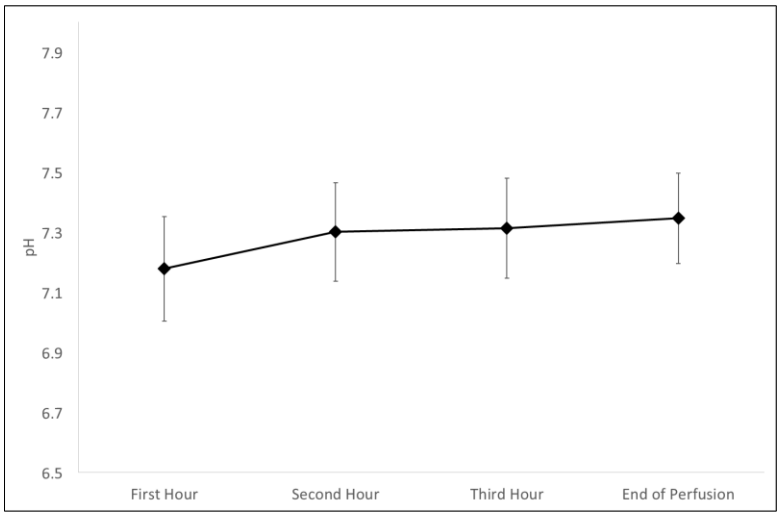


Figure 22: pH during perfusion for all successfully transplanted NMP livers

Time Point	Mean pH	Standard Deviation
First hour	7.178	0.174
Second hour	7.301	0.165
Third hour	7.313	0.166
End of perfusion	7.347	0.150

Table 33: pH during perfusion for all successfully transplanted NMP livers

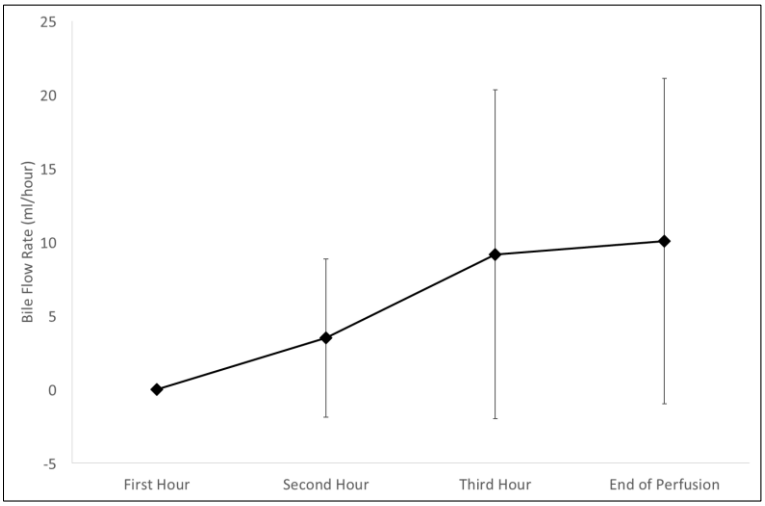


Figure 23: Bile flow during perfusion for all successfully transplanted NMP livers

Time Point	Mean flow (ml/hour)	Standard Deviation
First hour	0	0
Second hour	3.486	5.358
Third hour	9.166	11.160
End of perfusion	10.050	11.036

Table 34: Bile flow during perfusion for all successfully transplanted NMP livers

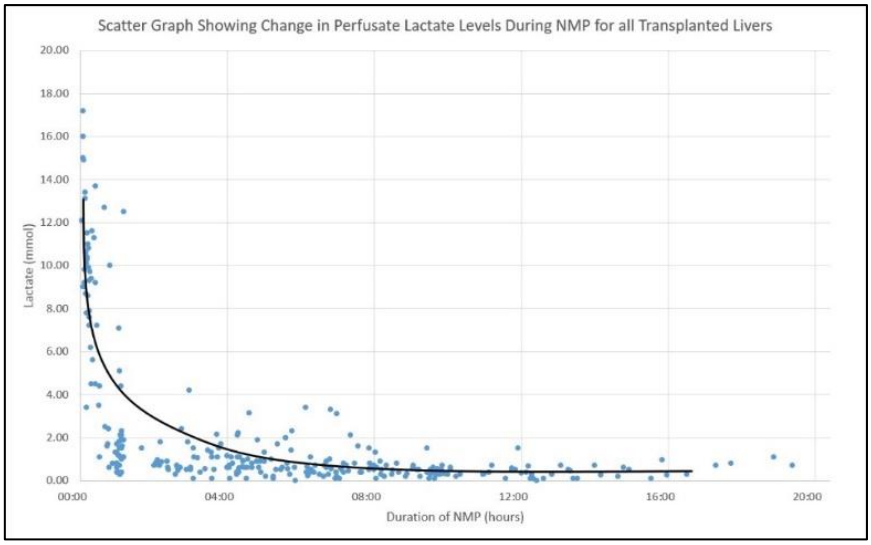


Figure 24: Change in perfusate lactate during NMP for all transplanted livers

One NMP liver developed PNF. This liver was persistently acidotic with lactate > 4 mmol for the duration of NMP. No other liver with these characteristics was transplanted.

Following transplant, 28 livers displayed minimal preservation injury (MPI; peak AST < 250 IU/l) and, at the other extreme, 25 showed evidence of severe preservation injury (SPI; peak AST > 1,000 IU/l). The donors in these groups were well-matched in all characteristics other than sex (Table 35).

	Minimal preservation injury (peak-AST<250 IU/L; n = 28)	**Significant preservation Injury (peak-AST>1000 IU/L; n = 25)**	**p-value**
Donor type (DBD/DCD)	19/9	20/5	0.365
Donor age (years)	56.2 ± 3.42	51.0 ± 3.02	0.260
Donor sex	9 M; 19 F	18 M; 7 F	0.006
ET-DRI	1.83 ± 0.22	1.76 ± 0.16	0.598
NMP duration (mins)	592.8 ± 51.10	633.7 ± 52.49	0.579
Recipient age	52.2 ± 2.11	56.0 ± 2.77	0.276
Recipient sex	17M; 11F	19M; 6F	0.257
MELD	15.0 ± 1.09	13.5 ± 1.28	0.392

Table 35: Donor and recipient demographic details for minimal and significant preservation injury livers. Continuous variables were analysed using an unpaired Student's t-test and categorical variables using Fisher's exact test. Data are mean ± s.e.m

During NMP, there was a difference in baseline perfusate alanine aminotransferase (ALT) (MPI 171 IU/l versus SPI 669 IU/l; *P* = 0.005) and lactate dehydrogenase (LDH) (MPI 1,073 IU/l versus SPI 1,838 IU/l; *P* = 0.01) between the two groups. Levels of these enzymes, as well as γ-glutamyltransferase (γGT), increased more rapidly during the first 8 h of NMP in the SPI group (ALT, an increase of 56 IU/l versus an increase of 461 IU/l, *P* < 0.001; LDH, an increase of 483 IU/l versus an increase of 980 IU/l, *P*

= 0.06; γGT, an increase of 23 IU/l versus increase of 104 IU/l, *P* = 0.004). MPI livers showed a reduction in measurable levels of haemolysis (haemolysis index) as NMP progressed, in contrast to SPI livers in which the levels of haemolysis rose (MPI, a decrease of 0.04 U versus SPI, an increase of 0.09 U; *P* = 0.03). Bile production was greater in the MPI group (MPI 13.1 ml/h versus SPI 7.8 ml/h; *P* = 0.03). Lactate clearance was similar in each group. Post-reperfusion syndrome was less common in the MPI group (MPI 0% (0 out of 28) versus SPI 24% (6 out of 25); *P* = 0.007). One NMP liver with perfusate transaminases in excess of 20,000 IU/l was transplanted successfully. Full perfusate analysis is shown in the Table 36 below.

	15 mins			60 mins			End			Difference End – 15mins		
	Peak AST <250 U/L	Peak AST >1000 U/L	P value	Peak AST <250 U/L	Peak AST >1000 U/L	**P value**	Peak AST <250 U/L	Peak AST >1000 U/L	**P value**	Change Peak AST <250 U/L	Change Peak AST >1000 U/L	**P value**
Haemolysis Index (median)	0.25	0.19	**0.727**	0.18	0.21	**0.960**	0.11	0.38	**0.072**	-0.04	0.09	**0.0278**
Urea (mean ± SEM)	3.4 ± 0.19	3.4 ± 0.23	**0.994**	5.0 ± 0.34	5.0 ± 0.34	**0.997**	18.9 ± 1.99	18.0 ± 1.43	**0.708**	15.5 ± 1.93	14.5 ± 1.37	**0.6880**
Median Bilirubin (range)	2 (2)	2 (2-10)	**0.034**	2 (2-5)	2 (2-28)	**0.026**	2 (2-108)	4.5 (2-121)	**0.102**	0	2.5	**0.1443**
ALT	170.5 (64-1811)	669 (58->20000)	**0.005**	193.5(78-2306)	570 (78->20000)	**0.006**	268.5 (106-4107)	1334 (266-16772)	**<0.001**	56	461	**<0.0001**
Median Alk Phos	7 (5-21)	7 (5-32)	**>0.999**	7 (5-45)	10 (5-105)	**0.113**	40 (5-394)	71.5 (8-322)	**0.051**	32	61.5	**0.0682**
Median GGT	4 (4-30)	4.5 (4-92)	**0.262**	5 (4-103)	12 (4-110)	**0.132**	35.5 (4-190)	108 (8-759)	**0.008**	23 (0-183)	104 (4-667)	**0.0039**
LDH	1073 ± 180	1838 ± 245	**0.015**	1491 ± 256.6	1884 ± 207.8	**0.257**	1479 ± 157.2	2610 ± 187.6	**<0.001**	482.8 ± 110.6	980.1 ± 278.7	**0.0591**
CRP	8.3 ± 0.87	8.6 ± 1.47	**0.857**	22.1 ± 2.83	23.0 ± 3.63	**0.853**	147.4 ± 24.39	191.5 ± 36.07	**0.300**	131.9 ± 27.28	189.2 ± 42.74	**0.2445**
Lactate	10.5 ± 0.61	10.7 ± 0.78	**0.852**	1.7 ± 0.39	3.0 ± 0.78	**0.106**	0.5± 0.09	0.9 ± 0.29	**0.146**	-8.6 ± 0.64	-7.7 ± 0.83	**0.3672**

Table 36: Comparison of NMP perfusate analyses between MPI (peak AST < 250 IU/l; n = 28) and SPI (peak AST > 1,000 IU/l; n = 25) groups. A D'Agostino–Pearson normality test was performed to assess data distribution. Parametric data were analysed using an unpaired Student's t-test and non-parametric data were analysed using a Mann–Whitney U-test. Parametric data are presented as mean ± s.e.m. and non-parametric data are presented as median and range.

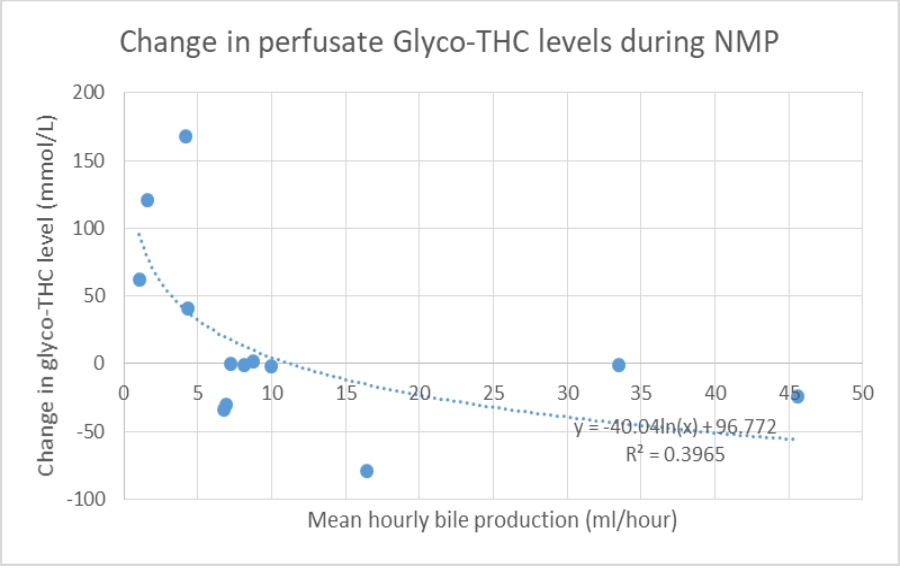
4.9 Bile salt analysis

Perfusate and bile samples were collected during the perfusion of 13 consecutive NMP livers at the time points shown below:

Time (hours)	Perfusate	Bile
1	X	
4	X	X
8	X	X
12	X	X

Table 37: Time-points for collection of perfusate and bile

The full ESI-MS results for analysis of the perfusate and bile are shown in Appendix 8. It was clear from an early stage of analysis that changes in bile acid concentrations in the perfusate and bile appeared to be related to bile production. When the change in perfusate bile salt concentration over the course of a 12 hour normothermic perfusion was plotted against mean hourly bile production (see graph below), it was apparent that an approximate threshold of 5ml/hour bile production was where the trend tended to cross the x-axis. Livers which produced less than 5ml/hour of bile during NMP showed a progressive accumulation of bile salts in the perfusate over 12 hours. Meanwhile, those which produced more than 5ml/hour showed no net change or a fall in perfusate bile salt concentrations.



Graph 3: Scatter graph showing the change in perfusate levels of glyco-trihydroxycholanoic acid during 12 hours NMP in all livers (n=13). R=0.630; t=2.688; p=0.021

To illusatrate this further, graphs 4 – 11 in Appendix 8 depict the differences in the manner in which perfusate levels of each bile salt change in livers with good bile production (>5ml/hr) compared with those with poor bile production (<5ml/hr)

The full results have therefore been arranged according to mean hourly bile production. Table 40 (Appendix 8) reports the results for livers with good bile production (>5ml/hour; n=9) and Table 41 reports results for those with poor bile production (<5ml/hour; n=4). These tables also contain basic donor information and mean hourly bile production for each liver.

Pearson's correlation coefficient was used to determine the degree of correlation between bile production, changes in perfusate bile acid levels and changes in bile acid levels in the bile over the course of a 12 hour perfusion. It can be observed that there are differences in the trends of change in perfusate bile acid concentrations according to the amount of bile produced by the liver. Therefore Pearson correlation coefficient was used to investigate the possible relationship between bile production and the change in perfusate bile salt concentration during NMP and showed this relationship to be of significance ($p<0.05$; graph 3 and graph 12-14).

In the bile produced during NMP, all bile acids were present including the primary derivative of sodium taurocholate, tauro-trihydroxycholanoic acid, which was present in the highest concentration at all time points. There was no change in bile salt concentrations with different rates of bile production. Indeed, the concentration of bile salts in the bile tended to remain constant regardless of the volume of bile produced.

5 Discussion

At the time of submitting this thesis, much of the work described above has already been published [95]. The full trial manuscript can be found in Appendix 12.

5.1 Clinical trial outcomes

The study represents the first randomised controlled trial to compare any form of machine perfusion technology with conventional static cold storage in human liver transplantation. The trial demonstrated significant reductions in peak AST and EAD rates in NMP livers; this is of clinical relevance as both are clinically accepted biomarkers for long-term graft and patient survival [79, 81]. These benefits are consistent with previous animal work [36] and the phase-I clinical study [38] that preceded this trial, both of which showed post-transplant AST reductions in NMP livers. No differences were seen in graft or patient survival. A much larger trial would be required to test this outcome. It is notable that these reductions in peak AST and EAD rates were achieved in the context of improved organ utilization and longer preservation times, both of which have implications in terms of addressing the donor shortage and logistical barriers that currently limit liver transplants.

The AST differences are in the context of longer functional warm ischaemia times (fWIT), longer preservation times and fewer organ discards in the NMP arm, suggesting that NMP may be achieving the desired objective of increasing organ utilization without compromising outcomes. If these findings were translated into clinical practice, the increase in organ utilization would have substantial implications for waiting list mortality, which is currently approaching one in five patients [2].

5.1.1 DCD outcomes

In contrast to DBD donors, the DCD donation process necessarily entails a period of warm ischaemia. It can be difficult to predict the extent of the damage that an organ may suffer as a consequence of this process. The utilisation of these organs is therefore often limited by concerns about the impact that this damage could have on graft function and survival after transplantation. In particular, DCD

livers have a higher rate of PNF, EAD and ischaemic cholangiopathy than those from DBD donors. In an attempt to mitigate for the harmful effects of the DCD donation process, DCD livers tend to only be accepted from donors that have fewer risk factors (e.g. younger and previously fitter) than their DBD counterparts. Furthermore, restrictions are placed on the acceptable duration of warm ischaemia prior to asystole to which a liver can be exposed before it is deemed untransplantable; this is usually up to 30 minutes of exposure to a systolic blood pressure <50mmHg or oxygen saturations <70% [37]. In addition, the recipients of such livers are generally selected to be the less severely ill, so that they are better placed to tolerate the early physiological, metabolic and biochemical insult associated with receiving a marginal organ.

It is notable that the greatest benefit of normothermic preservation was seen in DCD livers. For the reasons described above, these donors represent a largely untapped source of organs, comprising 42% of UK deceased donors, but only 21% of transplanted livers [12]. Allowing for the limitations of small group analyses, in this study NMP DCD liver primary outcome data were superior to those of both DCD and DBD livers preserved using SCS. In fact, the primary outcome of DCD NMP livers was superior to that of DBD livers preserved by NMP. This may be due to the selection bias, both of donors (lower threshold to decline DCD donors) and recipients (fitter patients selected for higher-risk organs).

If it transpires that the NMP preservation process removes much of the risk from a DCD liver it would have substantial implications for many of the sicker recipients on the liver transplant waiting list. At present, their frailty means that they are only deemed eligible to receive a liver from a low risk donor e.g. young, DBD with no co-morbidities. Given that these characteristics are uncommon in the donor population, by restricting the organs considered suitable for the sickest recipients it has the paradoxical effect of prolonging their time on the waiting list, which in turn leads to further deterioration in their health.

5.1.2 Preservation time

The introduction of University of Wisconsin solution (UW) for cold preservation of in the late 1980s was expected to herald an era of 24 hour organ preservation with improved outcomes after transplantation [17]. However, it quickly became apparent that UW was not the panacea that had been hoped with poorer outcomes seen as preservation times increased, especially in the context of the increasingly marginal donor organs transplanted. As a consequence it is now widely accepted that the preservation time for an SCS DBD liver should not exceed 10 hours, whilst for a DCD liver it should not exceed 8 hours.

With a median preservation time of almost 12 hours, compared with under 8 hours in the SCS arm, the much longer preservation times seen in the NMP group are perhaps the most striking finding in this trial. These longer preservations were not planned, but were all within the maximum perfusion time defined in the protocol. Indeed, although there was no stipulation in the trial protocol that the preservation times should be matched, the importance of maintaining equipoise in the study was repeatedly stressed to investigators.

As the trial progressed and clinicians gained experience of NMP, it became clear that many surgeons derived a sense of reassurance from observing the stable behaviour of the liver during normothermic perfusion. In some cases the theatre operating schedule was adjusted according to the preservation method. For example, instances included allowing an elective hepatobiliary case to proceed whilst the organ was on the device, so extending preservation time by several hours. In other cases, DCD livers preserved with NMP were delayed to allow SCS DBD livers to be transplanted first. Although not encouraged, there is no reason to believe that this behaviour (which was not universal) actually compromised the overarching outcomes of the trial. Indeed, any bias that may have occurred would have had the effect of diminishing the differences seen between the two arms of the trial.

It is likely that the extended preservation time that NMP allows also contributed to the better organ utilisation seen in this group. Occasionally organs that have been accepted for transplantation are

eventually declined due to a prolonged preservation time. This may occur due to a lack of access to the operating theatre (for example if other operating lists are over-running). If, as appears to be the case, NMP can safely extend preservation times without compromising outcomes, this will have implications for operating department planning as well as organ utilization.

5.1.3 Organ Utilisation

There were over 50% fewer discarded organs in the NMP group (11.7% NMP versus 24.1% SCS), resulting in approximately 20% more transplanted livers (121 NMP versus 101 SCS). The reasons for discard of NMP organs is shown in Appendix 6. The SCS discard rate of 24.1% was higher than the 17% reported in UK registry data [14], and may reflect the high proportion of DCD livers enrolled in the trial; the discard rate of retrieved DCD livers in the UK is 30% [12].

It appears highly likely that most of the additional transplanted livers in the NMP group were from higher risk donors. Similar numbers of livers with marginal characteristics (fWIT > 30mins or moderate/severe steatosis) were enrolled in both trial arms (43 SCS vs 47 NMP) but larger numbers of these were discarded in the SCS arm. There was also a corresponding difference in the livers that went on to be transplanted with NMP DCD livers experienced longer median functional warm ischaemia times (21 (17-25) minutes NMP versus 16 (10-20) minutes SCS; p=0.003) and, overall, a larger number of moderately and severely steatotic NMP livers being transplanted (29 NMP versus 12 SCS).

This reported difference in organ utilization is likely to be an underestimation of the full potential impact that NMP could have on transplant numbers. The trial stipulated that only livers considered transplantable according to standard practice could be enrolled. For the full extent of improved organ utilization to be measured, NMP would need to be applied to livers outside of standard acceptance criteria. Organs would need to be randomized to NMP or SCS before being offered for transplant; this should form the basis of a future trial. A logical primary outcome for such a study would be functional utilisation, combining graft utilisation with 12-month graft survival as a composite endpoint. An

increase of 20% or more in the number of transplantable donor livers would have a transformative effect on the mortality on liver transplant waiting lists around the world.

5.1.4 Post-reperfusion characteristics

The haemodynamic characteristics of the NMP recipients following reperfusion were measurably superior to those of SCS recipients (Table 18), in line with previously reported findings [96]. This was in association with lower on-going vasopressor requirements in the NMP group after the liver had been implanted (Table 19).

This greater haemodynamic stability also translated into lower post-reperfusion lactate levels. Lactate is a product of anaerobic respiration which is cleared from the body by the liver. Thus, these lower levels in the period after reperfusion could be a reflection of either: i) better systemic perfusion as a consequence of improved cardiovascular behaviour; or ii) better synthetic liver function enabling lactate to be cleared more effectively. The magnitude of the reperfusion syndrome is a factor in determining the eligibility of the sickest patients for high-risk organs, due to the limited capacity of such patients to tolerate cardiovascular instability; NMP might therefore increase the options for the most urgent patients.

The improved intra-operative behaviour of the NMP liver recipients did not translate into a difference in ICU stay, hospital stay or need for renal replacement therapy between the two groups. Previous reports have shown a correlation between peak AST and renal replacement therapy [97] although such a finding was not apparent in this study.

5.1.5 Device logistics

As well as demonstrating improved graft preservation, this trial tested the feasibility, usability and safety of NMP, a vital component of the evaluation of any new technology. It showed that the logistical challenges of NMP can be met successfully within clinical practice. Over 120 NMP livers were transplanted in seven transplant centres across four European countries. Despite better organ

utilisation in the NMP group it is notable that, for several of the discarded NMP organs, device related issues (mostly user errors) were implicated in the decision to decline the organ (see Table 11). More details about some of these cases can be found in Appendix 9 and Appendix 10. As experience and expertise in the use of NMP increases it is likely that such events will become less frequent and so avert these unnecessary losses.

The additional complexity that the use of NMP adds to the organ retrieval process may mean that, for this technology to be incorporated into clinical practice, it will necessitate changes to the technical support and transport arrangements that are part of the retrieval process. It remains to be seen whether NMP is required for the full duration of an organ's preservation or can equally well be applied after a short period of SCS when the organ reaches the transplanting centre. Such a change would probably facilitate adoption of the technology as it would avoid the need for the device, materials and expertise required for NMP to be transported to the donor hospital. Instead, all of the necessary resources and logistics could be kept 'in-house' which would save time, personnel and money. Such arrangements would certainly simplify the logistics but may not be suitable for the most marginal organs [35]. A phase-II study to test this has recently completed enrolment in the UK (NCT03176433) and does suggest that an initial period of SCS is not detrimental to standard criteria livers (C. Ceresa unpublished data). These findings are in contrast to previous animal work in this area [36] although the pig DCD model used in this study was at the upper extremes of what would be considered compatible with survival after SCS transplant.

For this new technology to be supported by healthcare funders, a health–economic case is needed; such an analysis was initially planned in parallel with the clinical trial. However, the results of this study suggest that benefits will accrue not only from improved early graft function but also from improved organ utilisation and transplantation logistics. This was not anticipated when planning the health economic study that would accompany the trial. Thus, whilst comprehensive information was collected from recipients relating to their resource use prior to, during and after their transplant, no

information was collected about recipients who failed to receive a liver due it being discarded. Similarly, no information was recorded about operating department logistical costs. By enabling livers to be safely preserved for longer durations, NMP may enable transplants to be moved predominantly into daytime operating, with reduction in staffing costs and likely improvements in outcome. This may also prevent organs being discarded because the preservation time exceeds that originally anticipated. In addition, by increasing the number of transplantable livers and so reducing organ discard rates NMP may reduce waiting list times. The reduced wait for an organ will bring economic benefits—earlier transplantation is associated with lower morbidity and cost. It would be wise for any future NMP trial investigators to take these considerations into account when planning an accompanying health economic assessment.

5.1.6 Steps ahead

The effects of NMP demonstrated in this study are unequivocal with respect to the primary endpoint, implying a benefit in livers currently used for transplantation. However, the greatest benefit may be realized by applying this technology to livers outside current acceptance criteria, in order to transplant organs currently deemed untransplantable. Algorithms to assess organ viability, based on data obtained during NMP, will be essential if this potential is to be realized. Such algorithms have been used in the recently completed VITTAL study [48] in which high-risk livers that had been declined by all UK transplant centres were perfused following an initial period of SCS. If predefined viability criteria (Table 3) were met, then the organs went on to be transplanted. Should these criteria be shown to be reliably predictive, this will have major implications for the application of NMP in guiding organ utilisation. Future trials may be needed to formally test the size of the effect of NMP on organ utilisation; for this it will be necessary to randomise livers at the time of organ offering rather than the time of retrieval. Organ utilisation, or organ utilisation with 12-month graft survival (functional utilisation) would be a logical primary endpoint for a study of this sort.

If, as this trial suggests, NMP can enable a greater proportion of livers to be transplanted safely, this will inherently lead to an increase in organ availability, which in turn may lead to smaller transplant waiting lists. At present, the indications for liver transplantation UK are fairly restrictive. For example, Patients with hepatocellular carcinoma (HCC) on a background of cirrhosis will only be listed if they meet the Milan criteria [98] (one tumour < 5cm or up to three tumours the largest of which is <3cm). It is well described that patients with larger lesions could benefit from transplantation [99] but at present these patients are not eligible for consideration for a transplant in the UK due to the necessity to ration organs because of their limited availability. A increase in the number of available organs should enable an easing of these restrictions and so increase the treatment options for patients with HCC.

Similarly, groups in Norway have described favourable survival rates after liver transplantation for patients with unresectable colorectal liver metastases and no extra-hepatic sites of disease [100]. Such a treatment is not an option for patients with unresectable liver metastases in the UK due to the rationing of organs, but could become an option in the future if organ numbers were to increase.

The final indication that is not currently considered in the UK is cholangiocarcinoma [101]. Some centres in North America will perform liver transplants for this disease, although usually using organs from living donors. This is not currently accepted practice in the UK, except in exceptional circumstances when a suitable living donor is available.

From Spring 2020 new legislation will come into effect in the UK in which all adults in England will be considered to have consented to be an organ donor when they die unless they had recorded a decision not to donate (‘opt out’ system). It is expected that this will increase the number of organ donors nationally and so, when combined with the effects of NMP, could have a substantial cumulative effect. If organ availability increases sufficiently, it may be the case that the indications for liver transplantation can be expanded to include those circumstances described above.

High-risk organs (for example, those with steatosis) may benefit from therapeutic interventions delivered during NMP: several groups are exploring potential strategies, including stem cell treatments, de-fatting agents and immunological modification of the organ.

This study describes the formal clinical evaluation of a novel technology in liver transplantation, and could herald the start of a new era of intervention during organ preservation. It represents a first, necessary step in demonstrating that NMP is feasible, safe and effective in clinical practice; the fact that the study has definitively met its primary endpoint should now enable the exploration of the technology's wider potential.

Most of the livers transplanted in this trial were enrolled at UK trial sites. The results are perhaps most applicable to this population of transplant patients although, since the completion of enrolment, the system of organ allocation has actually changed [102]. Since April 2018, DBD livers are now allocated on a named patient basis according to who will derive greatest benefit from each particular donor organ. Previously organs were allocated to individual centres who could select recipients using their own internal criteria. It is still not entirely clear how this new allocation system will affect organ utilisation or transplant numbers. Some centres have expressed concern that cold ischaemia times could increase if organs are allocated to patients located a long distance away or that inadequate weighting is given to certain patient groups e.g. those with HCC. It may well be that these potential logistical challenges can be overcome through the beneficial effects of NMP. In particular, if organs are required to travel further distances NMP could be used to mitigate the effects of the longer ischaemia time associated with longer transport distances. For patients with HCC who, under the new organ allocation system are given low priority, it may be that the best way to ensure a timely liver transplant is through the use of DCD organs, which are not allocated to a specific named patient. This study has shown that the greatest benefit of NMP is in the DCD group and so it may enable these organs to be used effectively for recipients with HCC.

Whilst the clinical component of this work is complete, Work Package 7 of the Consortium for Organ Preservation in Europe (COPE) is still active completing a comprehensive proteomic analysis of all perfusate and tissue biopsy samples collected and stored in the biobank. Details of these samples can be found in Appendix 2. This will include measurement of perfusate levels of IL7, TNF and vWF (planned secondary outcome measures from the present trial) as part of a much wider library of approximately 1000 proteins.

As described above, the health economic assessment that was completed as part of this trial did not anticipate that much of the benefit of NMP would be derived from improved transplant logistics and organ utilisation. The information that has been collected was therefore of limited use. This aspect of the work was overseen by Work Package 8 of COPE and it is likely that a report of the findings will be submitted to the European Commission in 2020.

5.1.7 Areas for improvement

Whilst this study was successful in meeting its primary outcome and many secondary outcomes, there are still shortcomings that should be addressed if future similar work is planned.

5.1.7.1 Primary outcome

The challenges of selecting a primary outcome for a liver transplant efficacy staudy have already been discussed. It is widely agreed by the liver transplant community that a concensus needs to be reached regarding an endpoint that the majority of clinicians would regard as meaningful. In the United States, the FDA have mandated that machine perfusion trials need to use early allograft dysfuction as their primary outcome measure. This sort of clear guidance is helpful as it helps to establish what standards need to be met is national health care providers are to endorse the application of this new technology.

5.1.7.2 Consent and coercion

Concerns about the last-minute consenting of some trial participants have already been discussed. When instances of inappropriate consent became apparent, the individuals concerned were verbally

reprimanded but no additional action was taken. In retrospect, it may have been more appropriate to take additional action.

The enrollement of patients into a clinical trial for which they have not been correctly consented is a serious breach of the GCP code and can constitute coercion. It would not have been unreasonable to halt recruitment into the study by either the individual concerned or the institution at which they were based. This could be used as an opportunity for remedial training and assessment to ensure GCP standards were met before recruitment could re-commence. As part of this process, qualitative improvement (QI) methods could be used. This would entail the formal evaluation of consent technique (such as through the audiovisual recording of a consent process) before a researcher could begin consenting patients for inclusion in a study. The embedding of such methods in the design of a clinical trial is well described [103] and is used to prevent the specific circustances arising that were described above. It also ensures consistency within and across trial sites for many potentially subjective aspects of the clinical trials process.

5.2 Predicting organ quality

This study sheds some light on which perfusion parameters may be used to assess organ quality: bile production, acid–base stability, lactate clearance, perfusate transaminase levels, and falling measurable haemolysis all correlate with the degree of preservation injury evident after transplant.

There was a clear inverse relationship present between the rate of lactate clearance and the rate of pH normalisation in the early hours of NMP. Once these parameters had both plateaued, this relationship disappeared, with pH fluctuating independently of lactate levels. Thus the maintenance of a normal pH appears to be indicative of organ quality independently of the lactate levels.

There was an important difference in perfusate transaminase levels between livers with evidence of minimal or significant preservation injury at all time points during NMP. In addition, the trajectory of the rise in transaminase levels was steeper in poorer quality organs with median levels approximately

doubling from 669 IU/L to 1334 IU/L during a typical 10 hour perfusion. Alanine aminotransaminase (ALT) is located only in hepatocytes. When liver parenchymal injury occurs this enzyme, along with aspartate aminotransferase (AST), is released from the cytoplasm into the blood, or perfusate during NMP. In liver transplantation, the correlation between elevated transaminases and graft survival is well described not only for AST in isolation, but also through it being a key component in most composite scores of early graft function [79, 104, 105]. During NMP, its potential role as a predictor of organ quality and viability has been previously described in animal models, although this is the first time it has been shown to be of relevance in a human liver transplant setting.

The final parameter that was able to discriminate between good quality and poor quality organs was the degree of haemolysis present in the NMP circuit. The shear stress associated with the passage of red blood cells in the perfusate through the plastic tubing, centrifugal pump, oxygenator, pinch valves and reservoir filter that make up the NMP circuit all inevitably causes a significant degree of haemolysis. However, in good quality organs the measurable level of haemolysis actually fell by over 50% during a typical 10 hour perfusion from 0.25 to 0.11. This is in contrast to poorer quality organs where the haemolysis index doubled from 0.19 to 0.38. It is reasonable to assume that the NMP circuit was inducing the same degree of haemolysis for both these groups of livers; the only variable in the circuit was the liver itself. The role of liver Kupffer cells in scavenging damaged red blood cells from the circulation is well described [106, 107]. It is likely that in good quality organs this function is sufficiently well preserved that, despite on-going haemolysis in the circuit, the organ is able to clear damaged red cells at a greater rate than they are produced. In poorer quality organs that have suffered parenchymal injury as a consequence of the organ donation process, this Kupffer cell function appears to be compromised leading to a progressive accumulation of lysed red cells in the pefusate. The measurement of change in the haemolysis index during NMP likely provides an indication of Kupffer cell function.

It is not possible from the present study to draw conclusions about markers that may predict organ viability. All but one of the livers transplanted in the NMP group functioned postoperatively, including one NMP liver with perfusate transaminase levels in excess of 20,000 IU/l and 18 livers with minimal bile production. The single case of primary non-function in the NMP group did have characteristics that were not seen in any of the other transplanted livers (see Appendix 11). In addition to a failure to make bile or maintain a normal pH, it was also the only transplanted liver that failed to clear lactate. One of the liver's core functions is the oxidation of lactate to pyruvate in a reaction catalysed by lactate dehydrogenase. It is possible that an inability to carry out this function is indicative of a degree of parenchymal injury from which the organ is not able to recover. Data from much larger numbers of NMP transplants (typically from a registry) would be required to determine if this is the case as well as to identify other specific markers of viability. At present, lactate clearance is the only parameter that is a necessity for an organ to be considered viable as part of the Birmingham criteria, although it is not included as part of the Cambridge groups viability parameters [45].

5.2.1 Implications for Perfusion of Other Abdominal Organs

Perfusing organs at body temperature necessitates the use of an oxygen carrier, usually in the form of packed red cells. The passage of erythrocytes through a mechanical circuit inevitably results in haemolysis with the potential for the lysed cells fragments to occlude small vessels and cause end organ ischaemia. It is this process which has largely limited the application of NMP to other abdominal organs, or at least limited the duration over which it can be applied. Relative to other organs, the liver is well adapted for ex-vivo perfusion. The results above would suggest that, in the case of a functioning liver, haemolysis during ex-vivo perfusion is not problematic as the lysed cell fragments can be scavenged by the Kupffer cells, resulting in a fall in measurable haemolysis levels as the perfusion progresses.

The pioneers of normothermic kidney perfusion, Sarah Hosgood and Prof Michael Nicholson, have reported promising results from the use of normothermic reconditioning for up to two hours prior to

kidney implantation [108]. Beyond this duration there is evidence of glomerular injury due to haemolysis. Fortunately, two hours of perfusion appears to be sufficient to identify markers of organ viability [109] which may, in turn, enable the utilisation of greater numbers of organs [110]. The biomarkers identified during renal perfusion are not as sophisticated as those described in the present study. Whilst they may reflect organ viability they do not provide information relating to quality.

If ex-vivo kidney perfusion is to realise its full potential then prolonged preservation is a necessity. This is partly due to the logistical benefits that can be derived from extending preservation times, which has impacts on theatre planning and organ utilisation. A further critical benefit of longer preservation is that it provides a mode for ex-vivo interventions to be delivered and its effects to be assessed. Given the challenges posed by haemolysis, it may be that using novel acellular oxygen carriers, such as Hemopure™ may offer a solution. This has be trialled successfully in human liver perfusions [111] and some groups have started to explore its potential in kidneys. Most recently, the Oxford groups have published the first report of a successful 24 hour ex-vivo normothermic kidney perfusion [112]. There are plans for their circuit to move into a phase I clinical study which, if successful, would have implications for using normothermic renal perfusion to deliver therapeutic interventions.

Attempts to apply normothermic perfusion to the pancreas during its preservation have achieved only limited success. The pancreas is extremely sensitive to prolonged cold ischaemia which, at reperfusion, causes an intense inflammatory response that manifests as pancreatitis. Overcoming this has proven challenging [113]. This is related to the difficulties of obtaining discarded human pancreases that are still viable and also due to uncertainty about the optimal perfusate composition. To determine the perfusate mixture that was required to enable successful liver perfusion required countless perfusions of porcine livers during the development of NMP technology. Such work in the pancreas is still in its infancy with a group in Leicester recently reporting the successful normothermic perfusion of nine porcine pancreases [114]. It is likely that a usable clinical device for pancreas NMP is still many years away.

5.3 The importance of bile

A number of previous studies in large animal models and discarded human livers [32, 40, 115] have suggested that bile production measured during NMP is an important marker of quality and, potentially, viability. Given that there were 18 NMP livers that produced minimal or no bile during perfusion and yet still functioned well after transplant, the present clinical trial would suggest that bile production is not a specific marker of organ viability. It may, however, reflect organ quality.

Livers which sustained significant injury during the organ retrieval and preservation process, as evidenced by higher post-transplantation transaminase levels, produced significantly less bile than those which had sustained only minimal preservation injury (MPI 13.1ml/hr versus SPI 7.8ml/hr; p=0.03). All of these organs ultimately functioned after transplant and so it cannot be claimed from these results that bile production reflects organ viability, although it does appear to correlate with the extent of organ injury.

The mechanisms behind differences in bile production can be inferred from the results of the analysis of bile acids in the perfusate and bile during NMP. It is clearly apparent that, in livers with poor bile production, there is a progressive accumulation of bile acids in the perfusate as the perfusion progresses. In contrast, there is a fall in perfusate bile acid levels seen in livers with good bile production. This correlation implies that preservation injury affects the ability of the hepatocytes in the liver sinusoids to take up bile acids from the circulation. This may be as a result of injury to the sodium-taurocholate co-transporting polypeptide (NTCP) [60] or the organic anion transporting polypeptides (OATP) located in the sinusoidal membrane. There is good evidence in rodents that ischaemia induces down-regulation of the expression of the NTCP and OATP proteins [116]. Should this be the case in human livers it would certainly account for the inverse relationship between preservation injury and bile acid uptake from the perfusate during NMP.

Given that over 75% of bile which is produced by the liver is generated through a bile salt dependent process [57], the inability of injured livers to take up bile salts from the perfusate would inevitably lead to poorer bile production during NMP.

Thus it seems reasonable to postulate that the ischaemic insult suffered by the liver during the organ donation process causes either direct damage or down-regulation of the NTCP and OATP proteins. This impairs the ability of sinusoidal hepatocytes to take up bile acids from the perfusate which has the downstream effect of reducing the amount of bile acid secreted by the hepatocytes into the biliary canaliculi. This in turn reduces the production of the bile-salt dependent component of bile. It is through this chain of events (Figure 24) that bile production reflects the degree of injury a liver has sustained during organ retrieval and so explains why the volume of bile produced during NMP correlates with organ quality.

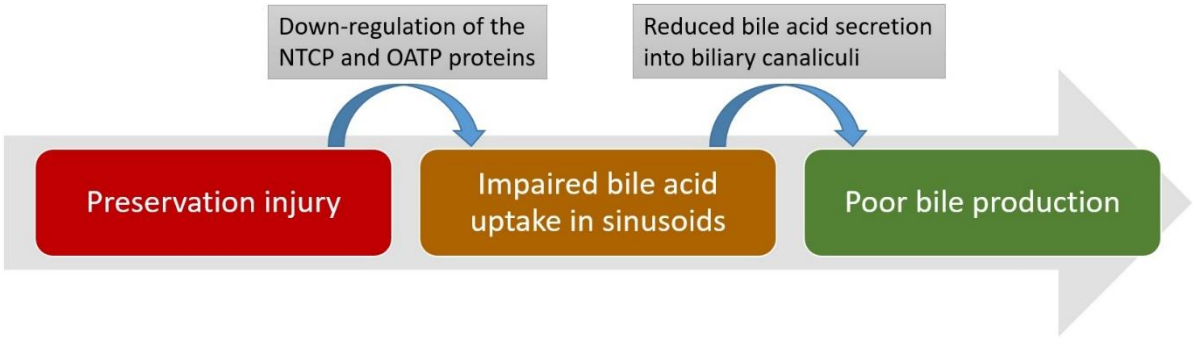


Figure 25: Postulated mechanism through which liver injury causes impaired bile production during NMP. Key: NTCP - sodium taurocholate co-transporting polypeptide; OATP – organic anion transporting polypeptide

The concentration of bile salts in the bile that is produced by the liver during NMP is maintained regardless of the amount of bile excreted. This would suggest that the function of the bile salt export pump (BSEP) is more resilient in the face of ischaemic injury such that its ATP dependent secretion of bile acids into the biliary canaliculi against a concentration gradient is preserved despite sufficient injury to impair function of the NTCP and OATP proteins.

As important as these findings is the clear observation that human livers are able to utilise a bovine source of sodium taurocholate. The primary derivative of sodium taurocholate, tauro-

trihydroxycholanoic acid, was present in the perfusate and in the bile at sustained high concentrations throughout all the perfusions. Its concentration was notably an order of magnitude higher than that of the other endogenous bile salts. Whilst this did not appear to cause hepatocyte or cholangiocyte injury, it may still be the case that the NMP perfusate is being over supplemented with bile salts. It has been previously suggested that excessive secretion of bile salts in the bile may induce biliary injury [117]. In the present study only one of the 13 livers under investigation showed evidence of non-anastomotic strictures on MRCP at 6 months. The liver in question was from a DBD donor, had satisfactory bile production (8.1ml/hr) and did not show evidence of significant preservation injury after transplantation. There were no biochemical changes to suggest biliary stasis and the liver continues to work well 2 years after transplant.

It is notable that other groups are perfusing and transplanting NMP livers without providing additional bile salts [45] and still observing that bile is produced by these livers. These perfusions tend to be less than 10 hours in duration and so may be brief enough that endogenous bile salt stores are sufficient for the bile production requirements of the liver, as was observed in the previous pig studies [62]. Further work is needed to determine if the degree of sodium taurocholate supplementation can be reduced or if it is even necessary.

5.4 Biliary strictures

Perhaps the greatest limitation to widespread utilization of DCD livers is the high rate of clinically important non-anastomotic biliary strictures (NAS) which lead to a high rate of graft failure. NAS are also termed ischaemic cholangiopathy, defined as multiple diffuse strictures in the biliary tree in the absence of hepatic artery thrombosis.

The blood supply to the biliary tree is derived almost exclusively from the hepatic artery, with no significant contribution from the portal vein. By implication this would suggest that the provision of oxygenated blood is important to maintain the health of the biliary tree and so these tissues are likely to be vulnerable to hypoxia. It is hypothesised that the warm ischaemic insult which occurs during the

DCD donation process can cause irreversible damage to the biliary tree which later manifests as diffuse strictures, subsequently leading to recurrent cholangitis and graft loss. Due to this potential risk, most transplant centres will not use livers with a fWIT greater than 30 minutes [37] or organs from donors that have suffered prolonged ischaemia during the course of their illness.

Reported rates of NAS in DCD transplants vary from 10 to 30% [69, 118], but these figures relate to clinically apparent biliary strictures. These usually present with deranged liver function tests or cholangitis, prompting further imaging from which the diagnosis of ischaemic cholangiopathy is made. Prior to this study, all reports of the incidence of biliary strictures related to those which were clinically apparent [69, 73, 118, 119]. The radiological incidence of both anastomotic and non-anastomotic strictures in asymptomatic liver transplant patients was unknown and thus the significance of a radiological diagnosis of NAS was also not clear, although the comparison between the groups is of interest.

The rate of NAS in the NMP DCD group (3/27; 11.1%) was lower than in SCS (5/19; 26.3%) livers, despite longer functional warm ischaemia times. Although this difference did not reach statistical significance, this may be a function of sample size; the trial was not powered for this outcome. Two of these patients were re-transplanted within 1 year due to ischaemic cholangiopathy, whilst almost all of the remaining patients with radiological evidence of NAS had normal liver function at one year. In DBD organs the radiological rates of NAS were similar in NMP and SCS livers (7.4% NMP versus 5.4% SCS; p=0.678). None of these patients developed clinically apparent evidence of cholangiopathy. The absence of any correlation between a radiological finding of NAS and clinical evidence of liver dysfunction questions the relevance of performing a protocol MRCP at six months. Alternatively, it is possible that MRCP is a very sensitive marker of biliary injury and therefore a useful surrogate marker of an important clinical outcome. The longer term follow-up of these patients will shed light on the importance of a radiological diagnosis of biliary stricturing in patients with normal graft function, and the role of MRCP as an endpoint in future trials.

Anastomotic biliary strictures were defined as a more than 70% narrowing of the luminal diameter with proximal dilation of the common bile duct. It is notable that the imaging from almost half the patients in this study fulfilled this criteria although very few of them had clinical evidence of biliary obstruction (9 NMP versus 11 SCS livers required interventions for clinically relevant anastomotic strictures; Table 27). This incidence of anastomotic strictures which required intervention fits with previously reported rates of approximately 6.6% at 1 year [64]. The marked disparity between the radiological rate of anastomotic strictures (overall NMP 43.2% versus SCS 45.9%) and those requiring intervention, again, questions the relevance of a radiological finding of an anastomotic stricture in the absence of any corresponding clinical abnormality.

Overall these findings would suggest that performing a protocol MRCP scan at 6 months after liver transplantation is of limited value in predicting stricture formation in the first year. Longer term outcomes will shed more light on the overall predictive potential.

6 Conclusions

This first randomised trial to compare any form of machine preservation technology with conventional cold storage in liver transplantation has furthered our understanding of normothermic perfusion beyond just the context of efficacy. It has shown NMP to be able to safely extend preservation times and offered a glimpse into the potential increases in organ utilisation that this technology could herald. Fundamental to this is the ability to assess organ quality during NMP.

However, as with many clinical studies, there are almost as many questions unanswered as have been answered. There was no measurable difference in many parameters that will be key to making the economic case for NMP. No difference was seen in resource utilisation or graft and patient survival at 1 year despite the important findings described above.

In coming years the analysis of the countless samples collected during this trial and stored in the COPE biobank will shed light on the mechanisms underlying the effects of NMP. First amongst these studies was the work reported here investigating the role of bile salt supplementation during NMP. For the first time it was demonstrated that the human liver is able to utilise non-human sources of bile acids. Whether this supplementation is always necessary is still unclear although it does seem possible that the amount of additive could be reduced. There also appears to be a viable hypothesis to explain how organ injury impacts on bile production, although further molecular work will be needed to confirm this.

Similarly, analysis of samples from the biobank provided valuable information regarding potential markers of organ quality. It was not possible to determine markers of organ viability; it is likely that information from much larger numbers of NMP transplants will be needed to answer this question. Further proteomic analysis of the same samples is planned in the coming years which may answer some of these questions and reveal further markers with a better predictive potential for organ quality.

This clinical trial also provided a valuable opportunity to explore the potential value of routine MRCP imaging in liver transplantation. The marked disparity between the radiological findings and clinical picture raises questions about the role of imaging in clinical practice. Of course, this could change as the longer term biliary outcomes from these patients become known.

Perhaps most importantly, this work will now open the door to exploring the wider potential of NMP. The ability to maintain a liver ex-vivo in a functioning state offers a vehicle through which interventions can be tested without risk to a patient. Already a large numbers of groups around the world have started this process and demonstrated that livers can be infected [120], split [121], de-fatted and even decellularised during NMP. Our group are exploring the potential to immunomodulate the liver during NMP and also planning further trials to more definitively determine the true potential impact of NMP on organ utilisation. The work described in this thesis is just the beginning.

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9 Appendix 1: Trial documents

9.1 Patient Information Leaflet

PATIENT INFORMATION SHEET

A multicentre randomised controlled trial to compare the efficacy of *ex-vivo* normothermic machine perfusion with static cold storage in human liver transplantation – COPE WP2

REC Ref. No.: 14/LO/0182
ISRCTN No.: 39731134

Introduction
We would like to invite you to take part in this research study. Before you decide you need to understand why the research is being done and what it would involve for you. Therefore we have provided the following information - please take time to read it carefully, and discuss it with others, such as friends, family or your GP, if you wish. We will do our best to explain and provide any further information you may ask for now or later.

Part 1 tells you the purpose of this study and what will happen to you if you take part.
Part 2 gives you more detailed information about the conduct of the study.

PART 1

What is the purpose of the study?
This study has been designed to assess the potential benefit of a new machine for **storing** a donor liver, between the time it is removed from the donor and transplanted into a recipient. The new machine uses a novel method to keep the liver healthy during storage that enables the liver to be transported at normal body temperature. It is hoped that, by improving the conditions in which the donor liver is stored, this would enable a greater number of organs to be made available for transplantation, so reducing the waiting list time and reducing the risks of the operation itself for future patients.

The study will involve 260 participants from the liver transplant waiting lists in four European countries. The purpose of this study is to find out how effective this new storage method is compared to the standard method currently used in transplant centers in the UK and Europe. This will be done by taking measurements of the function of the liver both during storage and after the liver has been transplanted (further information can be found on page 4).

What is the device that is being tested?
OrganOx Limited is a late-stage medical device development company that was founded in April 2008 as a spin-out from the University of Oxford.

The OrganOx *metra is* a new device for preserving a donor liver using technology that maintains the organ in a fully-functioning state, rather than cooling it down in an ice-box as is standard practice. The device perfuses the donor liver with blood (from the Blood Transfusion

Service), oxygen and nutrients, as well as a number of medications, at normal body temperature to mimic ideal physiological conditions and preserve the organ for up to 24 hours. It also provides information regarding the blood flow, synthetic and metabolic function of the liver whilst being perfused which may assist the surgeon in assessing the organ's suitability for transplantation. The transplant procedures and after-care are not changed from the routine procedures currently performed at your transplant centre.

Currently this machine is not approved for use in the NHS.

What are the alternatives for treatment?
The current standard method of storing and transporting a liver for transplant is to store it in an ice-box.

If you choose not to take part in this study, you will receive the same donor organ but stored using standard cold (ice-box) preservation.

How will treatment be allocated?
If you are eligible to participate in this study, and you choose to participate, your transplant liver will be randomly assigned (by chance, like the flipping of a coin) to be stored using the new perfusion machine, or using the standard method of storage in an ice-box.

Why have I been chosen?
We are asking adult patients (aged older than 18 years) who are currently on the liver transplant waiting list at the participating transplant centres. You are being asked if you would like to take part in this research because you are on the waiting list to receive a liver transplant and, in the opinion of the doctors looking after you, there are no other medical issues which would prevent you from taking part in the study.

Do I have to take part and what will happen if I don't want to carry on with the study?
No. It is entirely your decision as to whether or not you take part in the study. If you decide not to take part this will not affect your medical care in any other way, or your relationship with the medical and nursing staff looking after you. If you decide not to take part, your chances of receiving a liver transplant offer will not be affected.

If you decide to take part you will be invited to complete and sign a consent form.

If you do decide to take part, you are free to withdraw at any time and without giving a reason. If you do withdraw, this will not affect your medical care in any other way, or your relationship with the medical and nursing staff looking after you. If you decide on the day of your transplant that you do not wish to participate in this study then your transplant will still go ahead using conventional storage methods.

What are the Exclusion Criteria?
You would not be able to take part in the study if:

- You are younger than 18 years old,
- You are undergoing transplantation of another organ as well as the liver,

- You have rapid onset, severe liver injury with failure of liver function (acute/fulminant liver failure),
- Your medical team feels for any reason you are not suitable to take part. Your medical team will discuss this with you.

What will happen to me if I take part and what do I have to do?

Agreeing to participate in the study:

You have been given this Patient Information Sheet to read and keep, and you will have the opportunity to ask any questions you may have. If you decide to take part in the study you will be asked to sign and date a consent form in advance of your liver transplant procedure.

If you are interested in participating in the study, please return the reply slip on the bottom of the invitation letter. One of the Study Coordinators will then contact you by phone or by email to confirm that you would like to discuss participation in this study at a routine clinic appointment at your transplant centre. A confirmation letter and local directions and travel instruction will be sent to you at this time.

At your routine outpatient appointment, you will have the opportunity to discuss this study with one of the medical team involved with running this study (including the transplanting surgeon). They will be able to answer any further questions you may have. You will be asked if you want to consent to participating in this study at this meeting, you can consent at this appointment or are free to go home and discuss this further with family and friends and consent at a later appointment.

What happens when a liver is found for me?

When a donor liver is identified for you, the Transplant Coordinator will confirm that you are still willing to take part in the study, and you will be asked to initial a form to confirm you are still happy to take part. At this point you are free to withdraw your consent or confirm your consent to continue in the trial.

The total duration of the study is approximately 24 months from the date of your transplant. During this time we will record some of your blood test results taken after your operation and also keep a note of any problems you have during your recovery.

There will also be a few extra tests done specifically for this study. These will involve 2 extra blood tests, 2 urine samples and several biopsies from the donor liver being taken for research purposes at the time of your operation. An additional blood test will also be taken at the same time as your normal post-operative blood tests each day for the first week after your operation. Approximately 6 months after your transplant you will be required to have one additional MRI scan in order to study the liver. You will be asked to complete a short questionnaire when you are first put on the waiting list for a liver transplant and then again at each follow-up visit approximately 30 days and 6 months after your transplant, which will ask about your daily activities and quality of life.

Details of the visits are as follows:

Screening Visit

You will be contacted by the clinical study nurse or one of the doctors when you are at the hospital either as an outpatient or an inpatient. The study will be explained and you will have the opportunity to ask any questions. If you decide that you wish to take part, then you will be asked to sign the consent form, but if you wish to have more time to consider whether to take part, then we will arrange to contact you again after a suitable interval. If you do agree to take part, the clinical study nurse or one of the study doctors will collect certain details from your medical records, including age, gender, ethnicity and medical details. You will also be asked to complete a short (two-page) questionnaire about your daily activities and current quality of life.

Transplant (Study Visit 1)

At this visit you will undergo your liver transplant operation according to the current standard procedures at our transplant centre. The only difference will be that your donor organ may have been stored using the new method. Study personnel will record details of your hospital stay and any complications, and document the results of blood tests that are taken as part of your normal post-transplant care.

On discharge from hospital you will be given a "Post-Transplant Card", which will provide you with the relevant contact telephone numbers of the study team, should you wish to speak to anyone about your participation in the study, ask any further questions or discuss any medical issues. You will also be given a "resource use log" on which we would like you to record any hospital admissions, clinic visits or visits to your family doctor during the 6 months that you are taking part in the study.

Study Visit 2 – 30 days post–transplant

At the second visit, you will have a blood sample taken, the equivalent to a teaspoon, which will be analyzed to assess the function of your new liver. The tests are the same as those needed for routine follow-up but with the some additional tests. You will also be asked to repeat the short (two-page) questionnaire about your daily activities and current quality of life. Items collected on your "resource use log" will be recorded.

Study Visit 3 – 6 months post-transplant

At the third and, for the purposes of the study, your final visit, the same blood tests will be carried out as at the previous visit. In addition you will have a special MRI (magnetic resonance imaging) scan to look at the liver. This scan takes around 45 minutes and requires you to lie still, but does not involve any injections, medications or radiation. You will also be asked to repeat the short (two-page) questionnaire about your daily activities and current quality of life. Your "resource use log" will be collected from you at this visit.

Long-term follow up – 12 and 24 months post–transplant

At 12 and 24 months after your transplant we will collect data from your medical records about the function of your new liver. We will also record whether you have experienced any other health problems related to your transplant during the intervening period. All of this information will be collected from your routine follow-up assessments recorded in your medical notes and will not require you to be seen by the research team in person.

What are the risks of the device/procedures?
Research on this device has been carried out over several years in experimental settings, which suggest that the method of machine perfusion is effective and safe. A safety study at King's College Hospital, London and the Queen Elizabeth Hospital, Birmingham has demonstrated the safety of the perfusion device in twenty patients so far, and the medical risks are believed to be no different from standard transplant surgery currently performed at your transplant centre. The medical risk in taking blood samples is minimal but may cause minor pain and bruising.

The Magnetic Resonance Cholangiopancreatography (MRCP) examination is a type of MRI scan. The MRI examination poses almost no risk to the average patient when appropriate safety guidelines are followed. Some patients require sedation to help them lie still for the duration of the scan. If sedation is used there are risks of excessive sedation, including airway obstruction, apnea and hypotension. These are not uncommon during sedation and health professionals, who are suitably trained to detect and manage these problems, will be present. The direct health care team will monitor your vital signs to minimize this risk. Although the strong magnetic field is not harmful in itself, implanted medical devices that contain metal may malfunction or cause problems during an MRI scan. If you do have any metal implants it is important to let the radiology department team know as this may mean it is not safe for you to have the scan.

There is the possibility that the MRCP examination will show a medical problem unrelated to the study. Should this happen, the results will be shared with both yourself and the doctors responsible for your care to enable appropriate action to be taken.

What are other possible disadvantages and risks of taking part?
If there are concerns about the liver that you are scheduled to receive as part of this study, the operation will not proceed. This decision will be made by the surgeon responsible for your operation, independently of the study. Liver transplant surgery and management after surgery carry major risks. All of the risks associated with your transplant procedure will be fully explained to you by your health care team and transplant surgeon.

What are the possible benefits of taking part?
We cannot promise the study will help you but the information we get from this study may help improve the treatment of people needing a liver transplant in the future. We are not able to predict whether the results of this study will show any benefit over the standard organ storage method although the research that has been carried out in the laboratory suggests that this new method of organ preservation may aid maintenance of the liver prior to transplantation. If this is confirmed, then this trial may enable more organs to be transplanted with lower risk for future patients.

Safety study
From January to December 2013, a safety study was carried out in King's College Hospital, London and the Queen Elizabeth Hospital, Birmingham in which twenty patients received a liver transplant using a liver that had been preserved using the OrganOx *metra* liver preservation machine. All of the patients made a good recovery from the operation and have been discharged from hospital. Analysis of the data from this study suggests that livers which are preserved using the OrganOx *metra* suffer less damage and show improved function after being transplanted. However, because this study was designed mainly to investigate the safety of this technology, it is not possible to draw any final conclusions from it. We hope that our current trial, described in this leaflet, will be able to provide a clear answer regarding the potential benefit of this technology.

This concludes part 1. If you are interested in participating in the trial please read part 2 before making your final decision.

PART 2

What if relevant new information becomes available?
Sometimes, during the course of a research project, new information becomes available about the device being studied. If this happens, your study doctor will tell you about it and discuss whether you want to, or should, continue in this study. If you decide not to carry on, your doctor will make arrangements for your care to continue. If you decide to continue in the study you may be asked to sign an updated consent form. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

What if there is a problem and something goes wrong?
The University of Oxford, as sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm arising from the negligence of the University, or that of a collaborator in this research, and with that harm arising as a direct consequence of your participation in this trial.

The NHS will owe a duty of care to those undergoing clinical treatment, with Trust Indemnity available through the NHS Litigation Authority Scheme

What if I have any other concerns and want to speak to someone?
If you wish to complain about any aspect of the way you have been approached or treated during the course of this study, you should contact.

Chief Investigator: Prof Peter Friend **Telephone Number**: +44 (0) 1865 223872.

Or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on:

Telephone Number: +44 (0) 1865 572224 **E-mail:** ctrg@admin.ox.ac.uk

Alternatively, in the UK you can contact the Patient Advice and Liaison Service (PALS) at your transplant centre. This is a service that offers support, information and assistance to patients, relatives and visitors. They can also provide help and advice if you have a concern or complaint that staff have not been able to resolve for you. Their number is below: (for Essen, Leuven and Barcelona the wording of this section will be site specific)

Study Site PALS Telephone Number: XXXXXXXXXXXX

Who do I contact for more information?
If you would like any more information or you have any study related concerns, please contact:

Local Investigator: XXXXXXXXX **Telephone Number:** XXXXXXXXX

Will my taking part in this study be kept confidential?
Yes – the information we collect about you will be strictly confidential. If you agree to take part in this study, you will be given a unique study number, which is used as an identifier in our database and makes you anonymous to everyone except your research study nurses, study doctor and study administrator.

The database is stored on a secure server at the Surgical Intervention Trials Unit (SITU) at the University of Oxford. The unit routinely handles sensitive information and procedures are in place to ensure the highest possible data security and integrity.

Responsible members of the University of Oxford, regulatory authorities or the host institution may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations.

Your data will be stored in compliance with the UK Data Protection Act 1998, but may be transferred for the purpose of processing to associated researchers within the European Economic Area, which has the same stringent data protection laws as the UK. Any data released will be in an electronic form and will be anonymous (i.e. contain no personal identification). None of your personal information or data that could identify you will be released. Anonymised data collected during the course of the study may be passed on to other organisations which may include commercial organisations.

You may withdraw your consent at any time. If you withdraw your consent, you will not be able to continue in the study, and no further information about you will be gathered after that date. However, any information already collected about you may still be used as described above.

If you give your consent to take part in this study, with your agreement, your General Practitioner (GP) will be notified that you are taking part.

What will happen to any samples I give?

During your inpatient stay, and when you return for the follow-up visits at approximately 30 days and 6 months after liver transplant, blood samples (5 ml, about the size of a teaspoon) will be collected and sent to the laboratories for analysis. These tests will determine how well

your new liver is functioning, and would be taken routinely even if you were not participating in the trial. Again any residual samples will be discarded as per routine hospital procedure.

With your permission, additional samples of blood and urine will be taken at the time of your operation to be processed and analyzed centrally at the University of Oxford. Similar blood samples will be taken at the same time as your normal post-operative blood tests each day for the first week after your operation. These samples will be transported for storage for use in current and future ethically approved studies investigating methods for predicting transplant outcomes and investigation of the mechanisms of transplant failure. You will be asked for separate consent for the collection and storage of these samples. If you do not wish for these samples to be stored for future studies, then only the routine samples described above will be taken.

Several very small samples (biopsies) of the transplant liver will be taken - before being placed for storage on the new machine, after storage, and just before the end of your transplant operation. These samples will be sent to the University of Oxford for analysis, and any residual material processed and stored for future ethically approved studies as above. These samples are taken to assess how healthy the liver is over the storage time. You will be asked for your consent for this material to be stored and used in future studies.

What will happen to the results of the research study?

When the study is finished, all the results will be analyzed and a report written. The results may also be published in a medical journal. All reports and publications will maintain confidentiality of your identity. In the future, the results of this study may be used for further analysis, which at this time cannot be anticipated. Any data released will be in an electronic form and will be anonymised i.e. contain no personal identification. None of your personal information or data that could be traced to you will be released.

If you would like a copy of the results, please tick the box on the consent form.

Is my GP informed that I am taking part in the study?
Yes – if you agree to take part, your GP will receive a letter and any results obtained.

Who is organizing and funding the research?
This study is being sponsored by Oxford University and is being organized by the Nuffield Department of Surgical Sciences in Oxford.

The trial is funded by a grant from the European Commission (EC) Seventh Framework Programme (FP7).

Will my study doctor be paid for including me?
Your transplant centre will receive payment according to the number of patients that take part in the study. This payment will cover the additional costs of your care associated with the study and cover the cost of extra administration, such as completing the forms we use to collect the information we need. No study doctor will receive any personal payments for their involvement in the study. Professor Peter Friend and other members of the study team will receive no payment or other incentive for their involvement in the study. Professor Friend has been involved in the invention and development of the new technology and receives

payment as Medical Director and Consultant to OrganOx Ltd, the manufacturer. He is also a shareholder in the company. However, he will not be directly involved in the recruitment of patients to the study or their clinical care.

Expenses and payments
You will not be paid for taking part in this study. There should be no extra cost to you as all the assessments needed for the trial will be done alongside your routine care. In the unlikely event that any extra visits are required it will be possible to reimburse reasonable travel expenses.

Who has reviewed the study?
This study has been reviewed and given a favourable ethical opinion by the London–Dulwich Research Ethics Committee.

Thank you for taking the time to read this.

9.2 Letter to patient

Dear ***[patient name]***

We would like to invite you to take part in our research study entitiled:

A multicentre randomised controlled trial to compare the efficacy of *ex-vivo* normothermic machine perfusion with static cold storage in human liver transplantation

This study has been designed to assess the potential benefit of a new machine for **storing** the donor liver, between the time it is removed from the donor and transplanted into the recipient. The new machine uses a novel method to keep the liver healthy during storage that enables the liver to be transported at normal body temperature.

The study will involve 260 participants from the liver transplant waiting lists in four European countries. The purpose of this study is to find out how effective this new storage method is compared to the standard method of ice-box storage currently used in transplant centers in the UK and Europe. It is hoped that, by improving the conditions in which the donor liver is stored, this would enable a greater number of organs to be made available for transplantation with lower risk to future patients.

You are being asked if you would like to take part in this research because you are on the waiting list to receive a liver transplant and, in the opinion of the doctors looking after you, you may be eligible to take part.

Further information on this study and what it would involve for you can be found in the enclosed patient information leaflet. Please read this leaflet carefully and, if you are interested in participating in the study, please complete the form overleaf and return it to us in the envelope provided. A member of our research team will be in touch to arrange to meet you at a routine outpatient appointment to discuss the study further.

Thank you in advance for considering taking part.

Yours sincerely,

[Local investigator]

9.3 Letter to patient's GP

Dear Dr ***[doctor name]***

For your information, we are conducting a study entitled:

A multicentre randomised controlled trial to compare the efficacy of *ex-vivo* normothermic machine perfusion with static cold storage in human liver transplantation – COPE WP2

Your patient ***[patient name]***, of ***[patient address]*** has been entered into this study on ***[date]***, and is due to complete the study on ***[date]***.

The study is investigating the use of a novel device for perfusion of livers prior to transplant, using packed red cells as the main perfusion solution, at 37°C. Randomisation will compare this treatment to standard cold storage and transport in an ice-box. The study aims to recruit 260 participants from European Transplant Centres who meet the study inclusion / exclusion criteria and who volunteer and consent to participate in the study.

After the liver transplantation procedure, follow up visits will take place at 30 days and 6 months post-operatively. This will include liver function tests, quality of life assessment and a Magnetic Resonance Cholangiopancreatography (MRCP) scan 6 months post-transplant. Additional liver function and graft survival data will be collected at 12 and 24 months post-transplant.

Patients will be provided with a Study Contact Card on discharge, which will provide them with all the relevant contact numbers in the event of any urgent need to make contact with the study team.

During the period the patient is taking part in this study, it would be very helpful if you would inform us of any adverse events, significant changes in medical condition or new illnesses.

If during the course of the study, a new medical condition is discovered, we will of course inform you.

Please don't hesitate to contact me on ***[telephone number]*** if you require further information about the study or your patient's involvement.

Thank you in anticipation of your assistance.

Yours sincerely,

[Local investigator]

9.4 PATIENT CONSENT FORM

A multicentre randomised controlled trial to compare the efficacy of *ex-vivo* normothermic machine perfusion with static cold storage in human liver transplantation – COPE WP2

Participant ID:		**Date of Consent**	

Please initial all boxes.

I confirm that I have read and understood the Patient Information Sheet dated 13th January 2014 (Version 1.0), for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that my participation is voluntary and that I am free to refuse to take part in this study or withdraw at any time without giving a reason and without it affecting my medical care or legal rights.	
I understand that if, at any point, I choose to withdraw from the study then all data collected up until the point of my withdrawal will be kept for use in the trial, unless I specifically state that I do not wish this to be the case.	
I understand that relevant sections of my medical notes and data collected during the study may be looked at by responsible individuals from the University of Oxford, or their designates from regulatory authorities or from the host institution, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records	
I understand that my study data will be handled, stored and destroyed in compliance with the Data Protection Act 1998.	
I understand that some of the countries to which my anonymised data may be transferred will be outside the UK (Europe), which offers the same level of data protection as the UK.	
I understand that anonymised data collected during the course of this study may be passed on to other organizations which may include commercial organizations.	
I agree to data from my medical records, recorded as part of my routine clinical care, being collected after the end of the study for the measurement of long-term outcomes.	
I agree to my general practitioner and local hospital care team being informed of my participation in this study	
I agree to take part in the above study	
Optional. I give permission for biopsy, blood and urine samples taken as part of this study to be gifted to the University of Oxford and stored for use in future research. I understand that I will not gain any direct personal benefit from this.	

Name of Patient (Consent 1)	DD/MM/YYYY Date	Signature
Name of Investigator (Consent 1)	DD/MM/YYYY Date	Signature
Name of Patient (Re-consent)	DD/MM/YYYY Date	Signature
Name of Investigator (Re-consent)	DD/MM/YYYY Date	Signature

10 Appendix 2: Biobank samples

Sample	Type	Time points	Notes
Perfusion Samples			
LT1	Liver Biopsy	On back table at retrieval	
P1	Perfusion Blood	15 mins after NMP start	
P2	Perfusion Blood	1 hour after start	
P3	Perfusion Blood	At the end of NMP	
Bi1	Bile	At the end of NMP	Volume produced also recorded
LT2	Liver Biopsy	At the end of preservation	
BD0	Common Bile Duct Biopsy	At the end of preservation	
Recipient Procedure Samples			
RB1	Recipient Blood	Prior to transplant	Taken after induction of anaesthesia
RU1	Recipient Urine	Prior to transplant	
RB2	Recipient Blood	1 hour after reperfusion	
RU2	Recipient Urine	1 hour after reperfusion	
BD1	Common Bile Duct Biopsy	1 hour after reperfusion	
LT3	Liver Biopsy	1 hour after reperfusion	
Post-operative Samples			
D1 – D7	Recipient blood	Once daily	Additional 10ml sample as part of daily blood tests

Table 38: Collection time points for all biological samples collected from each enrolled liver.

11 Appendix 3: Statistical analysis plan

Consortium for Organ Preservation in Europe

WP2

A multicentre randomised controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation

Statistical Analysis Plan
3.0 – 17Oct2016

Based on protocol version 3.0 (11st February 2016)

	Name	Title/Role	Signature	Date
Author	Virginia Chiocchia	Trial Statistician		
Reviewer	David Nasralla	WP2 Central Investigator		
Approver	Peter Friend	Chief Investigator		
Approver	Susan Dutton	OCTRU Lead Statistician		

Surgical Intervention Trials Unit (SITU)
Centre for Statistics in Medicine (CSM)
Oxford Clinical Trials Research Unit (OCTRU)
University of Oxford

Contents

GLOSSARY OF ABBREVIATIONS

ADE	Adverse Device Effect
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ATP	Adenosine Triphosphate
AUC	Area Under the Curve
BMI	Body Mass Index
BP	Blood Pressure
CA	Competent Authority
CET	Centre for Evidence in Transplantation
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COPE	Consortium for Organ Preservation in Europe
CRF	Case Report Form
CSM	Centre for Statistics in Medicine
DBD	Donation after brain death
DCD	Donation after circulatory death
DMC	Data monitoring committee
DPMP	Donors per Million Population per Year
DRI	Donor Risk Index
EAD	Early Allograft Dysfunction
EC	European Commission
ECD	Extended Criteria Donor
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ESOT	European Society for Organ Transplantation
ET-DRI	Eurotransplant Donor Risk Index
FP7	Seventh Framework Programme
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl Transpeptidase
GST	Glutathione S-Transferase
HCC	Hepatocellular Carcinoma
HD	Haemodialysis
HDF	Haemodiafiltration
HDU	High Dependency Unit
HF	Haemofiltration
HMP	Hypothermic Machine Perfusion
IFU	Instructions for Use
IL6	Interleukin 6
INR	International Normalised Ratio
IQR	Interquartile range
IRB	Institutional Review Board
ITU	Intensive Care Unit
ITT	Intention-To-Treat analysis
IUD	Intrauterine Device
IVC	Inferior Vena Cava
MAP	Mean Arterial Pressure
MEAF	Model for Early Allograft Function

MELD	Model for End Stage Liver Disease
MHRA	Medicines and Healthcare Products Regulatory Authority
MRCP	Magnetic Resonance Cholangiopancreatography
NHSBT	National Health Service Blood and Transplant
NMP	Normothermic Machine Perfusion
OCTRU	Oxford Clinical Trials Research Unit
PGD	Primary Graft Dysfunction
PH	Proportional Hazards
PIL	Patient Information Leaflet
PNF	Primary Non-Function
PP	Per-Protocol analysis
QUOD	Quality in Organ Donation
R&D	Research and Development
REC	Research Ethics Committee
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SC	Stratified Cox model
SCD	Standard Criteria Donor
SCS	Static Cold Storage
SD	Standard Deviation
SITU	Surgical Intervention Trials Unit
SME	Small and Medium-sized Enterprises
TMC	Trial Management Committee
TNF	Tumor Necrosis Factors
UK-DRI	United Kingdom Donor Risk Index
USADE	Unanticipated Serious Adverse Device Event
UW	University of Wisconsin
vWF	Von Willebrand Factor
WP	Work Package

11.1 Introduction

This document details the proposed presentation and analysis for the main paper(s) reporting results from *the European Union Seventh Framework Programme (FP7) funded multicentre randomised controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation (WP2).* The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

The analysis strategy will be available on request when the principal papers are submitted for publication in a journal. Suggestions for subsequent analyses by journal editors or referees, will be considered carefully, and carried out as far as possible in line with the principles of this analysis strategy; if reported, the source of the suggestion will be acknowledged.

Any deviations from the statistical analysis plan will be described and justified in the final report of the trial. The analysis should be carried out by an identified, appropriately qualified and experienced statistician, who should ensure the integrity of the data during their processing. Examples of such procedures include quality control and evaluation procedures.

11.2 Key personnel

Trial statistician(s):
Ms Virginia Chiocchia
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DMC Members:

(1) Josep Grinyo – Chair
    Location: Barcelona, Spain
    Expertise: Transplantation
(2) Chris Watson – Vice Chair
    Location: Cambridge, UK
    Expertise: Transplantation[1]
(3) Gabriel Oniscu – Member
    Location: Edinburgh, UK
    Expertise: Transplantation
(4) Susan Charman – Member
    Location: London School of Hygiene and Tropical Medicine, UK

[1] Chris Watson and Josep Grinyo are the Chair and the Vice-Chair, respectively, of the COPE DMC but Josep Grinyo will take the place of DMC Chair for this trial to ensure independence, due to Cambridge being a participating centre.

Expertise: Clinical trials and Medical statistics

(5) Marco Senzolo – Member (replaced Patrizia Burra)
Location: Italy
Expertise: Hepatology

TMC Members:

Professor Peter Friend – Chief Investigator (Chair)
Professor Rutger Ploeg – COPE Coordinator
Professor Constantin Coussios – Central Investigator
Mr Simon Knight – Central Investigator
Mr David Nasralla – Central Investigator
Professor Andreas Paul – Local Lead Investigator
Professor Jaques Pirenne – Local Lead Investigator
Professor Juan Carlos García-Valdecasas – Local Lead Investigator
Professor Mr Wayel Jassem – Local Lead Investigator
Mr Hynek Mergantal – Local Lead Investigator
Mr Andrew Butler – Local Lead Investigator
Mr Charles Imber – Local Lead Investigator
Mr Zeeshan Akhtar – WP7 Representative
Mrs Susan Dutton – OCTRU Lead Statistician
Miss Virginia Chiocchia – Trial Statistician

WP8 Members:

CET:

Peter Morris – Chair, Director
Simon Knight – Academic Clinical Lecturer
Liset Pengel – Epidemiologist
John O'Callaghan – Research Assistant

Clinical Trial Unit:

Susan Dutton – OCTRU Lead Statistician
Allyson Bradley – COPE Central Trials Manager
Virginia Chiocchia – Trial Statistician
Rajeev Kumar – Database Manager

11.3 Changes from previous version of SAP

Issue Date	Details
04Feb2015	First version of SAP based on Protocol version 2.0 14th October 2014.
22Mar2016	Second version of SAP based on Protocol version 3.0 11th February 2016 including the following changes: – Patrizia Burra removed as DMC member (Section 1.1) – Primary analysis timepoint updated from 6 months to 30 days following new protocol version (Section 3.2) – Mention of Data Management Plan in Section 4 removed as none present for the study – MELD and DRI formulas updated; eGFR equation added (Section 6.2) – Baseline characteristics table updated (Section 6.2) – Patient groups for analysis (Section 7) updated with specific protocol deviations added – Subgroup analysis (section 8.5) updated – Secondary outcomes analysis (Section 9 and subsections) updated and clarified – Exploratory analyses (Section 10.1) added
17Oct2016	Third version including following changes: – New hepatologist added in DMC memberships (Section 1.1) – Flow diagram refined (Section 6.1) – Baseline characteristics table and section updated with further characteristics and p-value reporting for recipient characteristics (Section 6.2); UK-DRI formula updated – Summary of intervention details (Section 6.5) updated – Secondary analysis and subgroup analysis of primary outcome updated (Section 8) – Secondary outcomes analysis (Section 9 and subsections) updated and clarified – Exploratory analyses (Section 10.1) updated

11.4 Background Information

The experimental precursor to the proposed normothermic perfusion system was developed 15 years ago and the method of perfusion of the isolated pig liver with autologous blood has since been extensively tested and refined, although the overall design of the perfusion circuit remains unchanged [31]. The circuit incorporates a centrifugal pump, membrane oxygenator, and heat exchanger. Arterial perfusion is directly pumped and the portal vein is perfused via a soft-shell reservoir using gravitational force. The addition of various substrates to the perfusion solution enables maintenance of metabolic function [62].

The initial preservation experiments were carried out to compare preservation by warm perfusion with conventional cold preservation [32]. Porcine livers were retrieved and stored for a period of 24 hours, either flushed with UW solution and placed in an icebox or attached immediately to the preservation circuit. Both groups of livers were then reperfused on the circuit for 24 hours (as a surrogate for transplantation) and markers of cellular injury and of synthetic and metabolic liver function were measured. These experiments demonstrated significant superiority of normothermic machine perfusion in terms of haemodynamic, biochemical and histological parameters

Subsequent experiments investigated the use of oxygenated, normothermic perfusion in an experimental setting that reflected the clinical situation of DCD donor organ retrieval [33]. Perfusion with normothermic blood was again compared with static cold storage after 60 min of warm ischaemia. Normothermic perfused livers demonstrated recovery of function by synthetic function, substrate utilisation and perfusion hemodynamics. Furthermore these livers displayed less cellular injury as shown by hepatocellular enzymes. In contrast, cold stored livers showed no evidence of viability during reperfusion and massive necrosis on histological examination.

It is recognised that the combination of warm ischaemia and conventional cold preservation leads to a poor outcome in DCD liver transplantation [6]. In the experimental setting, it is possible to institute warm perfusion with minimal exposure of the organ to cooling. However, in contrast, the logistics of clinical multi-organ retrieval in a distant donor hospital are complex and would be simplified by a period of cold preservation prior to normothermic preservation. This would enable the liver to be retrieved in the normal way, transported in an ice box and then attached to the perfusion machine once back at the base hospital. This scenario was simulated in the same experimental model by inserting a period of cold preservation prior to normothermic preservation [34]. Porcine livers were subjected to 60 minutes of warm ischaemia and then assigned to either normothermic preservation for 24 hours or cold preservation in University of Wisconsin solution for 4 hours followed by 20 hours normothermic preservation to achieve a total preservation time of 24 hours [34]. Livers that underwent normothermic preservation throughout had superior bile production, metabolic activity (base deficit and greater glucose use), and less hepatocellular damage (transaminase levels), and sinusoidal endothelial cell dysfunction (hyaluronic acid). The histology of livers that had been exposed to 4 hours of cold preservation before normothermia showed more necrosis and destruction of architecture. A similar study investigated 60 minutes of warm ischemia followed by 1 hour of cold preservation before 23 hours of normothermic perfusion [35]. This also showed evidence of increased hepatocellular injury, sinusoidal cell injury, but no detriment in terms of protein synthesis (factor V), bile production or histological features. These studies, therefore, demonstrated the need for the warm preservation device to be transportable so that normothermic preservation can be instituted with a minimal period of cooling at the time of organ retrieval.

In order to confirm these results in a preclinical model of organ transplantation, a series of liver transplants in a pig model was performed [36]. In these experiments pig livers were cold-preserved or warm-preserved (using the same machine perfusion methodology as before) for either 5 hours or 20 hours, followed by liver transplantation. As a model of DBD and DCD clinical scenarios, organs were cold-perfused *in situ* either at the time of cessation of circulation (as in a DBD organ donation) or after 40 and 60 minutes of warm ischaemia (simulating DCD organ donation). The two

preservation times were selected because 5 hours is comfortably within, and 20 hours substantially beyond, the limit of the conventional cold preservation technology in pigs (in which a generally accepted limit for survival is 12 hours). Similarly the 40 and 60 minute periods of warm ischaemia are considerably longer than would be acceptable in current clinical practice where warm ischaemia rarely exceeds 30 minutes. Indeed, success at 40 minutes would raise the realistic prospect of transplantation of donor livers from uncontrolled DCD donors.
There was no difference in outcome between the two groups at 5 hours of preservation. After 20 hours of preservation, there were significant advantages consistently in warm compared to cold preservation of both DBD and DCD organs. These advantages applied to postoperative enzyme release and animal survival. Notably, in the 20 hour warm-preserved groups, there was no difference in survival or postoperative transaminase levels in recipients of DBD compared to DCD (40 minute warm ischaemia) donor organs (86% versus 83%). At 60 minutes of warm ischaemia and 20 hours normothermic preservation, however, there were no survivors.
Analysis of haemodynamic, synthetic and metabolic parameters showed that those groups of livers that subsequently went on to successful transplant were predictable before transplantation on the basis of portal flow/pressure, acid-base homeostasis and several other biochemical parameters [36]. It may be concluded that normothermic perfusion, in this context, is not only a more effective means of organ preservation than conventional cold storage, but also that this method can be configured to provide an effective means of viability assessment [46].
The prototype version of the automated clinical investigation device has been tested and demonstrated to be effective during pre-clinical studies in which human livers, discarded as unsuitable for transplantation, were perfused for 24 hours. 13 such livers were perfused with human blood and the perfusion characteristics and control algorithms have been shown to be equally applicable to human as to pig livers (manuscript in preparation). More recently, the clinical trials device has been tested, using livers declined for clinical transplantation, and all key functional aspects of the device shown to be operational, including particularly transport to the donor hospital, automation and 24 hour perfusion.

Phase 1 clinical trial data

A phase 1 clinical trial was opened at King's College Hospital in 2012 and extended to the Queen Elizabeth Hospital, Birmingham in 2013. The first patient was transplanted with a normothermically-perfused liver in February 2013. As of December 27th 2013, the trial completed recruitment and transplanted the twentieth recipient with a liver preserved using the OrganOx *metra* device (in the configuration intended for the COPE study). In all these cases, perfusion parameters were stable with good acid-base maintenance. Postoperatively, all patients have made good recoveries.

11.5 Objectives

Hypothesis

Normothermic machine perfusion (NMP) is superior to static cold storage (SCS) of human liver allografts for reduction of preservation injury.

Primary objective

To compare the effect of NMP to SCS in the prevention of preservation injury and graft dysfunction, as measured by peak transaminase levels in the first week following transplantation.

Secondary objective

- To compare graft and patient survival between NMP and SCS livers
- To compare biochemical liver function between NMP and SCS livers

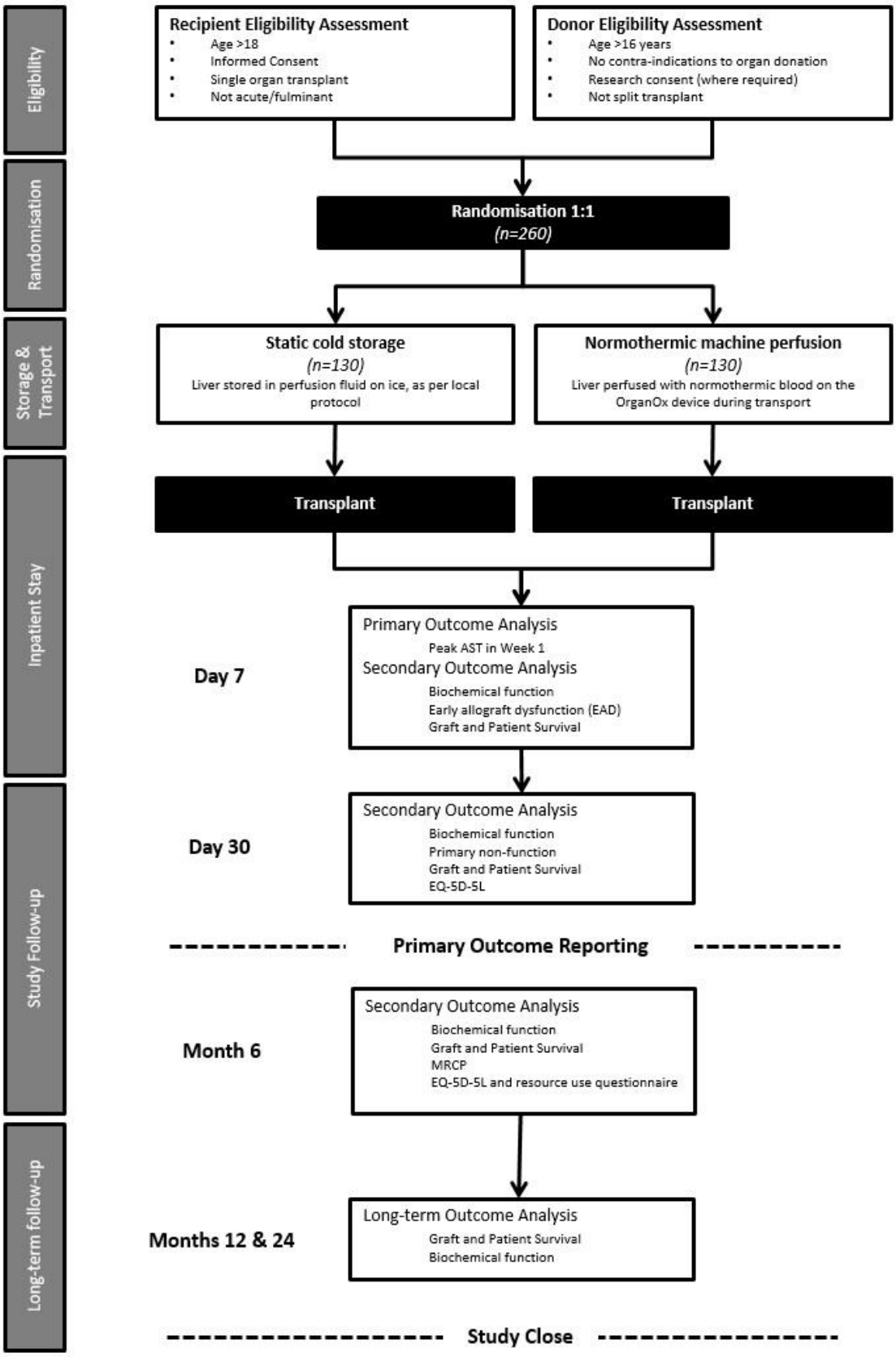
- To compare evidence of post reperfusion syndrome between NMP and SCS livers on implantation
- To compare evidence of ischaemia reperfusion injury between NMP and SCS livers
- To compare evidence of ischaemic cholangiopathy between NMP and SCS livers
- To assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation
- To assess the feasibility and safety of NMP as a method of organ storage and transportation
- To assess the health economic implications of normothermic liver perfusion

11.6 Study Design

This is a randomised controlled, non-blinded, clinical trial comparing static cold storage (SCS) to normothermic machine perfusion (NMP) for organ preservation prior to liver transplantation.
Following assessment of donor and recipient eligibility and confirmation of consent, the liver will be randomised to either NMP or SCS. At the end of preservation, the liver will be transplanted and the patient managed according to standard local practice and protocols.
Enrolled patients will participate in the study for 6 months, with outcomes assessed during the initial inpatient stay and at study visits at day 30 post-transplant and at month 6 post-transplant. Additional biochemical and survival data will be collected from routine clinical measurements taken in participating centres at 12 months and 24 months post-transplant.
Primary outcomes will be analysed and reported 30 days following enrolment of the last patient to the study. The study will close after the final patient has completed 24 months follow-up.
Anticipated flow of patients through the trial is depicted in Figure 26.

<u>Anticipated trial dates</u>

Date of start of recruitment:	May 2014
Date of expected end of recruitment:	June 2016
Date of expected primary analysis:	December 2016
Date of expected long-term follow-up analysis:	June 2018
Target number of subjects (livers):	220 transplanted livers (110 per arm) 260 randomised livers (130 per arm)
Participating Centres:	Addenbrooke's Hospital, Cambridge, UK King's College Hospital, London, UK Queen Elizabeth Hospital, Birmingham, UK Royal Free Hospital, London, UK Hospital Clinic Barcelona, Spain University Hospital, Essen, Germany University Hospitals, Leuven, Belgium



*Randomisation performed soon after organ retrieval by recipient centre

Figure 26. Flow of participants/livers through the trial

11.7 Eligibility

All eligibility criteria must be met at the time of randomisation.

11.7.1 Donor criteria

Inclusion: Donors over the age of 16 years. Liver allografts from donation after brain death (DBD), standard and extended criteria donors (SCD, ECD) and donation after circulatory death (DCD) donors.
Exclusion: Living donors; liver intended for split transplant; donor age <16 years; liver in which investigator is unwilling to randomise to either arm.

11.7.2 Recipient criteria

Inclusion: Adult patients (18 years or more), active on the waiting list for liver transplantation; able to give informed consent.
Exclusion: Age less than 18 years; acute/fulminant liver failure; transplantation of more than one organ (e.g. liver and kidney); refusal of informed consent; unable to give informed consent.

11.7.3 Donor assessment

The transplant recipient co-ordinator is usually an advanced practice nurse, one of whose roles is to facilitate calls for organ offers and co-ordinate all aspects of the transplant process. On receiving an organ offer, the local recipient co-ordinator/surgeon will ascertain baseline demographic information from the offering organisation to assess eligibility of the liver for inclusion in the trial.

11.7.4 Recipient assessment

All patients on the transplant waiting list in participating centres will have been screened for suitability for transplantation; further screening assessment is not required as part of the present trial. On offer of a suitable donor organ consent will be confirmed by signing and dating the informed consent form for a second time; this process may also be done with a documented phone call. The online randomisation tool will require confirmation that the recipient meets the inclusion criteria for the trial and require the entry of baseline demographic information prior to randomisation and release of the randomisation code.
On admission to hospital, the recipient will be assessed for fitness to proceed to transplant according to local procedures. If a recipient is deemed unfit for transplant at the time of admission[2], they will no longer be active on the transplant waiting list and as such will be excluded from the trial. Any data collected from these individuals will not be included in the trial and will be deleted.

11.8 Treatment Interventions

On receipt the organ will be assessed and if not suitable for transplantation this will not go ahead.

11.8.1 NMP Group

If the liver is randomised to the NMP group, arrangements will be made to transport the OrganOx *metra* device to the donor hospital (see section 7.6). The recipient co-ordinator will also request that the donor co-ordinator arrange for 3 units of donor-type red blood cells to be cross-matched at the

[2] In UK, livers allocated to a recipient deemed unfit and so excluded from the trial are usually allocated to another recipient in the same hospital, so personnel will try to allocate them to the next patient who has consented.
In the other countries (participating centres) these livers will probably be lost from the trial.

However, patients unfit for transplantation are expected to be very few, probably less than 5%.

donor centre for use as the perfusate in the device. Following the routine retrieval procedure at the donor hospital the liver will be placed in ice-cold perfusion solution (according to local protocol) on the back-table, and prepared for cannulation. The procedure for preparing the device for use and placing the organ on the device is described in detail in the device instructions for use (IFU) document. The device is then transported to the recipient transplant centre. The procedure for removing the liver from the device is also described in the IFU. Implantation and reperfusion of the liver proceed as per the usual practice of the implanting centre. The duration of machine perfusion will be dictated by logistics and local policy, but should not be less than 4 hours or more than 24 hours.
If cannulation proves impossible, the liver will be transported using standard static cold storage as described below. Results will be analysed in the randomised group (intention-to-treat)[3].

11.8.2 SCS group

Following the routine retrieval procedure, the liver will be placed in ice-cold perfusion solution (according to local protocols) on the back-table, followed by storage in cold perfusion solution within an icebox. The organ will be transported to the recipient centre, and removed from storage prior to implantation for standard back-table preparation. The duration of cold storage will be dictated by logistics and local policy.

11.9 Sample Size

Data from 416 liver transplant recipients from University Hospital Essen demonstrate the geometric mean of peak AST to be 608.59 IU/L (the geometric mean is used as peak AST is non-normally distributed). 220 transplants (110 per arm) would have 90% power at 5% significance level to detect a 33% reduction (to 401.67 IU/L) in the geometric mean of peak AST.
2011/12 NHSBT data suggest that 12% of livers retrieved are not transplanted. Assuming losses of 15%, randomisation of 260 livers into the trial will be required to achieve adequate power.
Data from a study of hypothermic machine perfusion of human livers demonstrate an approximate 35% reduction in the peak AST with machine preservation when compared to historical controls undergoing static cold storage [24]. It is expected that normothermic machine perfusion will be at least as effective as hypothermic machine preservation in preventing reperfusion injury. In studies of a porcine model of DBD liver transplantation, normothermic machine perfusion led to a 52% reduction in peak post-transplant AST [36]. In a DCD model, the reduction was 73%.
Given the relationship between peak AST and primary non-function, graft and patient survival described above [81, 84], a 33% reduction in peak AST is likely to represent a clinically significant difference in outcome between the study arms.
It is recognized that a proportion of DCD donor livers randomized in the study will not proceed to donation due to the donor not arresting within the time defined by local protocols. These livers will be replaced in the study so that the numbers above reflect the number of livers actually retrieved.

The sample size calculation has been performed using R statistical software, whose output is shown below:

```
> exp(mean(log(peakast)))
[1] 608.5898
```

[3] In phase I trial "aberrant arterial anatomy", which prevents from performing cannulation, happened in 10-15% of cases but the surgeon was able to reconstruct it, so no liver was actually moved to SCS.
In phase III this is less likely to happen, but an estimated percentage cannot be provided.

```
> exp(mean(log(peakast)))*0.66
[1] 401.6693
> control <- exp(mean(log(peakast)))
> study <- exp(mean(log(peakast)))*0.66
> log(control)-log(study)
[1] 0.4155154
> sd(log(peakast))
[1] 0.9456289
> (log(control)-log(study))/sd(log(peakast))
[1] 0.4394065
> pwr.t.test(d=0.4394, sig.level=0.05, power=0.90, type="two.sample", alternative="two.sided")
Two-sample t test power calculation
n = 109.8137
d = 0.4394
sig.level = 0.05
power = 0.9
alternative = two.sided
```

The actual number of livers needed, assuming 15% of losses, was then calculated by hand based on the following formula:

$$N_A = \frac{N}{1-p} = \frac{220}{1-0.15} = 258.82 \sim 260$$

Where p is the proportion of losses, N is the total sample size and N_A is the total number needed to achieve N considering the loss rate.

11.10 Strategies for achieving adequate recruitment

The emergency nature of liver transplantation means that once a potential recruit is called in for a transplant there will only be a 3-4 hour window for the consent and screening process to occur. This does not allow sufficient time for the potential participant to consider the implications of participating in the study. For this reason, all patients who fulfil the entry criteria and who are on the waiting list for liver transplantation at the participating centres will be approached in advance of the study either during a routine clinic appointment, inpatient admission or in advance of discussion by letter. If the patient expresses interest in the study, a face-to-face meeting will be arranged during a routine admission or outpatient appointment. Detailed information will be given both verbally and in the form of a patient information sheet. The study coordinator and/or a medically qualified researcher will give information.

11.11 Randomisation

Full details will be stored in a separate Randomisation and Blinding Plan document to ensure adequate concealment of the schedule.

11.11.1 Sequence generation

Participants will be randomly assigned to NMP or SCS with 1:1 allocation as per a computer generated randomisation schedule using variable block randomisation using the following stratification factors: participating (recipient) centre and by donor type (DBD or DCD).

11.11.2 Allocation concealment mechanism

Allocation concealment will be ensured by use of central computerised randomisation (with telephone backup). Allocation will not be revealed until the patient has been recruited to the trial and donor and recipient baseline characteristics have been recorded. Random permuted block length will be used; block sizes will not be disclosed.

11.11.3 Implementation

Prior to study enrolment, the local investigator will confirm the availability of the NMP device. Once informed consent has been obtained from the potential recipient of an organ offer, the local investigators will login to an online data collection and randomisation tool. This will require confirmation of consent, as well as compliance with inclusion and exclusion criteria. Baseline recipient and donor characteristics will be recorded by the recipient co-ordinator and are required prior to release of the randomisation group. This will ensure that the patient is eligible for inclusion in the trial prior to allocation.

11.11.4 Blinding/Masking

Whilst it is not possible to blind the local investigators to the method of organ preservation, outcome assessors will be blinded where possible. This includes the histopathologist interpreting the biopsy specimens as well as the radiologist interpreting the 6-month MRCP images.
Note the primary outcome and many secondary outcomes are objective measures, so blinding is not a requirement.

Randomisation is expected to occur before retrieval of the liver, provided that both donor and recipient exclusion and inclusion criteria are met. However, note that it is expected that in some European participating centres this may occur at a slightly different time-point i.e. just after assessment of the organ upon retrieval. Therefore, in such cases there will be a smaller rate of liver withdrawn from the trial for reasons like DCD donor not proceeding and organ deemed not transplantable.

11.12 Primary and Secondary Outcomes

Primary outcome
The primary endpoint is defined as the difference in peak serum aspartate transaminase level (AST) within 7 days post-transplant between the two treatment arms. Serum AST will be measured daily during the first post-transplant week, and the peak level will be defined as the highest of these values (in IU/L). In order to ensure consistency, the first post-transplant measurement should be taken at 12 to 24 hours post-reperfusion.

A number of studies have demonstrated a relationship between peak AST in the early post-transplant period and patient survival, graft survival, early graft dysfunction and primary non-function following liver transplantation [81, 84]. Peak AST is also significantly elevated in liver allografts with histological evidence of moderate to severe perfusion injury [85, 86].

Secondary outcomes

Objective	Outcome Measures

To compare graft and patient survival between NMP and SCS livers.	4. Primary non-function: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes. 5. Graft survival at 30 days and 6, 12 and 24 months following transplantation. 6. Patient survival at 30 days and 6, 12 and 24 months following transplantation.
To compare biochemical liver function between NMP and SCS livers.	5. Serum bilirubin, GGT, AST, INR and creatinine daily at days 1-7 following transplantation then at day 30 and months 6, 12 and 24 following transplantation. 6. Daily serum lactate at days 1-7 whilst in high level (ITU/HDU) care 7. Early allograft dysfunction (EAD) [79]; defined by any one of: a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant b. INR >1.6 on day 7 post-transplant. c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant
To compare the physiological response to reperfusion between NMP and SCS livers	6. Post-reperfusion syndrome, defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than one minute during the first five minutes after reperfusion. This will be assessed in the context of vasopressor use [87, 88] 7. Length of stay in high level (HDU/ITU) care 8. Length of hospital stay 9. Need for renal replacement therapy (haemodialysis, haemofiltration, haemodiafiltration)
To compare evidence of reperfusion injury between NMP and SCS livers.	Histological evidence of reperfusion injury in post-reperfusion biopsies (taken immediately prior to abdominal closure). These will be compared to baseline pre-reperfusion biopsies (on removal of the liver from SCS/NMP) and graded using standard histological criteria [85, 89]
To compare evidence of ischaemic cholangiopathy between NMP and SCS livers.	Evidence of biliary stricturing on magnetic resonance cholangiography (MRCP) at 6 months post-transplant.
To assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation.	5. Perfusion parameters (logged automatically by the device): a. Arterial and caval pressures (in mmHg) b. Arterial, portal and caval flow rates (in mmHg) c. pO_2, pCO_2 and pH d. Blood temperature (°C), Glucose (mmol/L) and bile production (ml/h) 6. Perfusate ALT and AST at 15 minutes, 1 hour and the end of NMP 7. Perfusate IL6, TNF, vWF at 15 minutes, 1 hour and the end of NMP 8. In addition to these pre-specified outcomes, additional biological samples will be taken for the COPE WP7 bioresource at specified timepoints as detailed in protocol appendix A1.
To assess the feasibility and safety of NMP as a method of	4. Organ discard rate 5. Perfusate culture. At the end of preservation a sample will be taken for microbiological culture (cold preservation or warm perfusate).

organ storage and transportation.	6. Adverse event rates and severity, graded according to the Clavien-Dindo classification [90]. a. Recipient infection b. Biopsy proven acute rejection c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks) d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis) e. Reoperation rate f. Technical complications/device failures
To assess the health economic implications of normothermic liver perfusion.	Full health economic analysis utilising: 4. Logistical costs, measured using national unit costs where available. 5. Healthcare resource use; measured by a combination of hospital episode records and a patient-completed resource use log. 6. Quality of life by delivery of the EQ-5D-5L questionnaire at baseline, day 30 and month 6 post-transplant.

11.13 Outcomes Assessment Schedule

Activity	Pre-study Screening	Pre-study Baseline	Pre-storage	Pre-reperfusion	Post-reperfusion	Postoperative									Follow-up		
						D1	D2	D3	D4	D5	D6	D7	D10	D30	M6	M12	M24
Informed consent	X																
Meets inclusion/ exclusion criteria	X																
Randomisation		X															
Donor & recipient demographics		X															
Perfusion parameters/samples				X													
Surgical variables					X												
Graft biopsy			X	X	X												
CBD biopsy					X												
Serum AST						X	X	X	X	X	X	X		X	X	X	X
Serum Bilirubin						X	X	X	X	X	X	X		X	X	X	X
Serum GGT						X	X	X	X	X	X	X		X	X	X	X
Serum Creatinine						X	X	X	X	X	X	X		X	X	X	X
INR						X	X	X	X	X	X	X		X	X	X	X
Serum lactate*						X	X	X	X	X	X	X					
Primary non-function													X				
Graft survival						X	X	X	X	X	X	X	X	X	X	X	X
Patient survival						X	X	X	X	X	X	X	X	X	X	X	X

MRCP														X			
Quality of life (EQ-5D-5L)	X												X	X			
Resource use log													X	X			
Safety outcomes					X	X	X	X	X	X	X	X	X	X	X	X	

* Serum lactate will be recorded daily whilst the recipient is admitted to high level (ITU/HDU) care.

11.14 Data Management Responsibility

Data management responsibility is detailed in a separate data management plan prepared by the trial coordinator, the trial statistician and the database manager.

11.15 Quality Control and Data Validation

Source documents are where data are first recorded, and from which participants' eCRF data are obtained. These include, but are not limited to, hospital records, clinical and office charts, laboratory reports, pharmacy records, subject diaries or logs, microfiches, radiographs, correspondence, device accountability records, recorded data from automated instruments and copies or transcriptions certified after verification as being accurate and complete.
eCRF entries will be considered source data if the eCRF is the site of original recording (e.g. there is no other written or electronic record of the data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name. Signed consent forms will be kept at the site and not sent to the Trial Office.
The central database will be monitored for discrepancies and missing data. The surgical interventional trials unit (SITU) will be responsible for managing the database, and if such discrepancies are identified the trial manager will be responsible for identifying the problem and contacting the local centre to ensure resolution. The trial manager will be responsible for the production of reports to each participating centre containing information and details of missing data or missed visits requiring completion.

11.16 Data monitoring Committee and Interim Analyses

The trial has a data monitoring committee (DMC) which consists of five independent members, including clinicians with relevant expertise and a statistical expert, independent from the Investigators and the funding source. The DMC will periodically review (approximately 6 monthly) accruing data to safeguard the interests of the trial participants, potential participants and future patients and assess the safety of the interventions. The DMC will advise the Trial Management Committee and WP8 if, in its view, the study should be terminated due to major clinical disadvantages in one of the study arms.

Interim analyses of primary and secondary efficacy outcomes are not planned. They will only be performed if requested by the DMC on the grounds of participant safety.

A separate DMC charter will contain full details of the committee, its roles and reporting structure and details of interim analyses.

11.17 Descriptive Analyses

11.18 Representativeness of Study Sample and Patient Throughput

Participants will be adult patients active on the waiting list for liver transplantation at any of the participating transplant centres. Patients screened for eligibility, reasons for exclusion and the flow of livers and recipients through the study will be summarised in the following adapted flowchart.

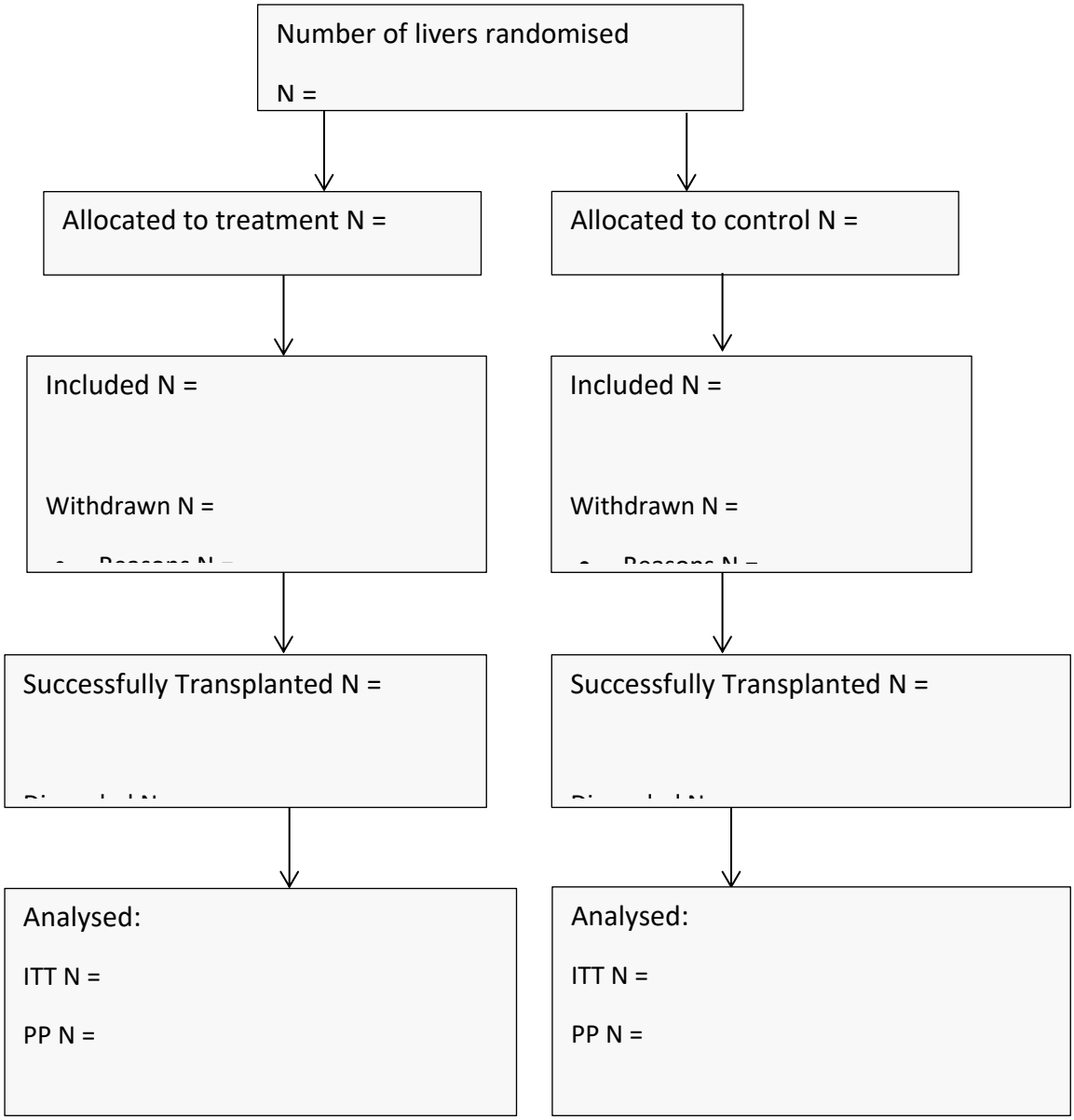


Figure 27. Adapted flowchart for WP2

11.19 Baseline Comparability of Randomised Groups

Participants will be described with respect to the stratification factors (participating centre and donor type) and to both donor and recipient demographics and other key prognostic factors at baseline, both overall and separately for the two randomised groups.

MELD Score will be described overall and separately by country due to variations in national donor rates causing likely differences in average MELD scores in different countries.

The formula to calculate MELD Score from serum creatinine, bilirubin and INR, developed and validated by Kamath and Kim [122] is the following:

$$MELD\ Score = 0.957 \times \ln\left[serum\ creatinine\left(\frac{mg}{dL}\right)\right] + 0.378 \times \ln\left[serum\ bilirubin\left(\frac{mg}{dL}\right)\right] + 1.120 \times \ln[INR] + 0.643$$

The resulting score will be multiplied by 10 and rounded to the nearest whole number.
UNOS has made the following modifications to the score [123]:

- If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0
- Any value less than one is given a value of 1 (i.e. if bilirubin is 0.8, a value of 1.0 is used) to prevent the occurrence of scores below 0 (the natural logarithm of 1 is 0, and any positive value below 1 would yield a negative result).

DRI will be described overall and calculated using both the UK-DRI and the Eurotransplant DRI formulas:

$$\textit{UK-DRI} = exp\big[2.3159 + (0.9106\ if\ DCD) - 0.01434 \times \big(height(cm)\big) + (0.3058\ if\ history\ of\ cardiac\ disease) + (0.2545\ if\ steatosis\ present) + 0.01222 \times \big(bilirubin(\mu mol/L)\big) + (0.1736\ if\ positive\ smoking\ history) + (0.6453\ if\ black\ ethnicity)\big]$$

There is currently no reference for this model as the background information is currently being prepared for publication. The formula was kindly provided to us by the Associate Director of NHSBT Statistics and Clinical Studies after the DMC Chair's recommendation of using this rather than the EuroTransplant DRI.
The UK-DRI formula is not applicable to livers from outside the UK. So for livers from Essen, Leuven and Barcelona the Eurotransplant DRI formula will be applied instead:

$$\textit{ET-DRI} = exp\big[0.960\big((0.154\ if\ 40 \le age < 50) + (0.274\ if\ 50 \le age < 60) + (0.424\ if\ 60 \le age < 70) + (0.501\ if\ 70 \le age) + (0.079\ if\ COD = hypoxia) + (0.145\ if\ COD = CVA) + (0.184\ if\ COD = other) + (0.411\ if\ DCD) + (0.105\ if\ different\ retrieval\ team)\big) + 0.06((latest\ lab\ GGt(U/L) - 50)\ /\ 100)\big]$$

The above formula has been adapted for the purposes of this study from the one developed by Braat et al [124].

The calculation of eGFR is based on the CKD-EPI Creatinine equation [125] as follows:

$$eGFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018[if\ female] \times 1.159[if\ black]$$

Scr is serum creatinine in mg/dL;
κ = 0.7 for females
κ = 0.9 for males;
α = –0.329 for females
α = –0.411 for males.

Serum creatinine can be recorded in mg/dL or in µmol/L. However, it will be reported and used in mg/dL for the purposes of analysis so all values will need to be converted $\left(\text{mg/dL} = {}^{\mu\text{mol/L}}/_{88.4}\right)$.

Numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with lower and upper quartiles) for continuous variables will be presented.

There will be no tests of statistical significance nor confidence intervals for differences between randomised groups for stratification factors and donor characteristics. However, due to the nature of the study (the randomisation unit is the donated liver and not the participant) and to a potential differential discard rate between the two groups, an appropriate statistical test will be applied for recipient characteristics and a p-value reported to demonstrate that the final recipients were well matched.

Stratification factors	**NMP (N =)**	**SCS (N =)**	**Total (N =)**
Centre*			
Addenbrooke's Hospital, Cambridge, UK			
King's College Hospital, London, UK			
Queen Elizabeth Hospital, Birmingham, UK			
Royal Free Hospital, London, UK			
University of Barcelona, Spain			
University Hospital, Essen, Germany			
University Hospitals, Leuven, Belgium			
Donor type*			
DBD			
DCD			
Donor demographics	**NMP (N =)**	**SCS (N =)**	**Total (N =)**
Gender*			
Male			
Female			
Age^			
Ethnicity*			
Caucasian			
African-Caribbean			
Other			
Cause of death			
CVA			
Hypoxia			
Trauma			
Other			
BMI^			
UK-Donor risk index^			
ET-Donor risk index^			
Recipient demographics at randomisation	**NMP (N =)**	**SCS (N =)**	**Total (N =)**
Transplant centre*			
Addenbrooke's Hospital, Cambridge, UK			
King's College Hospital, London, UK			
Queen Elizabeth Hospital, Birmingham, UK			
Royal Free Hospital, London, UK			
University of Barcelona, Spain			
University Hospital, Essen, Germany			

University Hospitals, Leuven, Belgium

Gender*

Male

Female

Age^

Cause of Liver Failure*

Alcoholic

Auto-Immune Hepatitis

Drug Induced

Hepatitis B

Hepatitis C

Hepatocellular Carcinoma on background of Cirrhosis

Hepatocellular Carcinoma without Cirrhosis

Metabolic

Non-Alcoholic Fatty Liver Disease

Non-Alcoholic Steato-Hepatitis

Other Cancers

Primary Sclerosis Cholangitis

Primary Biliary Cirrhosis

Other

BMI^

MELD score^

UK

Essen, Germany

Leuven, Belgium

Barcelona, Spain

eGFR^

**Frequency and percentages are displayed*
^Median, IQR and range are displayed

11.20 Comparison of Losses to Follow-up

The numbers (with percentages) of losses to follow-up will be summarised at each stage. Those lost to follow-up will be reported and compared (where appropriate) between the NMP and SCS groups with absolute risk differences (95% confidence interval [CI]). Any deaths (and their causes) will be reported as described in the secondary outcomes analysis section.

11.21 Description of Available Data

It is anticipated very few patients will be lost to follow-up, but it is likely that not all measurements at all time points will be recorded for every recipient. Methods to handle missing data are described in section 8.4.

HRQL questionnaire, EQ-5D-5L, will be collected at baseline (pre-study screening) and at each early follow-up visit following liver transplantation (day 30 and month 6). Details of completed forms will be provided for each follow-up point, overall and separately for the two treatments and will include the numbers of forms expected, received and the compliance rate as in the following table.

Time Point	Overall		
EQ-5D-5L	Expected	Received	Compliance
Baseline			
Day 30			
6 months			

11.22 Description of Intervention

A summary of the treatment received will be provided, particularly the warm ischaemic time, cold ischaemic time, duration of machine perfusion, total preservation time, implantation time, perfusion parameters (logged automatically by the device) and any procedural complications. They will be reported as frequencies and percentages or median and ranges, as appropriate.
The duration of the total preservation will also be compared between the two groups using non-parametric tests. This is defined as time from "cross clamp time" to "time of arterial/portal reperfusion" (whichever occurs first) in the recipient.
Perfusion parameters collected will be:

1. Arterial and caval pressures (in mmHg)
2. Arterial, portal and caval flow rates (in mmHg)
3. pO2, pCO2 and pH
4. Blood temperature (°C), Glucose (mmol/L) and bile production (ml/h).

Perfusate ALT and AST and perfusate IL6, TNF, vWF will be collected at 15 minutes, 1 hour and the end of NMP.
Pre and post-reperfusion vasopressor and total duration of operation will also be reported as described above.
Protocol deviations (see Section 7) and any conversion from NMP to SCS will be reported with reasons for not receiving the assigned treatment.
Fisher exact test or Chi-squared test will be used to test the association between compliance and treatment group.

11.23 Unblinding of Randomised Treatments

As the endpoints are objective the study is open-label but, where feasible, outcome assessors will be blinded, e.g. histopathologist interpreting the biopsy specimens and radiologist interpreting MRCP imaging.

11.24 Reliability

Calculations performed by the computer will be checked by hand calculations for a minimum of 5% or 20 patients, randomly sampled, where appropriate.

Missing value codes will be checked for consistency and the proportion of missing values per variable will be presented. Patterns of missing data will be explored. Any missing value imputation used will be checked to ensure the missing values have been imputed within the limits of the data.

11.25 Patient Groups for Analysis

All livers/patients who undergo preservation and transplant will be analysed.

Intention-to-treat analysis (ITT): liver analysed in the groups to which their liver is randomly assigned, irrespective of whether the assigned method of preservation is actually used.

Per protocol analysis (PP): Liver analysed as treated; therefore, in the groups of the intervention they actually received. This will be carried out as a sensitivity analysis and will also exclude patients who had the following protocol deviations:

- Machine preservation time <4 or >24 hours;
- Intervention/machine failure that resulted in the treatment not being carried out as per protocol.

Also, in case of change of transplant centre (stratification factor), in the PP analysis the liver will be analysed according to the centre where it was actually transplanted.

11.26 Analyses to address primary aims

It is anticipated that the analysis will be undertaken using STATA, SAS, R or other validated statistical software.
The trial is expected to complete recruitment within 24 months. Primary outcome analysis time point will be 30 days after the last liver has been transplanted.

11.27 Definition of Primary Outcome

Clinically significant response to treatment is defined as a 33% difference in peak serum aspartate transaminase level (AST) within 7 days post-transplant between the two treatment arms.

$$\frac{Peak\ Serum\ AST(SCS) - Peak\ Serum\ AST(NMP)}{Peak\ Serum\ AST(SCS)} \geq 0.33$$

For these analyses of the primary outcome a P-value of 0.05 (5% level) will be used to indicate statistical significance. Exact P values will be presented to three decimal places.

11.28 Statistical Methods Used for Analysis of Primary Outcome

The distribution of peak serum AST between the two arms will be assessed by a Normal plot for each treatment group. If this appears to be normally distributed the difference in peak serum AST between the two groups will be compared by using ANOVA with adjustment for stratification factors: participating (recipient) centre and donor type (DBD and DCD).
If peak serum AST has departure from Normality the first approach will be transformation. If the data cannot transform to Normal distribution, the difference in peak serum AST between arms will be analysed using Mann-Whitney U test. The difference will be presented along with 95% CI for the difference in medians. If a non-parametric analysis needs to be performed there will be no adjustment for stratification factors or other covariates.

11.28.1 Secondary Analysis of Primary Outcome

The analysis comparing the difference in peak serum AST between the two groups using ANOVA will be repeated with additional adjustment for important prognostic factors.

11.29 Adjustment of P values for Multiple Testing

There is no multiple testing as only a single primary outcome is considered. Therefore significance levels used will be 0.05 and 95% confidence intervals will be reported.

Interim analyses of primary and secondary endpoints will not be carried out unless requested by the DMC. In this case p-values of 0.001 will be used for significance.

11.30 Missing Data

All randomised patients completing the 30 days follow-up assessment will be regarded as having completed the primary study. All patients will be encouraged to complete study follow-up, and all reasonable efforts will be made to ensure completeness of follow-up. Measures include ensuring that assessments are made, where possible, at routine hospital visits rather than additional appointments, and that patients do not incur extra financial costs (e.g. travelling costs) as a result of study participation.

It is understood that study participants may withdraw consent for study participation at any time irrespective of their reasons. The investigators may also withdraw a recipient from the study in order to protect their safety and/or if they are unwilling or unable to comply with the required study procedures. We will keep all data accrued to the point of withdrawal unless the participant requests otherwise, as is stipulated in the trial consent form. In the event a patient withdrawing from the trial, the reason for withdrawal must be documented on the eCRF. Such patients will be asked whether they consent to data accrued before the date of withdrawal being included in the trial analysis. A narrative analysis of withdrawals will be performed.

As the primary outcome is the peak serum AST within 7 days post-transplant and there is a very strict protocol for post-operative procedure in place in every centre, it is expected that there will be no/few missing data. Even in case of missing daily values, the peak of available values will be taken, which is expected to occur within the first 36 hours post-transplant. Therefore, no imputation methods will be employed.
Strategies to handle missing data are, therefore, expected to be used only for secondary outcomes analyses or in long-term follow-up (12 and 24 months) analysis and only if the missing rate is 5% or more.

11.31 Pre-specified Subgroup Analysis

Subgroup analyses will be performed for donor type (DCD vs. DBD), donor risk index (ET-DRI) and MELD Score. Value intervals for ET-DRI will be 'low', 'medium' and 'high' based on the 33rd percentiles. Value intervals for MELD Score will be <9, 10-19, 20-29, 30-39, ≥40 based on the UNOS standard reference ranges[93].
The study is not powered to detect differences in the subgroups and should only be regarded as hypothesis-generating. Interaction methods will be used to look for consistency of treatment effect across the different subgroups and reported using forest plots.

11.32 Treatment by Centre Interaction

Consistency of effect will be assessed across the 7 centres by examination of the within centre effects. We are not expecting, however, differences as centre is a stratification factor. Any differences between centres will be explored graphically using forest plots as reported above and no formal test will be undertaken as there will be little power for a test of interaction.

11.33 Sensitivity Analysis

Sensitivity analyses will be carried out for the primary outcome using the per protocol population.

11.34 Analysis to address secondary aims

The secondary aims will determine the effect of NMP compared to SCS on graft and patient survival, biochemical liver function, physiological response to reperfusion, reperfusion injury and ischaemic cholangiopathy. They also will assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation and to assess the feasibility, safety and health economic implications of NMP as a method of organ storage and transportation.

Binary outcomes will be reported in terms of proportions along with 95% confidence intervals and will be assessed using chi-squared test. Logistic regression will be performed to adjust for potential confounders and the treatment difference will be reported using odds ratios with relevant 95% confidence intervals. Continuous outcomes will be reported as mean with standard deviation (SD) or as median and interquartile range (IQR), and compared using the T-test if normally distributed, or by the Mann-Whitney U. The treatment difference will be reported in terms of difference in means together with 95% confidence intervals. Time-to-event outcomes will be analysed using survival analysis methods, including Kaplan-Meier and Cox proportional hazards regression model with calculation of hazard ratios. The proportional hazards (PH) assumption will be tested and if non-proportionality is present appropriate methodology will be used such as stratified Cox (SC) model and extended Cox model containing time-dependent variables. Median survival times will be reported together with 95% confidence intervals.
Outcomes will be reported with 95% confidence intervals and p-values to 3 decimal places. A p-value of less than 0.05 will be regarded as statistically significant.

Primary timepoint for secondary outcomes analysis is at 6 months with long-term follow-up at 2 years.

1.1 Primary Non-Function

Primary Non-Function (PNF) is defined as irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes.

Presence of PNF will be compared across the two randomised groups by performing unadjusted and adjusted analysis as described above to take account of the stratification factors and important prognostic factors such as peak AST, DRI and MELD Score.

11.35 Graft and patient survival

Graft survival is defined as time (in days) from transplant to graft failure. Patients who die with a functioning graft will be censored at their date of death. Patients who are still alive with a functioning graft at the end of the study will be censored at their last known date alive with functioning graft.

Patient overall survival is defined as time (in days) from transplant to patient death. Patients still alive will be censored at their last known alive date.[126]

Graft survival and patient overall survival will be compared across the two groups using the methods described above reporting at 6 months and at long-term follow-up time points. Cox proportional hazards regression model will be performed both in a univariate and multivariate framework adjusting for stratification factors and known or suspected prognostic factors such as donor and recipients demographics (BMI, DRI, indication for transplant, MELD Score).

11.36 Biochemical liver function

Liver function will be explored by assessing biochemical components of the blood serum. These are a number of serum biochemical tests: Biliburin, Gamma glutamyl transpeptidase (GGT), Aspartate transaminase (AST), International normalised ratio (INR), creatinine and lactate dehydrogenase.
All of these tests are interpreted using reference ranges.
The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the International Sensitivity Index (ISI) value for the analytical system used.[127]

$$INR = \left(\frac{PT_{test}}{PTnormal}\right)^{ISI}$$

There are circumstances where the INR is medically corrected. Although this is expected to be very uncommon, cases of INR being medically corrected will be recorded and this value used instead of the ordinary one. Proportion of INR medically corrected will also be reported overall and by treatment arm.
As Bilirubin, GGT, AST, INR and creatinine are measured daily at days 1-7 following transplantation and serum lactate daily at days 1-7 whilst in ITU/HDU care, they will be assessed using a number of different methods: their average value as well as area under the curve (AUC) will be calculated and compared across the two treatment arms.
Bilirubin, GGT, AST, INR and creatinine will also be measured at day 30 and month 6, 12 and 24 following transplantation. A mixed model for repeated measurement will be used at 24 months to compare each of these measures across the two groups.

11.36.1 Early Allograft Dysfunction

Early allograft dysfunction (EAD) has been defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function [79]:

a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant
b. INR >1.6 on day 7 post-transplant.
c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant

In case of missing data in any of the three variables required to define the EAD, this will be considered as present if any of the available values satisfies the specified criteria. In case of Bilirubin and/or INR missing due to the patient being discharged before Day 7 post-transplant, this will be considered as absence of EAD as the recipient would not be discharged earlier if the graft was not functioning. Subjects with missing data due to patient death or graft failure will be excluded from this analysis as expected to be few in numbers in the first 7 days post-transplant.

Presence of EAD will be compared across the two groups using the methods described above to adjust for potential confounders such as DRI and MELD Score.

11.37 Physiological response to reperfusion

Post-reperfusion syndrome is defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than a minute during the first five minutes after reperfusion.

$$\frac{MAP_{baseline} - MAP_{5mins}}{MAP_{baseline}} > 0.30 \text{ mmHg } for > 1min$$

Need for renal replacement therapy indicates the requirement of treatments for renal failure such as haemodialysis, haemofiltration and haemodiafiltration.
Differences in proportions of both post-reperfusion syndrome, need for renal replacement therapy abd post-reperfusion lactate levels between groups will be assessed by performing unadjusted analysis as described above.
Duration of renal replacement therapy at Day 7 will also be compared between the two groups using appropriate tests for continuous outcomes as described above.

Length of hospital stay and length of stay in HDU/ITU care will be summarised as median together with interquartile range and will be compared between the two groups using Kruskal-Wallis test. This will then be used as a resource for the health economic analysis (see Section 9.9).

11.38 Reperfusion injury

Graft biopsies will be taken immediately prior to abdominal closure and examined for evidence of reperfusion injury. These biopsies will be compared to the baseline biopsies prior to organ reperfusion (on removal of the liver from SCS/NMP) and graded according to standard histological criteria [85, 89]. The trial histopathologist, who will be blinded to the method of perfusion, will assess all biopsies.

Evidence of reperfusion injury will be compared across the two treatment groups by performing unadjusted analysis as described above.

11.39 Ischaemic cholangiopathy

All study participants will undergo magnetic resonance cholangiopancreatography (MRCP) with T2-weighted turbo-spin echo sequences at 6 months post-transplant unless contraindicated. The trial radiologist, who will be blinded to the preservation method, will assess all MRCPs. Evidence of ischaemic cholangiopathy will be taken as the presence of extra-anastomotic biliary structuring in the absence of hepatic artery thrombosis [128, 129].

Proportions of MRCP evidence of ischaemic cholangiopathy will be compared across treatment arms by performing unadjusted analysis as described above.

11.40 Perfusion parameters and biomarkers

Perfusion parameters will be collected and used to give a summary of the intervention, as described in section 11.22.

Biomarkers will also be collected to be used with perfusion parameters as described in the Exploratory Analyses section (11.44).

11.41 Feasibility and safety of NMP

Organ discard rate and adverse events rates and severity will be assessed as described in the Safety Analysis section (11.46).

11.42 Resource Use and Cost Data – only include if written by Economists

These will not be analysed by the statistician. All the planned health economic analyses will be detailed in a separate document held by the trial health economist.

Summary of cost-effectiveness analysis:
The trial health economist will perform an economic analysis with the objective of estimating average costs and effectiveness in each arm of the study. This will inform a cost-effectiveness analysis using a health service perspective and incremental cost effectiveness ratios (ICER's) will be reported. Quality adjusted survival will be obtained by administration of the EuroQol EQ-5D-5L questionnaire. Quality of life data will be collected at baseline (pre-transplant, at time of consent) and at each study follow-up visit following liver transplantation (day 7, day 30 and month 6).
Costs will be estimated based upon measured resource use and national unit costs. Resources will include machine and disposables costs, immunosuppression and other drugs, inpatient hospital stays (including intensive care days), radiological investigations, biopsies and other procedures, outpatient visit and visits to the family doctor. Resource use will be identified from case report forms, hospital episode statistics/insurer claims and from patient self-reporting using a simple log/questionnaire (to assess out-of hospital resource use). These questionnaires will be kept by the patient during the study and collected at the final study visit. Resource use will be transferred to an eCRF, and the original document kept at the participating centre as source material.

11.43 Additional Analyses

11.44 Exploratory analyses

Pre-specified exploratory analyses

Histological and molecular markers collected whilst the liver is perfused on the device will be combined with perfusion parameters to develop a composite liver grading scoring system and to assess their ability to predict clinical outcomes i.e. viability assessment.

A model for the assessment of early allograft function was recently developed [104] and also showed to be associated with patient and graft survival. MEAF stands for Model of Early Allograft Function and is calculated by applying the formula below using the peak ALT and INR value from the first 3 post-operative days and the bilirubin value from the third postoperative day. The final MEAF score is the sum of the 3 values obtained rounded to the nearest integer.

$$Score\ ALT_{\max 3POD} = 3.29 \Big/ \left(1 + exp\left(-1.9132 \times \ln\left(\max_{3POD} ALT\right) - 6.1723\right)\right)$$

$$Score\ INR_{\max 3POD} = 3.29 \Big/ \left(1 + exp\left(-6.8204 \times \ln\left(\max_{3POD} INR\right) - 0.6658\right)\right)$$

$$Score\ Bilirubin_{3POD} = 3.4/\left(1 + exp(-1.8005 \times \ln(Bilirubin\ 3POD) - 1.0607)\right)$$

$$MEAF = Score\ ALT_{\max 3POD} + Score\ INR_{\max 3POD} + Score\ Bilirubin_{3POD}$$

This will be compared by treatment arms (NMP vs SCS) using ANOVA adjusting for Donor Type (DBD, DCD) and centre if the MEAF Score is normally distributed. In case MEAF Score has departure from Normality the first approach will be transformation. If the data cannot transform to Normal distribution, the difference in MEAF Score between arms will be analysed using Mann-Whitney U test. If a non-parametric analysis needs to be performed there will be no adjustment for other factors.

Another recent publication reported that high AST levels on day 3 correlates with patient and graft survival [80]. The relationship of patient and graft survival with the AST on day 3 will be explored using survival analysis techniques as described in Section 9.

Other exploratory analyses will be performed to explore the reasons for any differential discard rate between the two arms.

Additional Exploratory Analysis Not Specified Prior to Receiving Data

Any analyses not specified in this statistical analysis plan will be exploratory in nature and a significance level of 0.01 will be used to declare statistical significance. 99% confidence intervals will be presented.

11.45 Blinded analysis

This is an open label trial. Blinded review of the data will not be carried out.

11.46 Safety Analysis

Safety outcomes to be reported will be:

- Organ discard rate (number of livers not suitable for transplantation)
- Adverse event:
  - Recipient infection (defined as a positive microbiological culture result)
  - Biopsy-proven acute rejection episodes
  - Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks)
  - Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
  - Reoperation rate
  - Technical complications/device failures

Rates of adverse events and organ discards will be compared between NMP and SCS groups by examination of 95% confidence intervals for the difference in incidence. Their severity will be graded according to the Clavien-Dindo classification. Discarded organs will also be distinguished between declined (if accepted in a different hospital) and actually discarded.
The number of patients with any serious adverse events will be compared by assessment of the difference in incidence with 95% confidence interval. The number of serious adverse events per patient will be reported.

12 Appendix 4: Clavien-Dindo grading system for surgical complications

Grade	Definition
I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic or radiological intervention
II	Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusionsand total parenteral nutritionare also included.
III	Requiring surgical, endoscopic or radiological intervention
IIIa	Intervention not under general anesthesia
IIIb	Intervention under general anesthesia
IV	Life-threatening complication (including CNS complications) requiring ICU management
IVa	Single-organ dysfunction (including dialysis)
IVb	Multi-organ dysfunction
V	Death of a patient

Table 39: Clavien-Dindo grading system for the classification of surgical complications. Key: CNS - central nervous system; ICU - intensive care unit

13 Appendix 5 – technique for performing bile salt analysis

13.1 Electrospray ionisation-mass spectrometry

Electrospray ionisation is a technique in which a high voltage is applied to a liquid to produce an aerosol known as an electrospray. It is useful for the analysis of macromolecules, such as bile salts, because it overcomes the potential for these molecules to fragment when ionised.

To generate the electrospray it is necessary for the test sample to be relatively volatile. This is usually achieved through the addition of a volatile organic compound to the solution e.g. methanol acetonitrile. The volatile mixture is forced through a steel capillary tube with a very high charge differential across it. This causes the solution to break up into charged droplets of analyte molecules.

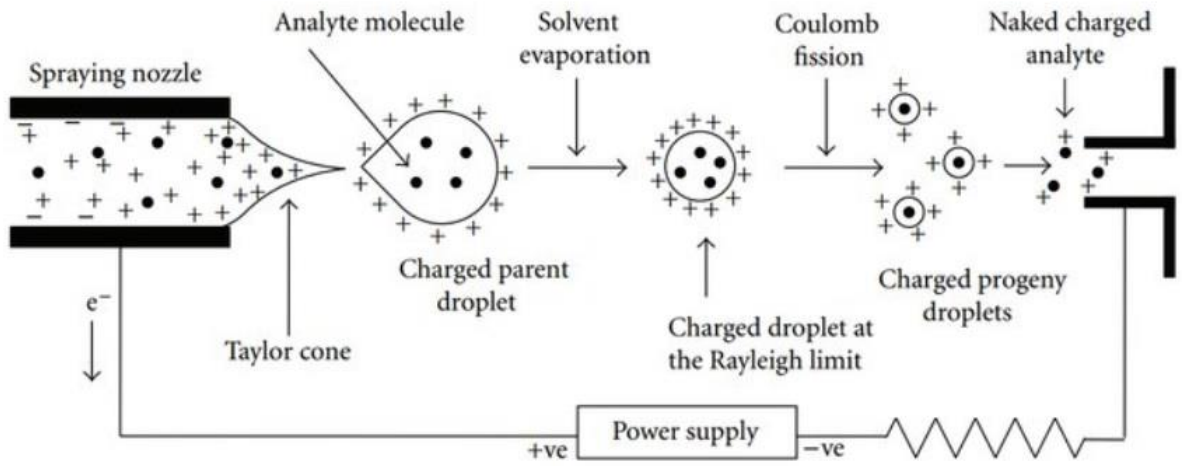


Figure 28: Schematic representation of the electrospray ionisation process showing the break-up of a volatile mixture into an aerosol of charged molecules as they pass along a high voltage differential capillary tube

Eventually the solution evaporates leaving only the naked charged analyte which exits the capillary into the mass spectrometer.

Mass spectrometry is a well-established method of sample analysis used across many different disciplines. In ESI-MS, the ionised molecules produced by ESI exit the charged capillary tube into the mass spectrometer. Here the ionised particles are separated according to their mass through a process of acceleration and deflection.

Acceleration is achieved through the use of negative plates. Positive ions accelerate towards these plates at a speed dependent on their mass, with lighter molecules moving more quickly than heavier ones.

Deflection is achieved through the use of a magnetic field. Following the acceleration process the ions are exposed to a magnetic field which deflects them. The extent of this deflection is dependent on the mass of the ions. The combined effect of the two processes of acceleration and deflection means that ions with different masses will pass through the spectrometer at different speeds.

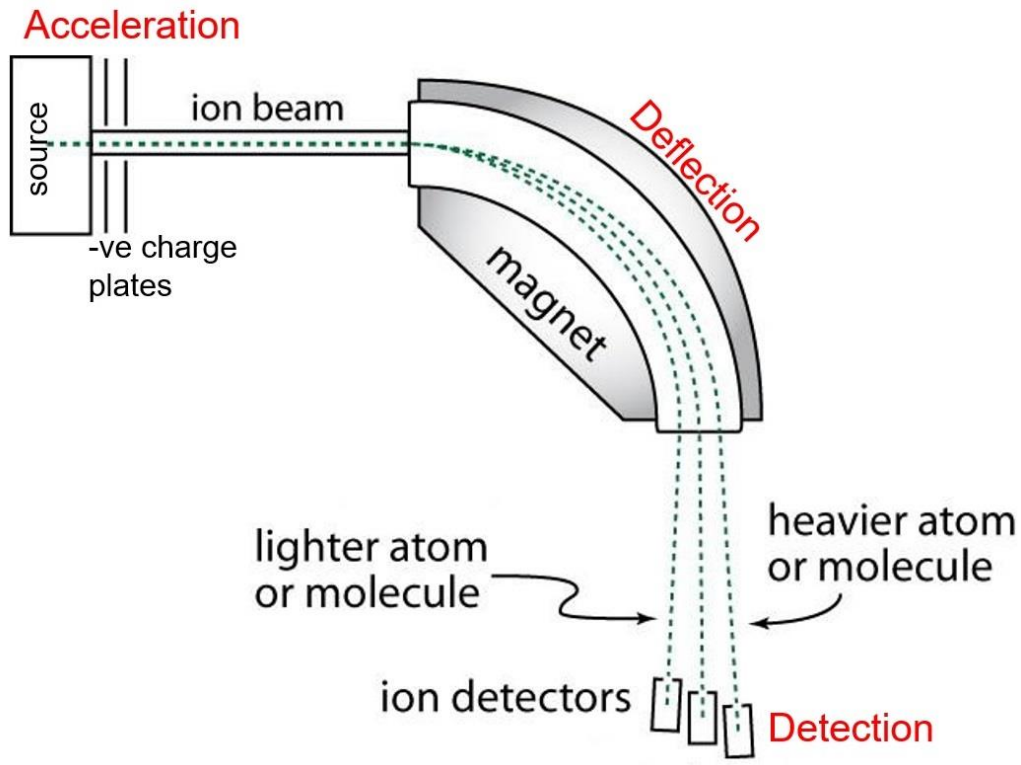


Figure 29: Schematic representation of the passage of charged particles through a mass spectrometer

At the end of their passage through the spectrometer the ions reach an electron multiplier: a detector that is able to measure the ionic charge of the particles. The relative time-points at which the particles reach the detector reflects their mass. This information about the mass and charge of the particles is used to determine the mass-to-charge (m/z) ratio of each molecule. This is then combined with the relative abundance of each different measured mass-to-charge ratio to produce a graphical representation known as a spectrum.

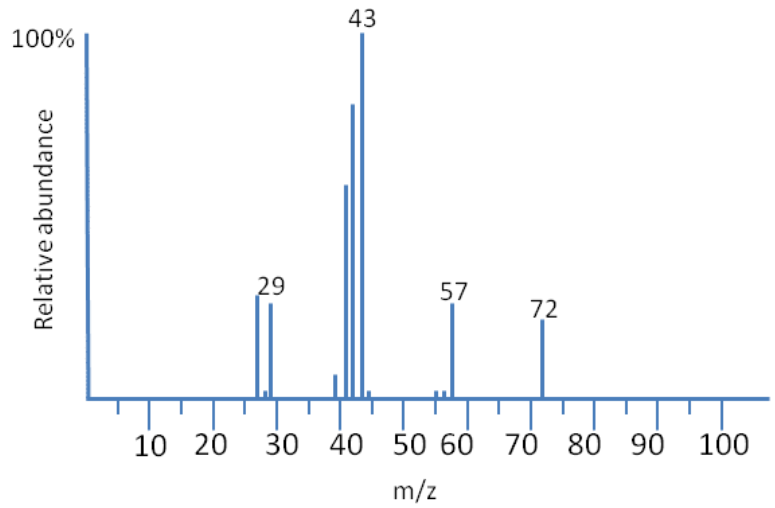


Figure 30: Example of a spectrum generated by ESI-MS

Negative ion electrospray ionises conjugated bile acids producing molecule-H- ions which can then be scanned using mass spectrometry to give a profile of bile salts e.g. glycine, taurine, sulphate and glucuronide conjugates. This can reveal abnormally increased peaks of primary bile acids and compounds characteristic of bile acid synthesis defects. Isomers cannot be separated, so deoxycholic, chenodeoxycholic and ursodeoxycholic acids and salts must be designated. In the absence of chromatographic separation (which is not a part of ESI), many other compounds in the solution such as drugs or peptides can interfere with this process. Therefore profiling was further refined by performing three cycles of tandem mass spectrometry, with each cycle being more selective in the specific mass-to-charge ratio being measured. This filtered out all but the glycine and taurine conjugates in each scan function. Thus it was possible to characterise the major bile salt fractions more specifically.

13.2 Sample preparation

Bile salt analysis was performed by personnel in the Sheffield Children's Hospital Department of Clinical Chemistry following standardised ESI-MS procedures, as described below:

1 Before analysis, samples were thoroughly thawed and shaken before 50µl duplicate aliquots were assayed.

2 50µl of sample was pipetted (2 x duplicates) into a 1.5ml polypropylene 'flip top' microtube (Eppendorf). 50µl water duplicates were included as blanks.

3 50µl of D4-Taurocholic Acid internal standard was added.

4 250µl acetonitrile was added.

5 This extraction mixture was vortexed for 30 seconds, allowed to stand for 15 minutes and vortexed again.

6 Tubes were then spun in a 'microfuge' twice for 5 minutes at 12,000rpm.

7 100µl supernatant was transferred to a multiwell plate for injection into ESI-MS. 100µl water was added to each well.

8 All samples were analysed by 3 ESI-MS injections

9 The spectrum generated from the analysis was assessed for peaks relating to the known mass-to-charge ratios for the following bile salts: glycol-dihydroxycholanoic acid (G-DHC; m/z 448), glyco-trihydroxycholanoic acid (G-THC; m/z 464), tauro-dihydroxycholanoic acid (T-DHC; m/z 498), and tauro-trihydroxycholanoic acid (T-THC; m/z 514).

Initial analysis was performed of five samples containing only the bovine sodium taurocholate in solution. This was used to determine whether it was detectable using the ESI-MS technique and assess which mass-to-charge peak would reflect its abundance in solution.

14 Appendix 6: Reasons for NMP discards

Discarded Liver No	Device Error	Device User Error	Poor Perfusion Parameters	Donor Malignancy	Donor Cirrhosis	Poor In-situ Perfusion	Prolonged Donor Warm Ischaemia	Liver Size	Steatosis	Further Details
1	N	Y	N	N	N	N	N	N	Y	Poor perfusion due to IVC cannula positioning. Safely converted to cold storage and discarded due to steatosis.
2	N	N	N	N	Y	N	N	N	Y	Appearances consistent with cirrhosis in donor with known hepatitis C.
3	N	N	Y	N	N	N	N	N	N	Poor hepatic artery flow during NMP with increasing lactate.
4	N	N	N	Y	N	N	N	N	N	Incidental lung tumour found at retrieval
5	N	N	N	N	N	N	Y	N	Y	Warm ischaemia time greater than 30 minutes in DCD donor.
6	N	N	N	N	N	Y	Y	N	Y	Warm ischaemia time greater than 30 minutes in DCD donor. Poor in situ cold perfusion.
7	N	N	Y	N	N	N	N	N	Y	Persistently raised lactate >6mmol after 6 hours NMP.
8	N	N	N	Y	N	N	N	N	Y	Colonic tumour found at retrieval.
9	N	N	N	N	N	N	N	N	Y	60% steatosis on biopsy.
10	N	N	Y	N	N	N	N	N	N	Persistent acidosis with lactate >6mmol after 8hours NMP.
11	N	N	N	N	N	N	N	Y	Y	Large steatotic liver, no size-matched recipient found.
12	N	N	Y	N	N	N	N	N	Y	Persistently raised lactate >3mmol with acidosis.
13	N	N	N	N	N	N	N	N	Y	Moderate steatosis on biopsy, surgeon decision to discard.
14	N	N	Y	N	N	N	N	N	Y	Poor hepatic artery and portal vein flow during NMP in a steatotic liver.
15	N	Y	N	N	N	N	N	N	Y	Excessive bleeding during NMP from phrenic veins and hepatic artery in a steatotic liver. Safely converted to cold storage and declined due to steatosis.
16	Y	N	N	N	N	N	N	N	Y	See supplementary material for narrative description of device error.

15 Appendix 7: Trial outcomes

	NMP ($n=121$)[a]	SCS ($n=101$)[a]	Effect (95% CI)[b]	*P* value
Peak AST				
ITT[c]				
Adjusted	488.1 (408.9–582.8)	964.9 (794.5–1,172.0)	0.5 (0.4–0.7)	0.0000
Unadjusted	484.5 (406.4–577.6)	973.7 (795.2–1,192.3)	0.5 (0.4–0.6)	0.0000
Test for interaction by donor type				0.012
Subgroup analysis by donor type				
DBD	526.2 (427.3–647.9)	880.2 (708.5–1,093.5)	40.2% (19.3–55.7%)	0.0009
DCD	389.7 (278.0–546.4)	1,458.1 (944.7–2,250.5)	73.3% (53.7–84.6%)	0.0000
PP analysis	498.6 (414.8–599.4)	982.9 (810.4–1,192.2)	0.5 (0.4–0.7)	0.0000
Secondary outcomes				
Discard rates[d]	16 (11.7%)	32 (24.1%)	−12.4% (−21.4 to −3.3%)	0.008
Primary non-function[e]	1 (0.8%)	0 (0.0%)	NA	NA
Post-reperfusion syndrome	15 (12.4%)	32 (33.0%)	−20.6% (−31.6 to −9.6%)	0.0002
Post-reperfusion lactate[f]	3.6 (2.6–4.2)	4.1 (3.2–5.0)		0.018
Early allograft dysfunction	12 (10.1%)	29 (29.9%)	0.263 (0.126–0.550)	0.0002
Biochemical liver tests[f] (average value over day 1–7)				
Bilirubin (μmol l^{-1})				
Days 1–7	38.5 (21.0–73.2)	49.1 (26.0–85.5)		0.029
30 days	13.0 (8.0–22.1)	13.0 (9.1–21.0)		0.479
6 months	9.1 (6.0–15.1)	9.1 (6.0–13.0)		0.671
AST (IU l^{-1})				
Days 1–7	167.5 (98.0–320.7)	318.5 (152–611.5)		0.0000
30 days	20 (14–35)	22 (15–40)		0.707
6 months	23 (18–33)	23 (18–37)		0.931
γGT (IU l^{-1})				
Days 1–7	268.1 (156.3–408.3)	301 (201.1–443.9)		0.157
30 days	178 (109.5–410.0)	200 (96.0–397.5)		0.949
6 months	47 (28–144)	47 (26–128)		0.452
INR				
Days 1–7	1.2 (1.2–1.4)	1.2 (1.2–1.4)		0.644
30 days	1.1 (1.0–1.2)	1.1 (1.0–1.2)		0.735
6 months	1.1 (1.0–1.2)	1.1 (1.0–1.1)		0.167
Creatinine (μmol l^{-1})				
Days 1–7	92.8 (60.1–121.1)	97.2 (67.2–143.2)		0.139
30 days	82.2 (66.3–104.3)	90.2 (72.5–121.1)		0.019
6 months	99.9 (81.3–117.6)	99.9 (83.1–134.4)		0.265
Lactate (mmol l^{-1})				
Day 1–7	1.3 (1.0–1.7)	1.1 (0.9–1.6)		0.130
Other outcomes				
Need for RRT (number (percentage) of patients)				
Day 1–7 after transplant	26 (21.5%)	19 (18.8%)	2.7% (−7.9 to 13.2%)	0.621
30 days	27 (22.3%)	20 (19.8%)	2.5 (−8.2 to 13.3%)	0.648
6 months	27 (22.3%)	21 (20.8%)	1.5% (−9.3 to 12.4%)	0.784
Duration of RRT day 1–7[f]	4 (2–6)	5 (4–6)		0.346
Length of hospital stay[f]	15 (10–24)	15 (11–24)		0.926
Length of ICU stay[f]	4 (2–7)	4 (3–7)		0.339
Graft survival at 1 year	0.950 (0.893-0.977)	0.960 (0.897–0.985)		0.707
Patient survival at 1 year	0.958 (0.902–0.982)	0.970 (0.909–0.990)		0.671

Table 40: Trial outcomes.

CI, confidence interval.

[a]Total number of livers transplanted and analysed overall. Primary outcome analysed on n = 220 due to unavailability of AST values during the first seven days after transplant. Specific outcomes may have different denominators due to some missing data. [b]Effect reported is: Percentage reduction (from geometric mean ratio) for peak AST; odds ratio for early allograft dysfunction; difference in proportions (%) for discard rates, post reperfusion syndrome and need for renal replacement therapy (RRT); not reported for outcomes for which medians are reported, for survival scores and for tests for interactions of subgroup analysis (only *P* values are reported). [c]Intention to treat (ITT) analysis was adjusted for donor type and transplant centre. [d]Denominators for the discard rates is the total number of livers retrieved (n = 270 (NMP, n = 137; SCS n = 133)). [e]Test not performed due to few events and no events in one arm. [f]Median and IQR are reported, a non-parametric Mann–Whitney *U*-test was used.

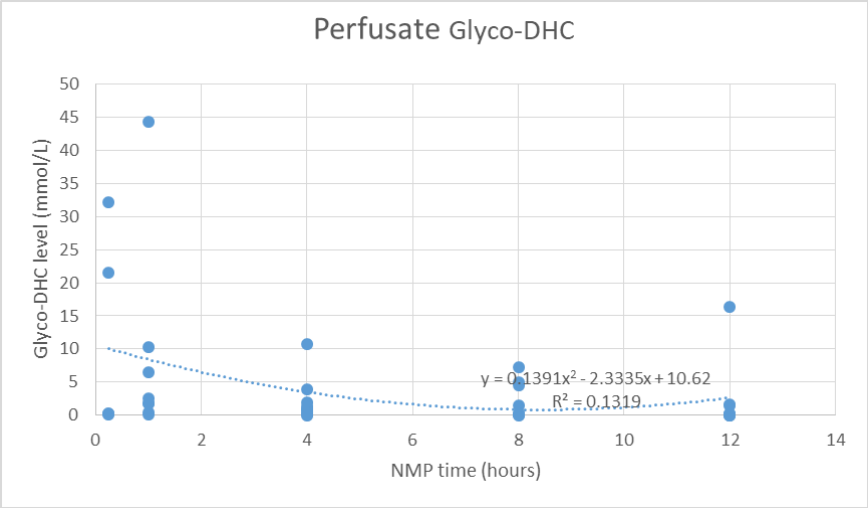
16 Appendix 8 – Bile salt analysis results

	Perfusate Results					Bile Results					
TRIAL ID	Sample Time (hrs)	Glyco-DHC mmol/L	Glyco-THC mmol/L	Tauro-DHC µmol/L	Tauro-THC mmol/L	Sample time (hrs)	Glyco-DHC mmol/L	Glyco-THC mmol/L	Tauro-DHC µmol/L	Tauro-THC mmol/L	Bile prod (ml/hr)
WP212020	1	1.85	0.69	0.71	8.88	4	1652	354	604	22699	8.122744
DBD	4	0.82	0.35	0.62	26.9	8	497	202	234	43419	
70 M	8	0	0.02	0.47	17.05	12	144	201	132	52971	
	12	0.1	0.08	1.13	28.16						
WP212026	0.25	0.2	0.42	0.71	5.22	4	525	665	722	30499	7.185629
DBD	1	0.14	0.51	0.52	5.18	8	139	473	236	38000	
61 F	4	0	0.26	0.61	5.38	12	145	629	200	42011	
	8	0.09	0.17	0.57	6.36						
	12	0.14	0.29	0.93	6.81						
WP213081	1	44.33	79.53	16.3	124.2	4	134	216	228	47648	16.40625
DBD	4	0.17	0.4	1.19	30.56	8	82	162	162	59408	
58 F	8	0.05	0.07	0.95	130.48	12	104	170	78	66110	
	12	0.22	0.37	1.36	60.84						
WP214010	0.25	21.51	37.22	8.3	194.05	4	1348	1106	924	23722	6.763285
DBD	1	0.44	0.27	0.34	50	8	1196	692	328	41646	
58 M	4	0.85	0.11	0.37	129.57	12	1076	838	322	51146	
	8	4.46	4.9	4.28	340.69						
	12	16.39	3.26	5.11	303.03						
WP214016	0.25	0.18	1.94	1.22	181.26	4	2008	1546	390	43890	10
DBD	1	1.74	1.17	0.51	6.88	8	472	250	280	21258	
56 F	4	1.35	0.74	0.44	8.11	12	74	118	58	21984	
	8	0.17	0.27	0.68	13.47						
	12	0	0.06	0.53	4.99						
WP214029	0.25	0.03	0.22	0.81	7.78	4	3334	2378	1614	5336	8.72093
DBD	1	6.47	6.6	0.58	6.41	8	398	516	116	27472	
43 F	4	3.93	4.26	0.86	44.59	12	206	452	96	33468	
	8	4.94	5.06	2.22	88.55						
	12	1.66	1.82	1.71	98.37						
WP214035	1	10.23	24.38	7.33	1082	4	274	160	386	23156	45.62738
DCD	4	1.91	6.94	0.21	11.1	8	1066	820	442	9168	
21 M	8	1.46	0.79	0.81	24.77	12	426	474	250	19872	
	12	0	0	0	53.63						
WP213087	1	2.48	1.16	1.12	37.62	4	289	415	459	29540	33.4728
DBD	4	0.4	0.96	0.56	8.08	8	82	202	128	31638	
42 F	8	0.32	0.34	0.28	18.34	12	20	148	46	45178	
	12	0.16	0.06	0.16	27.52						
WP214015	0.25	32.1	33.53	11.48	1671	4	1616	702	934	18732	6.916427
DBD	4	10.71	11.57	3.55	5.3	8	932	962	572	34444	
74 M	8	7.2	10.25	6.2	278.92	12	372	718	300	44270	
	12	1.5	3.21	2.26	219.54						

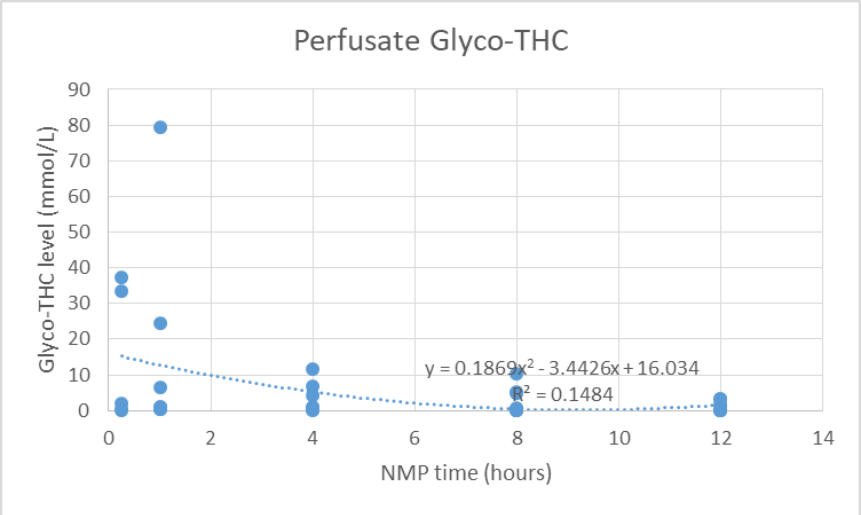
Table 41: Results from bile salt analysis of perfusate and bile from livers with good bile production (last column)

	Perfusate Results					Bile Results					Bile prod (ml/hr)
WP213084	0.25	0.13	0.16	0.6	43.28						4.172462
DBD	1	2.31	3.2	1.22	16.38						
74 M	4	14.44	19.61	5.96	284.75						
	8	95.66	150.14	48.29	7001	8	106	86	44	8208	
	12	82.64	167.87	53.78	10140	12	620	250	492	26952	
WP212024	1	5.93	9.93	4.05	31.71						1.584507
DCD	4	12.87	32.43	8.2	545.55	4	1030	649	917	6163	
75 M	8	20.89	91.28	16.93	2034.36	8	890	766	1058	34172	
	12	23.5	130.51	18.64	3135.55	12	465	517	732	82244	
WP213902	1	27.54	13.3	17.94	70.06						4.293381
DCD	4	6.8	3.13	1.9	216.8	4	3026	818	606	38024	
50 F	8	28.02	20.81	8	1117	8	2196	407	281	29218	
	12	32.85	53.71	12.46	3007	12	881	88	250	39012	
WP213080	1	0	0.15	1.42	8.79						1.041667
DCD	4	4.12	2.32	1.68	10.74	4	276	250	354	30348	
70 F	8	9.88	4.21	2.58	41.01	8	1318	889	459	65433	
	12	54.95	62.63	18.99	3979	12	1154	1110	318	73442	

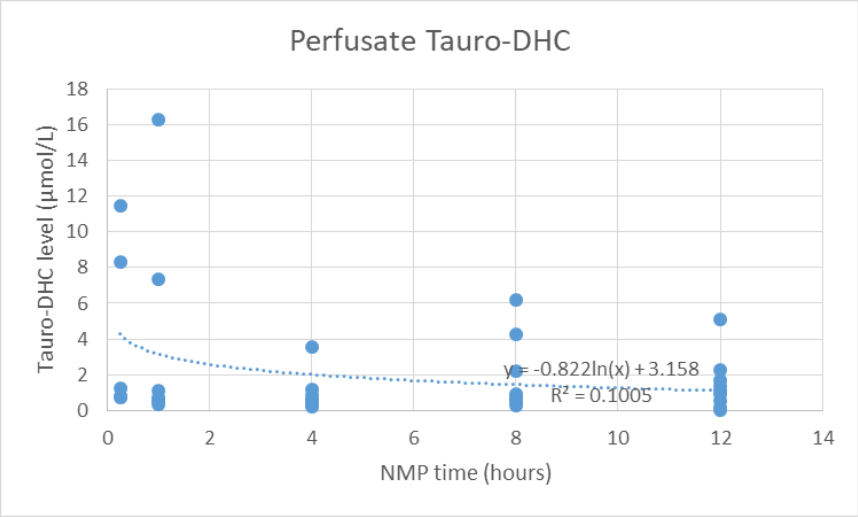
Table 42: Results from bile salt analysis of perfusate and bile from livers with bad bile production (last column)



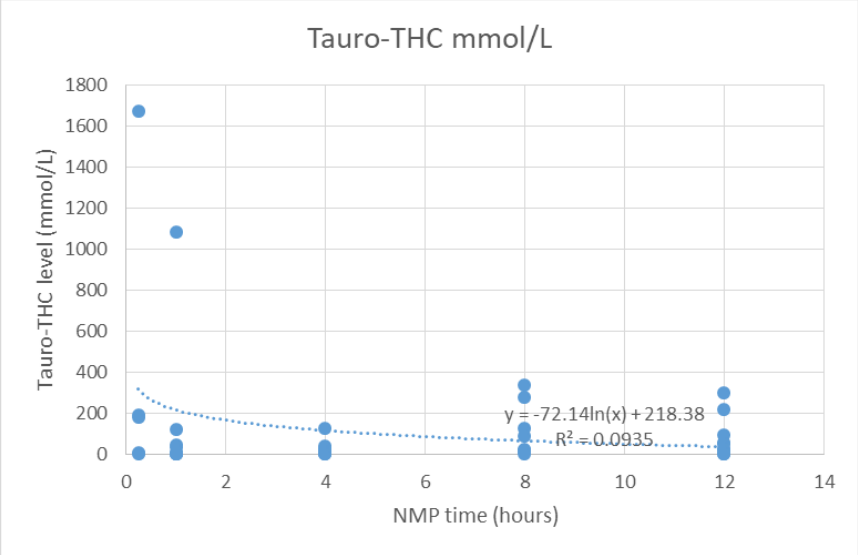
Graph 4: Scatter graph showing perfusate levels of glyco-dihydroxycholanoic acid during 12 hours NMP in livers with good bile production (>5ml/hour; n=9). R=0.363; t=0.675; p=0.548



Graph 5: Scatter graph showing perfusate levels of glyco-trihydroxycholanoic acid during 12 hours NMP in livers with good bile production (>5ml/hour; n=9). R=0.385; t=0.723; p=0.522



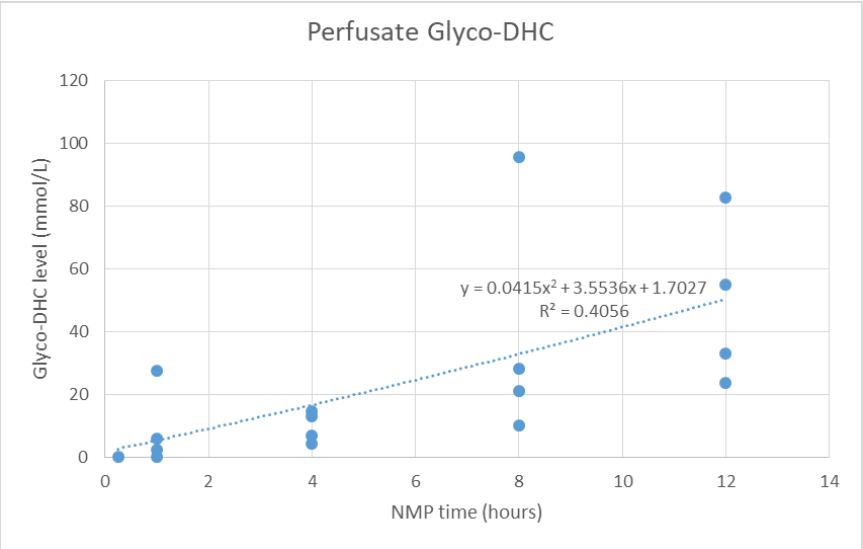
Graph 6: Scatter graph showing perfusate levels of tauro-dihydroxycholanoic acid during 12 hours NMP in livers with good bile production (>5ml/hour; n=9). R=0.317; t=0.579; p=0.603



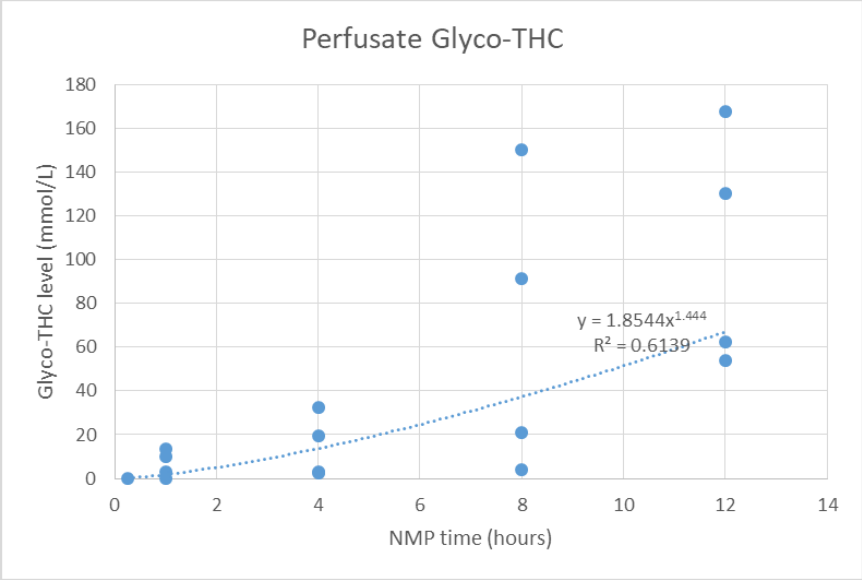
Graph 7: Scatter graph showing perfusate levels of tauro-trihydroxycholanoic acid during 12 hours NMP in livers with good bile production (>5ml/hour; n=9). R=0.306; t=0.556; p=0.617

17

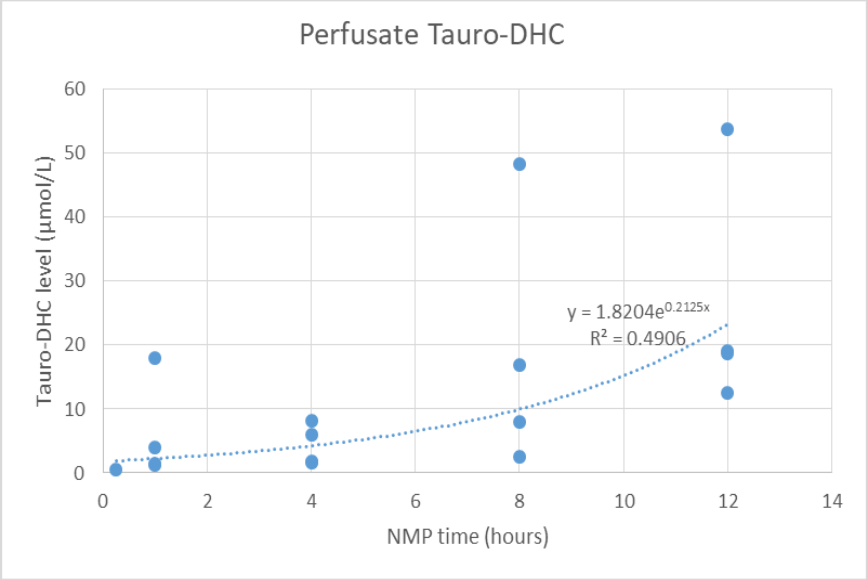
In livers with poor bile production bile acid levels tended to increase as the perfusion progressed:



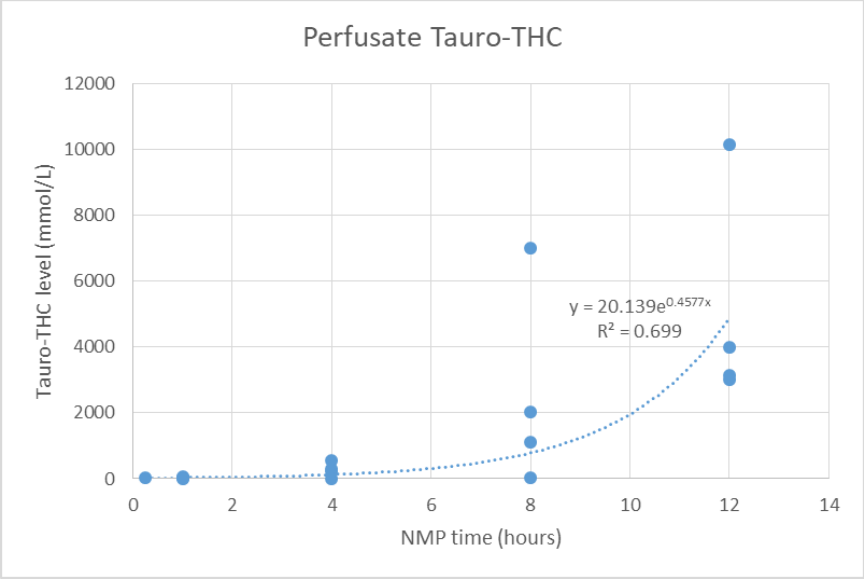
Graph 8: Scatter graph showing perfusate levels of glyco-dihydroxycholanoic acid during 12 hours NMP in livers with poor bile production (<5ml/hour; n=4). R=0.637; t=1.431; p=0.248



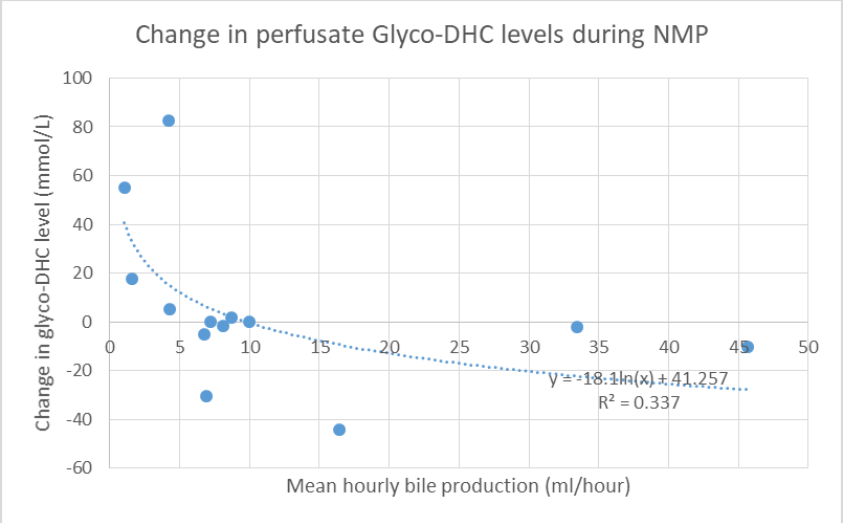
Graph 9: Scatter graph showing perfusate levels of glyco-trihydroxycholanoic acid during 12 hours NMP in livers with poor bile production (<5ml/hour; n=4). R=0.784; t=2.184; p=0.117



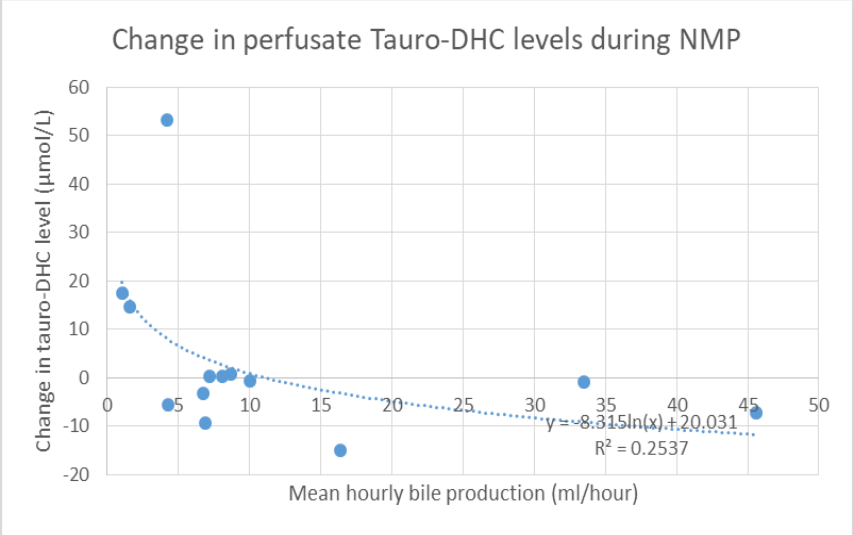
Graph 10: Scatter graph showing perfusate levels of tauro-dihydroxycholanoic acid during 12 hours NMP in livers with poor bile production (<5ml/hour; n=4). R=0.700; t=1.700; p=0.188



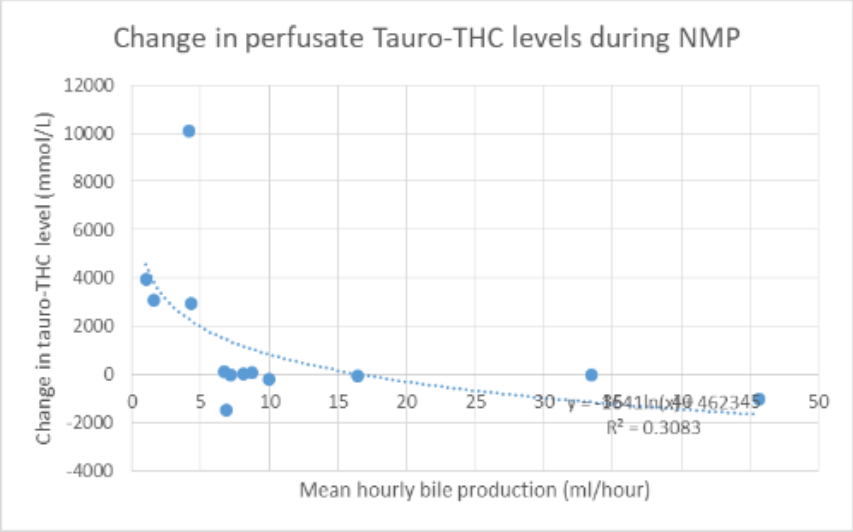
Graph 11: Scatter graph showing perfusate levels of tauro-trihydroxycholanoic acid during 12 hours NMP in livers with poor bile production (<5ml/hour; n=4). R=0.836; t=2.639; p=0.078



Graph 12: Scatter graph showing the change in perfusate levels of glyco-dihydroxycholanoic acid during 12 hours NMP in all livers (n=13). R=0.581; t=2.365; p=0.038



Graph 13: Scatter graph showing the change in perfusate levels of tauro-dihydroxycholanoic acid during 12 hours NMP in all livers (n=13). R=0.504; t=1.934; p=0.079



Graph 14: Scatter graph showing the change in perfusate levels of tauro-trihydroxycholanoic acid during 12 hours NMP in all livers (n=13). R=0.555; t=2.214; p=0.049

17 Appendix 9 – Device user error

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9th March 2015

Dear Professor Grinyo,

Below are the details regarding a Serious Adverse Event that was reported on 3rd March 2015 by the research team at Addenbrooke's Hospital, Cambridge, and resulted in the discard of a liver that was enrolled in the trial entitled A Multicentre Randomised Controlled Trial to Compare the Efficacy of Ex-vivo Normothermic Machine Perfusion with Static Cold Storage in Human Liver Transplantation (Ethics ref 14/LO/0182; MHRA ref. CI/2014/0007).

On 25th February 2015 a liver was randomised to normothermic machine perfusion as part of this Trial. The donor was a 49 year old DBD, Caucasian male with BMI 30. Pre-retrieval liver function was normal with ALT 37 IU/L and γGT 57 IU/L. The liver was noted to be significantly steatotic on visualisation.

The retrieval proceeded uneventfully and, following explant, the liver back-table was performed as is standard practice for a normothermic preserved liver. The hepatic artery, portal vein and inferior vena cava (IVC) were cannulated in preparation to connect the liver to the OrganOx *metra* device. The liver was then connected to the machine and perfusion commenced.

Almost as soon as the perfusion began it was apparent that this liver was not perfusing well. Poor outflow was recorded from the inferior vena cava with the following results:

- inadequate refilling of the portal perfusion reservoir and thus low portal vein flow
- poor flow and pressure measured in the hepatic artery

Attempts were made to rectify the situation by resetting the machine, controlling any bleeding and manipulating the cannulae, but with no improvement in IVC flow. Eventually, after 18 minutes of continued poor flow a decision was made to abort the perfusion. Due to the fact that the liver was already considered a marginal organ (due to steatosis) and it had now been subjected to a period of warm ischaemia on the machine, the decision was made by the transplanting surgeon that the liver would not be viable if it was now cold stored and so the organ was discarded.

To date, a total of 80 livers have been enrolled into this trial, 39 randomised to cold storage and 41 to normothermic perfusion. 12 livers in total have been discarded, 8 that were cold stored and 4 that were on the machine. Since the liver discard described above, a further 3 trial livers have been successfully transplanted that were preserved using normothermic perfusion. None of these have suffered a similar problem.

Following the adverse event described above, the machine in question was returned to OrganOx and underwent extensive testing but the machine performed well and it was not possible to replicate this

same problem. The machine data log has been thoroughly interrogated and does not show any identifiable device faults.

A review of the events from this perfusion suggests that the most likely underlying cause of the problems described is incorrect cannulation of the IVC preventing blood from draining out of the IVC into the perfusion circuit. This has been recorded as a Device User Error and checks have been put in place to ensure that, should a similar scenario arise again, the position of the IVC cannula will be reviewed.

Please let me know if you need any further information.

Best wishes,

David Nasralla
Clinical Research fellow in Transplant Surgery

18 Appendix 10 – Device error

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5th February 2016

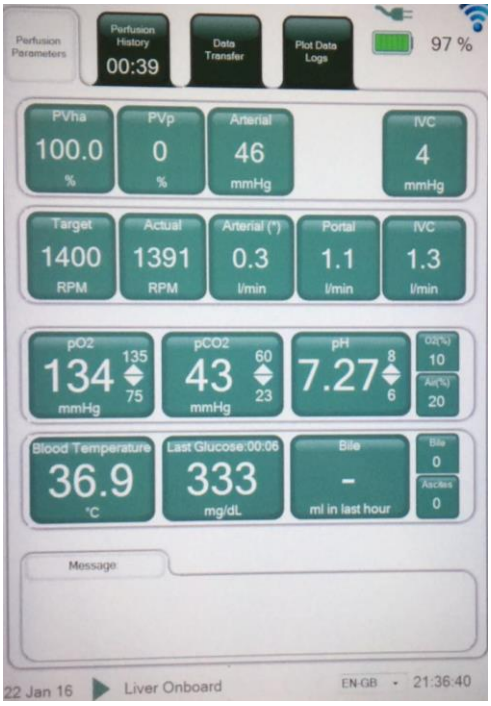
Dear Professor Grinyo,

Below are the details regarding a Serious Adverse Event that was reported on 29th January 2016 by the research team at University Hospitals, Leuven, Belgium, and resulted in the discard of a liver enrolled in the trial entitled A Multicentre Randomised Controlled Trial to Compare the Efficacy of Ex-vivo Normothermic Machine Perfusion with Static Cold Storage in Human Liver Transplantation (Ethics ref 14/LO/0182; MHRA ref. CI/2014/0007).

On 22nd January 2016 a liver was randomised to normothermic machine perfusion by the WP2 research team in Leuven. A member of the Oxford WP2 research team was requested to attend for the retrieval in Leuven due to a lack of staff being available and so this was provided. The donor was an 84 year old, DBD, Caucasian male in Roeselare Hospital, approximately 145km away from Leuven. The retrieval proceeded uneventfully with cross clamp at 21:10.

A standard liver backtable was performed; the liver had normal anatomy, was mildly steatotic and was cannulated and connected to the machine in the standard manner. Normothermic machine perfusion was commenced at approximately 22:00.

From the start of NMP the approximate flows through the liver were as follows: hepatic artery 300ml/min; portal vein 1.1L/min; IVC 1.3L/min (image right). The pH of the circuit reached 7.3 by 45mins perfusion with lactate falling to 0.4mmol/L by 1 hour perfusion.



However, it was apparent from early in the perfusion that the machine was failing to achieve a target hepatic artery pressure of 65-80mmHg, instead only reaching approximately 45-50mmHg. This in combination with a reported hepatic artery pinch valve closure of 100% (should usually read 88-93%) suggested a possible underlying device problem to account for the failure to achieve satisfactory pressure.

In an attempt to resolve this problem the perfusion was stopped and restarted (this should reset the pinch valve calibration). The cannulae positions were also assessed but appeared satisfactory.

The problem did not resolve with these simple interventions. Despite this the liver appeared healthy and all biochemical parameters (pH, lactate, glucose) as well as bile production were satisfactory. A decision was made to transfer to Leuven to make a further assessment of the organ. A message was sent to the transplanting surgeon (Mr Diethard Monbalieu) advising to wait until the liver had arrived before commencing surgery due to possible concerns about the organ.

The ambulance journey commenced and it was soon noticed that the power inverter in the ambulance was not working, so the machine was being powered only by its internal battery supply. The battery life was considered insufficient to last the entire journey to Leuven and so, once the battery had depleted to 50% it was decided that the safest action was to wait at a service station with the machine plugged in until a replacement ambulance could arrive.

Whilst waiting at the service station the user again examined the perfusion device when a further problem developed where air was noticed in the hepatic artery line (image right). Due to lack of sterile facilities this could not be investigated further at that time and the cause for it is still not known. Shortly after this the replacement ambulance arrived and the journey was completed to Leuven.

On arrival at University Hospital Leuven it transpired that the transplant surgery had commenced as the message to not commence surgery had not reached the transplanting surgeon until shortly after knife-to-skin. However, no irreversible steps had been taken during the surgery and the operation had been paused in anticipation of the liver's arrival. On assessing the organ there was no longer any air in the arterial line and the liver appeared to be perfusing homogeneously. The lactate was 0.2mmol, pH 7.31 with glucose 6.5mmol and continued bile production of 6-8ml/hr. AST levels were also monitored over a 60minute period and found to be stable at approximately 200 IU/L. However, arterial perfusion pressure remained approximately 40mmHg with the pinch valve displaying 100%. The machine was displaying arterial flow of 200-300ml/min. Prof Monbalieu used Doppler ultrasound to directly assess the arterial flow which demonstrated flow of only 50ml/min.

Due to the combination of the following factors, a decision was made to discard the liver:

- Organ was already considered marginal due to donor age (84 years old) and steatosis
- Concerns about air in arterial line
- Low arterial perfusion pressure
- Lower flows demonstrated on Doppler scan than were being displayed by the machine
- No irreversible steps had been taken in the recipient operation
- A alternative organ was available for the same recipient

The recipient was successfully transplanted with a different liver less than 24 hours later.

From a global experience of more than 150 normothermic liver perfusions using the OrganOx *metra* this is the first such incident of this type to have been reported. The OrganOx *metra* machine from Leuven was returned to the OrganOx offices shortly after this incident. Investigations revealed a

mechanical fault with the hepatic artery pinch valve. The device has been returned to the manufacturing warehouse for repair according to OrganOx specifications.

Please let me know if you need any further information.

Best wishes,

David Nasralla
Clinical Research fellow in Transplant Surgery

19 Appendix 11 – Primary non-function

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14th July 2015

Dear Professor Grinyo,

Below are the details regarding a Serious Adverse Event that was reported on 7th July 2015 by the research team at the Queen Elizabeth Hospital, Birmingham, and resulted in the death of a transplant recipient who was enrolled in the trial entitled A Multicentre Randomised Controlled Trial to Compare the Efficacy of Ex-vivo Normothermic Machine Perfusion with Static Cold Storage in Human Liver Transplantation (Ethics ref 14/LO/0182; MHRA ref. CI/2014/0007).

On 2nd July 2015 a liver was randomised to normothermic machine perfusion as part of this Trial. The donor was a 69 year old, DBD, Caucasian female, BMI 26.3 with type II diabetes in the Great Western Hospital, Swindon who had died as a result of a brain tumour. She had normal liver function tests with AST 49 IU/L, GGT 48 IU/L and Na 132 mEq/L. The retrieval proceeded uneventfully with aortic cross clamp at 00:31. Aortic and in situ portal perfusion were performed using 2.4L and 2.1L of University of Wisconsin solution respectively. The liver was explanted at 00:55 and was noted to be severely steatotic (image 1a and 1b).

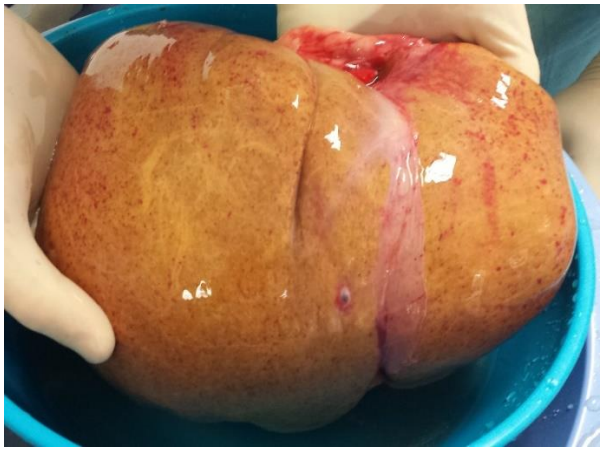


Image 1a

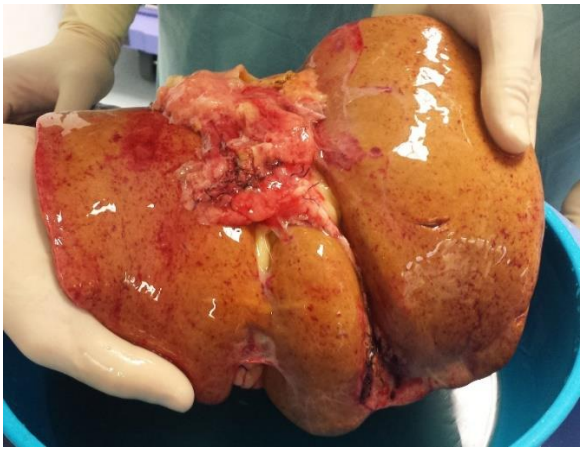
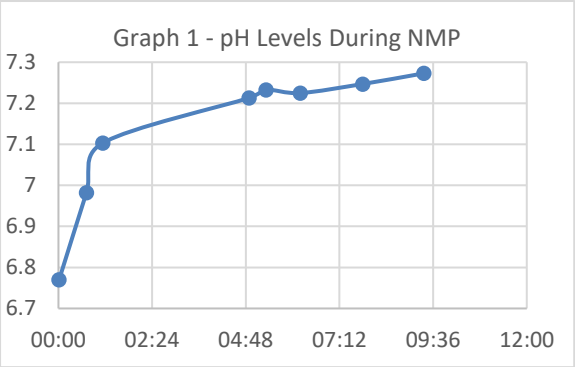


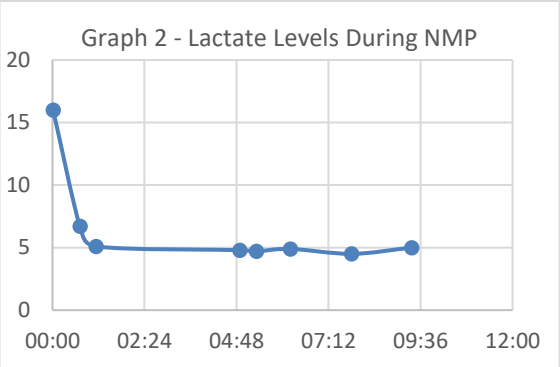
Image 1b

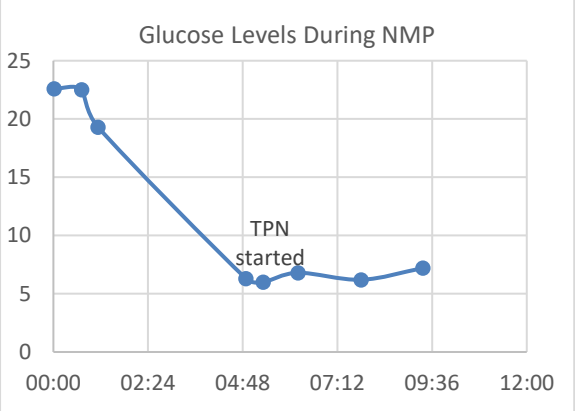
A standard liver backtable was performed; the liver had normal anatomy and was cannulated and connected to the machine in the standard manner. Normothermic machine perfusion was commenced at 02:04.

From the start of NMP the approximate flows through the liver were as follows: hepatic artery 300ml/min; portal vein 1L/min; IVC 1.3L/min. These were maintained throughout the perfusion. The initial pH of the circuit was very acidotic at 6.77, rising gradually to 7.1 by 1hour and then continuing to slowly increase towards 7.3 over the course of the 10 hour perfusion (graph 1). A total of 82.5ml of

sodium bicarbonate was required to support the organ in achieving this pH, more than double the usual dose required. An initial lactate was measured at 16mmol which by 1 hour had fallen to 5.1mmol. The lactate level remained between 4.5 – 5mmol for the rest of the perfusion (graph 2). Only minimal bile was produced. There appeared to be active glucose utilisation, with levels dropping from 22.6mmol to 6.3mmol over the first 5 hours, and then remaining between 6 – 7.2mmol with TPN supplementation (graph 3).







The transplanting surgeon was aware of the machine parameters as described above. The liver felt soft and appeared homogeneously perfused throughout.

The transplant recipient was a 68 year old male, BMI 25.5, MELD score 17, eGFR 45.9 with significant cardiac history whose indication for transplant was primary sclerosing cholangitis.

The transplant operation proceeded in an uncomplicated fashion. Eventually, after a total preservation of 11hrs 40mins, with 10hr 7min NMP, the liver was removed from the machine at 12:11 to be transplanted. A piggyback side-to-side IVC anastomosis was performed and the time of portal reperfusion was 12:35. At this point there was a significant post-reperfusion syndrome, with a significant increase in ionotropic requirement and mean arterial pressure drop from 100mmHg to 42mmHg. This responded to noradrenaline such that after approximately 20mins the patient appeared to stabilise. An initial post-reperfusion lactate measured at 12:55 was 6.1mmol. This dropped to 5mmol at 13:30. However, approximately 2hours later the patient became increasingly cardiovascularly unstable requiring higher dose ionotropes and with his lactate increasing to 12mmol. A decision was made not to close his abdomen and to transfer him to ITU.

Over the next 3 days the patient remained on ITU requiring respiratory and cardiac support with bloods on day 2 of: bili 110umol/L, AST 5101IU/L, ALT 3405IU/L, GGT 176IU/L, INR 1.2, lactate 14.6mmo/L and creatinine 101umol/L. The patient was placed on the super-urgent waiting list for re-transplant due to primary non-function but unfortunately passed away on 6th July 2015. The cause of death was recorded as multi-organ failure due to primary non-function of liver transplant.

Were this liver to have been cold stored, we believe it is highly likely that it would either not have been transplanted due to severe steatosis or, were it to have been transplanted, would not have functioned. Although interpretation of the perfusion parameters as a means of determining the viability of the liver is not part of the study protocol, there were signs that this liver was performing poorly during perfusion, particularly with respect to the failure to correct lactate or produce bile. Also, the requirement for bicarbonate was much greater than we have seen in other perfused livers in this trial. One of the purposes of the COPE trial is to determine which flow-dynamic and biochemical parameters recorded during normothermic perfusion correlate with organ quality and outcome after transplant. Whilst previous animal studies have suggested that pH and bile production are important predictors, we have observed that this has not consistently been the case so far in this trial. Although a number of livers have been transplanted successfully in the trial that either did not achieve a normal pH during perfusion or made only minimal bile, but this is the first liver where a decision has been made by the transplanting surgeon to transplant it in the context of a raised lactate.

We do not believe this outcome resulted from a 'problem' with the device or was a direct effect of normothermic perfusion. Indeed, no significant problems were experienced during the perfusion. Rather, we believe that this outcome was the result of a poor quality, severely steatotic organ being transplanted.

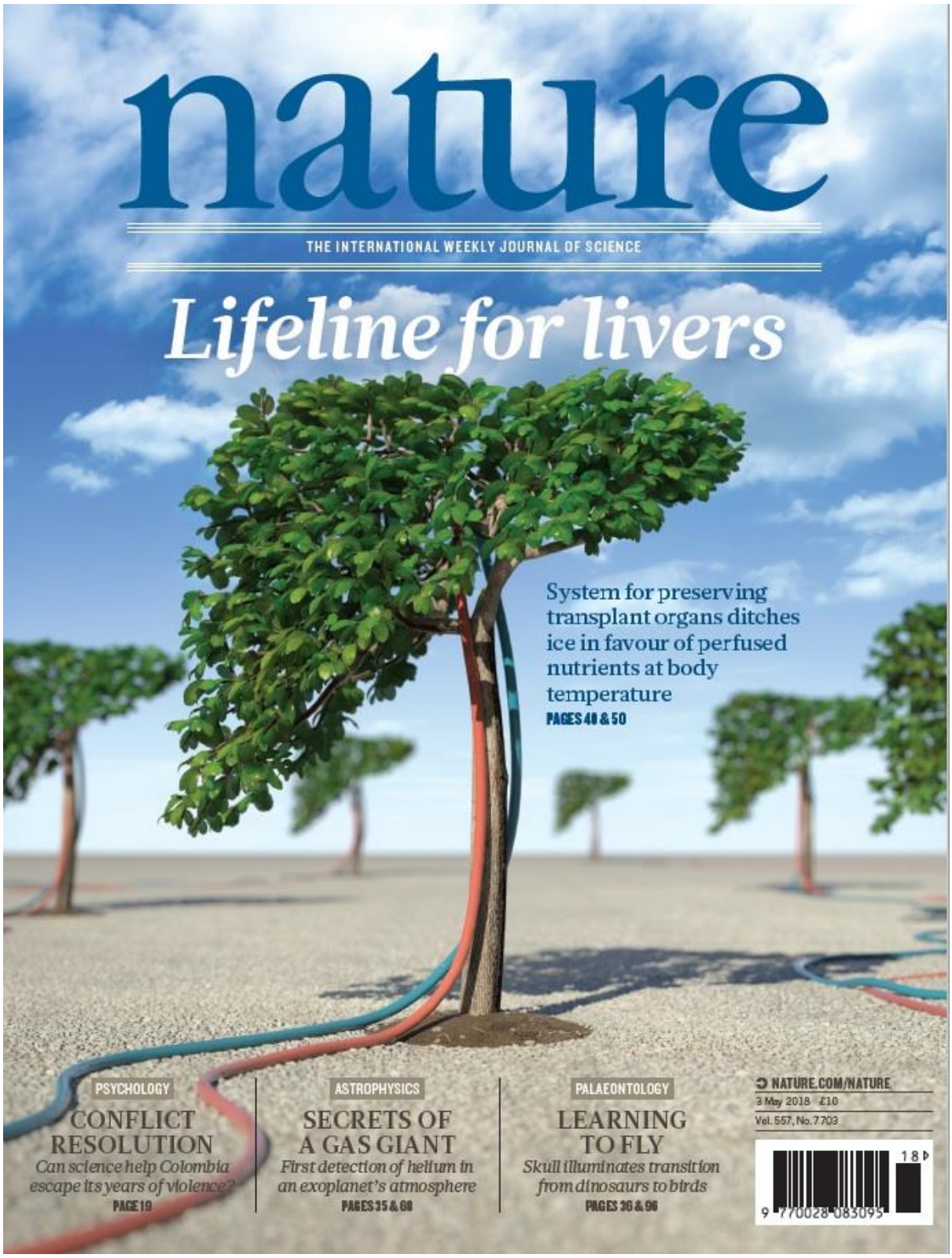
As a direct consequence of this event, the Chief Investigator is writing to all the local PIs to emphasise the need for extreme caution in making a decision to transplant an NMP liver that might not be transplanted if stored cold, particularly if the perfusion parameters are less than optimal.

Please let me know if you need any further information.

Best wishes,

David Nasralla
Clinical Research fellow in Transplant Surgery

20 Appendix 12 – Published trial manuscript



ARTICLE

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A randomized trial of normothermic preservation in liver transplantation

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Liver transplantation is a highly successful treatment, but is severely limited by the shortage in donor organs. However, many potential donor organs cannot be used; this is because sub-optimal livers do not tolerate conventional cold storage and there is no reliable way to assess organ viability preoperatively. Normothermic machine perfusion maintains the liver in a physiological state, avoids cooling and allows recovery and functional testing. Here we show that, in a randomized trial with 220 liver transplantations, compared to conventional static cold storage, normothermic preservation is associated with a 50% lower level of graft injury, measured by hepatocellular enzyme release, despite a 50% lower rate of organ discard and a 54% longer mean preservation time. There was no significant difference in bile duct complications, graft survival or survival of the patient. If translated to clinical practice, these results would have a major impact on liver transplant outcomes and waiting list mortality.

Liver transplantation is the accepted treatment for end-stage liver failure, with one and five year survivals in excess of 90% and 70%, respectively[1]. With increasing rates of liver disease[2], the supply of transplantable organs is no longer able to meet demand. Paradoxically, despite substantial waiting list mortality (for example, 21% in the UK), only 63% of UK deceased donor livers are transplanted[1]. Increasing numbers of deceased organ donors in many countries have not been matched by a corresponding rise in the number of transplantable organs. This is mainly because these additional donors tend to be high-risk—either declared dead by cardiovascular criteria (DCD), as opposed to brainstem death donors (DBD), or elderly with multiple co-morbidities (extended criteria donors). Such organs pose a greater risk to the recipient, with a higher probability that the liver will never function (primary non-function (PNF)) or that it will lead to later complications, particularly biliary stricturing.

Despite many advances in liver transplantation, the method of organ preservation has changed very little in almost 30 years[3]. The liver is flushed and cooled with specialist preservation fluid, then stored in an icebox. This process of static cold storage (SCS) has several limitations. Although SCS slows metabolism by 10- to 12-fold, substantial anaerobic activity continues even at ice temperature[4]. This leads to ATP depletion and accumulation of succinate and other metabolites. These lead to the generation of reactive oxygen species[5] that are the basis of ischaemia–reperfusion injury, when the organ is re-exposed to oxygenated blood at the time of transplantation. This damage, exacerbated by any prior injury, limits the maximum safe preservation time of the donor organ. Once cooled, the cessation of normal cellular activity also makes functional assessment impossible.

These shortcomings are particularly problematic in the higher-risk donor organs that form an increasing proportion of current liver transplant practice. The very severe ischaemia–reperfusion-related morbidity that characterizes transplantation of such organs is now a major limitation in meeting the demand for life-saving transplants. To combat the limitations imposed by cold storage, a change in preservation technology is required. In recent years, interest has developed in perfusion at physiological temperature (normothermic machine perfusion (NMP))[6–9].

During NMP, the liver is perfused with oxygenated blood, medications and nutrients at normal body temperature to maintain a physiological milieu. Evidence from animal models of both DBD and DCD liver transplantation[10,11] suggests that this improves the post-transplant survival of transplanted livers, and potentially enables the assessment of organ viability during preservation. The mechanism underlying these improved outcomes is at least partly related to the metabolic resuscitation of the organ that occurs with preservation under physiological conditions. This has been demonstrated through the replenishment of ATP levels[11], which in turn contributes to a reduction in the severity of the ischaemia–reperfusion injury that is experienced after transplant[5,10].

There is increasing interest in the clinical application of NMP, with several cases described in the recent literature[6,7]. In 2013, a phase-I study by our group[9], demonstrated the safety and feasibility of NMP in

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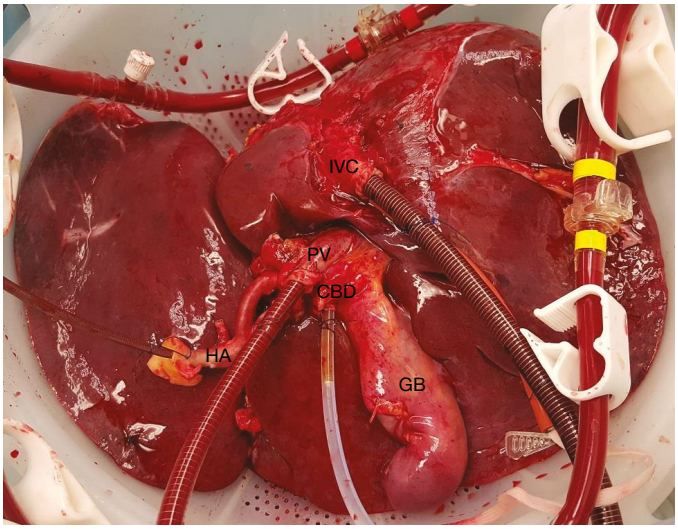

Fig. 1 | Image of liver during normothermic machine perfusion. The hepatic artery (HA), portal vein (PV), inferior vena cava (IVC) and common bile duct (CBD) are all cannulated. The gallbladder (GB) is also present although this was often removed during the retrieval process before NMP. This image has been used with consent from the family of the donor.

20 liver transplant recipients. This was used as the precursor to the present study which, to our knowledge, is the first randomized controlled trial to test the efficacy of machine perfusion against conventional cold storage in liver transplantation.

Livers from adult DBD or DCD donors were eligible for enrolment. Adult patients awaiting a liver-only transplant, excluding those with fulminant liver failure, were eligible. If a suitable liver was allocated to a consented recipient, the liver was randomized to either conventional SCS or NMP. In the SCS arm, the organ retrieval, storage and the transplant were conducted according to standard practice. In the NMP arm, following removal from the donor, the liver was attached to the OrganOx metra NMP device, where it was perfused throughout the duration of preservation (Fig. 1), until the transplanting surgeon was ready to implant it, at which point it was removed from the device. The remainder of the recipient's care followed standard practice.

Daily during the first postoperative week, and at day 10, day 30, month 6 and month 12, biochemical results were recorded as well as graft and patient survival data. At six months, a magnetic resonance imaging scan of the biliary tree (MRCP) was performed to assess evidence of biliary injury. Biological samples were collected and stored in a biobank from each liver and recipient enrolled in the study, for use in further mechanistic studies.

The primary endpoint was defined as the difference between the two treatment arms in the peak level of serum aspartate transaminase (AST) within seven days after transplant. This hepatocellular enzyme is a clinically accepted biomarker, predictive of graft and patient survival[12].

Recruitment

Between 26 June 2014 and 8 March 2016, 334 livers were randomized, with 64 livers subsequently excluded (Fig. 2). Following organ retrieval, a markedly different discard rate between the two trial arms resulted in 100 SCS and 120 NMP livers available for primary outcome reporting, with 101 SCS and 121 NMP livers available for secondary outcome analysis. This discrepancy in group size reduced the study power to 89.7%.

One NMP liver was cold stored due to an accessory left hepatic artery arising from the aorta preventing effective cannulation. Eight NMP livers received machine perfusion for less than four hours (for logistic rather than technical reasons). All of these organs are included in the NMP arm as part of the modified intention to treat analysis. For the per protocol sensitivity analysis, the eight livers perfused for less than four hours were excluded and the single NMP liver that was preserved using SCS was reassigned to the SCS group.

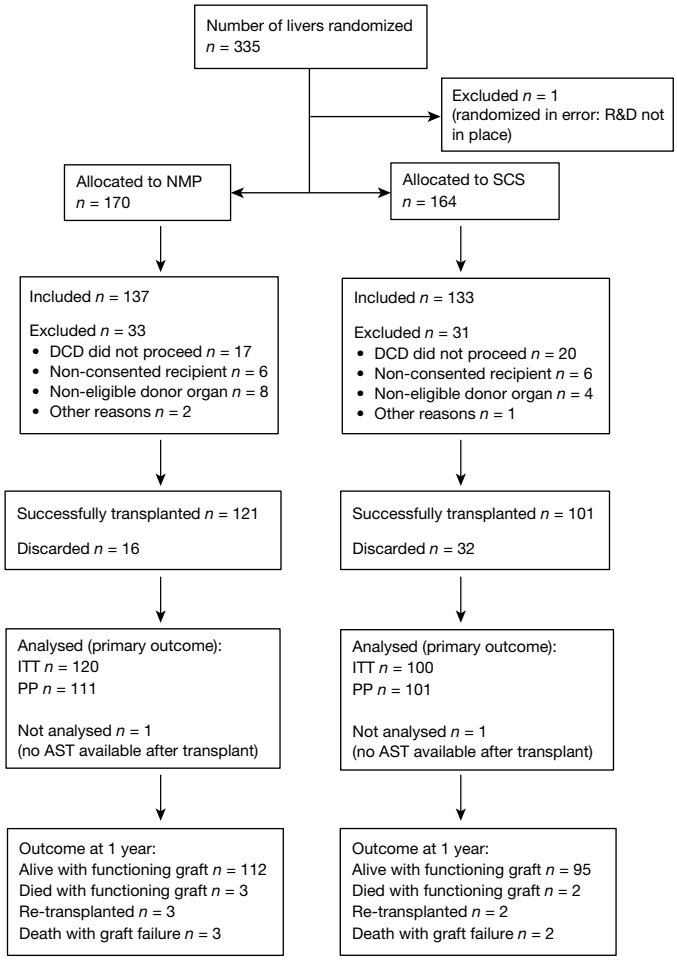

Fig. 2 | CONSORT diagram. CONSORT diagram depicting the outcome for all donor livers enrolled in the trial. ITT, intention to treat; PP, per protocol; R&D, research and development.

Donor, preservation and recipient characteristics

NMP and SCS donor and recipient groups were well-matched (Tables 1, 2). The discard rate was higher in the SCS arm (24.1%; 32 out of 133) than the NMP arm (11.7%; 16 out of 137; Extended Data Table 1). This difference was statistically significant (−12.4%, 95% confidence interval −21.4 to −3.3%; $P = 0.008$). One NMP discard was the result of a device malfunction in an already marginal organ (hepatic artery hypoperfusion due to pinch valve miscalibration; see Supplementary Information).

Functional warm ischaemia time applies only to DCD livers and was measured as the time from the onset of donor hypoxia (oxygen saturation < 70%) or hypoperfusion (systolic blood pressure < 50 mmHg) until the start of cold aortic perfusion in the donor. The median functional warm ischaemia time was longer for NMP than SCS livers (21 min versus 16 min; $P = 0.003$).

Total preservation time was measured from the start of cold aortic perfusion in the donor until graft reperfusion in the recipient. The median total preservation time was longer for NMP than SCS livers (11 h 54 min versus 7 h 45 min; $P < 0.001$). Within the NMP arm, there was no significant difference in median perfusion time between DBD and DCD livers (9 h 55 min DBD versus 8 h 45 min DCD; $P = 0.449$).

Post-reperfusion haemodynamics were documented in 218 cases: post-reperfusion syndrome was more common in the SCS (32 out of 97) than the NMP group (15 out of 121), a statistically significant difference (–20.6%, 95% confidence interval –31.6 to –9.5%; $P < 0.001$). This was despite reduced requirement for vasopressors in NMP livers in the post-reperfusion period (Extended Data Table 2a–c).

Table 1 | Donor demographic details

Stratification factors for all randomized livers	NMP ($n=170$)	SCS ($n=164$)
Donor type[a]		
DBD	107 (62.9%)	104 (63.4%)
DCD	63 (37.1%)	60 (36.6%)
Donor demographics for all retrieved livers	**NMP ($n=137$)**	**SCS ($n=133$)**
Gender[a]		
Female	54 (39.4%)	57 (42.9%)
Male	81 (59.1%)	76 (57.1%)
Missing	2 (1.5%)	0 (0.0%)
Age[b]	56 (45–67) (16–84)	56 (47–66) (20–86)
Ethnicity[a]		
African–Caribbean	3 (2.2%)	1 (0.8%)
Caucasian	131 (95.6%)	128 (96.2%)
Other	1 (0.7%)	4 (3.0%)
Missing	2 (1.5%)	0 (0.0%)
Cause of death		
CVA	74 (54.0%)	74 (55.6%)
Hypoxia	30 (21.9%)	32 (24.1%)
Trauma	17 (12.4%)	16 (12.0%)
Other	14 (10.2%)	11 (8.3%)
Missing	2 (1.5%)	0 (0.0%)
Body mass index[b]	26.26 (23.66–30.52) (16.42–46.65)	27.01 (23.74–30.56) (17.24–49.96)
Missing	2 (1.5%)	0 (0.0%)
ET-Donor risk index[b]	1.72 (1.47–2.09) (0.98–4.31)	1.72 (1.50–2.10) (1.06–3.49)
Missing	16 (11.7%)	19 (14.3%)

CVA, cerebrovascular accident.
[a]Frequency and column percentages are reported.
[b]Median, interquartile range (IQR, first brackets) and full range (second brackets) are reported.

Table 2 | Preservation and recipient demographic details

Preservation details for all transplanted livers	NMP ($n=121$)	SCS ($n=101$)	*P* value[a]
Functional warm ischaemia time[b] (min) (applies to DCD livers; $n=55$ (34 NMP, 21 SCS))	21 (17–25) (9–93)	16 (10–20) (2–32)	0.003
Cold ischaemia time prior to NMP (min)[c] ($n=120$)	126 (106.5–143.0) (49–218)	NA	
Machine perfusion time (min)[c] ($n=120$)	547.5 (372.5–710.5) (85–1,388)	NA	
Total preservation time from cross-clamp in donor to organ reperfusion in recipient (min)	714 (542–876) (258–1,527)	465 (375–575) (223–967)	0.0000
Steatosis assessed pre-preservation[d,e]			0.366
None or mild	91 (75.3%)	89 (88.2%)	
Moderate or severe	29 (24%)	12 (11.9%)	
Missing	1 (0.8%)		
Recipient demographics	**NMP ($n=121$)**	**SCS ($n=101$)**	***P* value[a]**
Gender[d]			0.717
Female	35 (28.9%)	27 (26.7%)	
Male	86 (71.1%)	74 (73.3%)	
Donor type[d]			0.209
DBD	87 (71.9%)	80 (79.2%)	
DCD	34 (28.1%)	21 (20.8%)	
Age[c]	55 (48–62) (20–72)	55 (48–62) (22–70)	0.713
Cause of liver failure[d]			0.782
Alcoholic	36 (29.8%)	29 (28.7%)	
Auto-immune hepatitis	2 (1.7%)	5 (5.0%)	
Hepatitis B	3 (2.5%)	2 (2.0%)	
Hepatitis C	4 (3.3%)	4 (4.0%)	
Hepatocellular carcinoma on background of cirrhosis	15 (12.4%)	16 (15.8%)	
Non-alcoholic steato-hepatitis	11 (9.1%)	11 (10.9%)	
Primary biliary cirrhosis	10 (8.3%)	3 (3.0%)	
Primary sclerosis cholangitis	18 (14.9%)	13 (12.9%)	
Other	22 (18.1%)	18 (17.8%)	
Body mass index[c]	26.18 (23.12–32.39) (18.02–50.99)	26.94 (24.36–30.42) (18.91–42.95)	0.626
Missing	0 (0.0%)	1 (1.0%)	
Retransplant[d]	12 (9.9%)	8 (7.9%)	0.605
MELD score[c] (calculated at time of transplant)	13 (10–18) (6–35)	14 (9–18) (6–29)	0.970
UK	13 (10–17) (6–33)	14 (9–18) (6–28)	
Essen, Germany	17 (14–19) (13–23)	15.5 (14–17) (14–17)	
Barcelona, Spain	16 (8–26) (8–35)	14 (9–16) (8–29)	
Leuven, Belgium	19 (13.5–25.0) (13–26)	16 (16–20) (9–27)	
eGFR[c]	87.36 (69.61–107.66) (33.45–156.43)	92.22 (69.72–104.24) (30.19–155.04)	0.928
Missing	4 (3.3%)	3 (3.0%)	
ET-Donor risk index[c]	1.70 (1.47–2.07) (0.98–4.31)	1.71 (1.50–2.01) (1.06–3.49)	0.610
Missing	13 (10.7%)	13 (12.9%)	

NA, not applicable. eGFR, estimated glomerular filtration rate.
[a]χ^2 tests and non-parametric Mann–Whitney *U*-tests were used for categorical and continuous variables, respectively. No adjustment for multiple comparisons were made.
[b]Functional warm ischaemia applies to DCD donors and is measured from the onset of functional warm ischaemia (systolic blood pressure < 50 mmHg or O_2 saturation < 70%) to cross-clamp.
[c]Median, IQR and full range are reported.
[d]Frequency and column percentages are reported.
[e]Measurement of the degree of steatosis was based on clinical assessment by the retrieval surgeon.

Peak AST (primary outcome)

Peak AST during the first 7 days after transplant was reduced by 49.4% in the NMP group compared to SCS when adjusted by centre and donor type (geometric mean ratio 0.506, 95% confidence interval 0.388–0.659; $P<0.001$). Unadjusted analysis (Student's *t*-test) and sensitivity analysis undertaken in the per-protocol population confirmed these results.

Subgroup analysis showed that the effect of NMP was different in the two donor types (test for interaction $P=0.012$), although it was statistically significant in both subgroups; the reduction in geometric mean peak AST was greater in DCD (73.3%, 95% confidence interval 53.7–84.6%; $P<0.001$) than in DBD livers (40.2%, 95% confidence interval 19.3–55.7%; $P=0.001$). Subgroup analyses for the model for end-stage liver disease (MELD) score and Eurotransplant-donor risk index (ET-DRI) showed no statistically significant differences (data not shown). See Extended Data Table 3a, b and Extended Data Fig. 1 for further analysis. See Table 3 for full outcome results.

Early allograft dysfunction

Data to assess early allograft dysfunction (EAD) rates were available in 216 recipients: the odds of developing EAD in the NMP arm (12 out of 119) were 74% lower than the SCS arm (29 out of 97; odds ratio 0.263, 95% confidence interval 0.126–0.550; $P<0.001$). A logistic regression model adjusted for donor type, MELD score and ET-DRI showed that the adjusted odds of EAD in the NMP arm were approximately 72% lower than in the cold storage arm (adjusted odds ratio 0.276, 95% confidence interval 0.124–0.611; $P=0.002$). The difference in EAD rates was partly a result of the difference in peak AST (described above), but also a reflection of differences in bilirubin. The median bilirubin level in the first week postoperatively was lower in NMP recipients (2.25 mg dl^{-1}, 95% confidence interval 1.23–4.28)) than in the SCS group (2.87 mg dl^{-1}, 95% confidence interval 1.52–5.00; $P=0.029$).

Biliary strictures on MRCP

An MRCP was performed on 155 (81 NMP, 74 SCS) of the 222 transplanted trial patients. There was no significant difference in the rate of non-anastomotic strictures for DBD (NMP 7.4% (4 out of 54) versus SCS 5.4% (3 out of 55); $P=0.678$) or DCD (NMP 11.1% (3 out of 27) versus SCS 26.3% (5 out of 19); $P=0.180$) livers. Only one patient in each trial arm developed clinically relevant evidence of ischaemic cholangiopathy in the first year after transplant, both of whom were re-transplanted.

Table 3 | Trial outcomes

	NMP ($n=121$)[a]	SCS ($n=101$)[a]	Effect (95% CI)[b]	*P* value
Peak AST				
ITT[c]				
Adjusted	488.1 (408.9–582.8)	964.9 (794.5–1,172.0)	0.5 (0.4–0.7)	0.0000
Unadjusted	484.5 (406.4–577.6)	973.7 (795.2–1,192.3)	0.5 (0.4–0.6)	0.0000
Test for interaction by donor type				0.012
Subgroup analysis by donor type				
DBD	526.2 (427.3–647.9)	880.2 (708.5–1,093.5)	40.2% (19.3–55.7%)	0.0009
DCD	389.7 (278.0–546.4)	1,458.1 (944.7–2,250.5)	73.3% (53.7–84.6%)	0.0000
PP analysis	498.6 (414.8–599.4)	982.9 (810.4–1,192.2)	0.5 (0.4–0.7)	0.0000
Secondary outcomes				
Discard rates[d]	16 (11.7%)	32 (24.1%)	−12.4% (−21.4 to −3.3%)	0.008
Primary non-function[e]	1 (0.8%)	0 (0.0%)	NA	NA
Post-reperfusion syndrome	15 (12.4%)	32 (33.0%)	−20.6% (−31.6 to −9.6%)	0.0002
Post-reperfusion lactate[f]	3.6 (2.6–4.2)	4.1 (3.2–5.0)		0.018
Early allograft dysfunction	12 (10.1%)	29 (29.9%)	0.263 (0.126–0.550)	0.0002
Biochemical liver tests[f] (average value over day 1–7)				
Bilirubin (μmol l^{-1})				
Days 1–7	38.5 (21.0–73.2)	49.1 (26.0–85.5)		0.029
30 days	13.0 (8.0–22.1)	13.0 (9.1–21.0)		0.479
6 months	9.1 (6.0–15.1)	9.1 (6.0–13.0)		0.671
AST (IU l^{-1})				
Days 1–7	167.5 (98.0–320.7)	318.5 (152–611.5)		0.0000
30 days	20 (14–35)	22 (15–40)		0.707
6 months	23 (18–33)	23 (18–37)		0.931
γGT (IU l^{-1})				
Days 1–7	268.1 (156.3–408.3)	301 (201.1–443.9)		0.157
30 days	178 (109.5–410.0)	200 (96.0–397.5)		0.949
6 months	47 (28–144)	47 (26–128)		0.452
INR				
Days 1–7	1.2 (1.2–1.4)	1.2 (1.2–1.4)		0.644
30 days	1.1 (1.0–1.2)	1.1 (1.0–1.2)		0.735
6 months	1.1 (1.0–1.2)	1.1 (1.0–1.1)		0.167
Creatinine (μmol l^{-1})				
Days 1–7	92.8 (60.1–121.1)	97.2 (67.2–143.2)		0.139
30 days	82.2 (66.3–104.3)	90.2 (72.5–121.1)		0.019
6 months	99.9 (81.3–117.6)	99.9 (83.1–134.4)		0.265
Lactate (mmol l^{-1})				
Day 1–7	1.3 (1.0–1.7)	1.1 (0.9–1.6)		0.130
Other outcomes				
Need for RRT (number (percentage) of patients)				
Day 1–7 after transplant	26 (21.5%)	19 (18.8%)	2.7% (−7.9 to 13.2%)	0.621
30 days	27 (22.3%)	20 (19.8%)	2.5 (−8.2 to 13.3%)	0.648
6 months	27 (22.3%)	21 (20.8%)	1.5% (−9.3 to 12.4%)	0.784
Duration of RRT day 1–7[f]	4 (2–6)	5 (4–6)		0.346
Length of hospital stay[f]	15 (10–24)	15 (11–24)		0.926
Length of ICU stay[f]	4 (2–7)	4 (3–7)		0.339
Graft survival at 1 year	0.950 (0.893-0.977)	0.960 (0.897–0.985)		0.707
Patient survival at 1 year	0.958 (0.902–0.982)	0.970 (0.909–0.990)		0.671

CI, confidence interval.
[a]Total number of livers transplanted and analysed overall. Primary outcome analysed on $n=220$ due to unavailability of AST values during the first seven days after transplant. Specific outcomes may have different denominators due to some missing data.
[b]Effect reported is: Percentage reduction (from geometric mean ratio) for peak AST; odds ratio for early allograft dysfunction; difference in proportions (%) for discard rates, post reperfusion syndrome and need for renal replacement therapy (RRT); not reported for outcomes for which medians are reported, for survival scores and for tests for interactions of subgroup analysis (only *P* values are reported).
[c]Intention to treat (ITT) analysis was adjusted for donor type and transplant centre.
[d]Denominators for the discard rates is the total number of livers retrieved ($n=270$ (NMP, $n=137$; SCS $n=133$)).
[e]Test not performed due to few events and no events in one arm.
[f]Median and IQR are reported, a non-parametric Mann–Whitney *U*-test was used.

Similarly, there was no significant difference in the rate of anastomotic strictures for DBD (NMP 40.7% (22 out of 54) versus SCS 41.8% (23 out of 55); $P=0.909$) or DCD (NMP 48.1% (13 out of 27) versus SCS 57.9% (11 out of 19); $P=0.515$) livers.

Hospital stay, graft and patient survival

There was no difference in median intensive care unit (ICU) stay (4 days NMP versus 4 days SCS; $P=0.339$), hospital stay (15 days NMP versus 15 days SCS; $P=0.926$) or the need for renal replacement therapy in the first postoperative week (2.7%, 95% confidence interval –7.9 to 13.2%; $P=0.621$).

One NMP liver developed PNF (see Supplementary Information). There were no PNF cases in the SCS arm. Overall 10 recipients died during follow-up, producing a one-year survival of 0.949 (95% confidence interval 0.890–0.977) in the NMP group and 0.958 (95% confidence interval 0.902–0.982) in the SCS group ($P=0.901$). Two deaths in the SCS group and three deaths in the NMP group were due to graft failure.

Graft survival at one year was 0.950 (95% confidence interval 0.893–0.977) and 0.960 (95% confidence interval 0.897–0.985) in the NMP and SCS groups, respectively ($P=0.695$). The causes of graft failure in the SCS arm were hepatic artery thrombosis ($n=3$) and ischaemic cholangiopathy ($n=1$). The causes of graft failure in the NMP arm were hepatic artery thrombosis ($n=2$), ischaemic cholangiopathy ($n=1$), non-thrombotic infarction ($n=1$), inferior vena cava occlusion ($n=1$) and PNF ($n=1$). (Extended Data Fig. 2a, b).

For more detailed analysis of trial outcomes please see Supplementary Information.

Perfusion characteristics indicative of organ quality

The following continuously monitored parameters (mean ± s.d.) by the third hour of NMP were measured for all livers that went on to be successfully transplanted (Extended Data Figs. 3, 4). The measured haemodynamic parameters were: hepatic artery flow ($280 \pm 120\,ml\,min^{-1}$) and portal vein flow ($1.11 \pm 0.2\,l\,min^{-1}$). The measured metabolic parameters were: pH (7.31 ± 0.17) and lactate clearance from $9.99 \pm 3.13\,mmol\,l^{-1}$ at 15 min NMP to $0.93 \pm 0.63\,mmol\,l^{-1}$ by 4 h NMP. The measured synthetic parameter consisted of bile production ($9.17 \pm 11.16\,ml\,h^{-1}$). Notably, 18 transplanted NMP livers produced no/minimal bile during perfusion. All but one of these functioned after transplant. There was no correlation between bile production and post-transplant liver function or later development of non-anastomotic biliary strictures.

One NMP liver developed PNF. This liver was persistently acidotic with lactate > 4 mmol for the duration of NMP. No other liver with these characteristics was transplanted.

Following transplant, 28 livers displayed minimal preservation injury (MPI; peak AST < 250 IU l^{-1}) and 25 showed evidence of severe preservation injury (SPI; peak AST > 1,000 IU l^{-1}). The donors in these groups were well-matched in all characteristics other than sex (Extended Data Table 4). During NMP, there was a difference in baseline perfusate alanine aminotransferase (ALT) (MPI 171 IU l^{-1} versus SPI 669 IU l^{-1}; $P = 0.005$) and lactate dehydrogenase (LDH) (MPI 1,073 IU l^{-1} versus SPI 1,838 IU l^{-1}; $P = 0.01$) between the two groups. Levels of these enzymes, as well as γ-glutamyltransferase (γGT), increased more rapidly during the first 8 h of NMP in the SPI group (ALT, an increase of 56 IU l^{-1} versus an increase of 461 IU l^{-1}, $P < 0.001$; LDH, an increase of 483 IU l^{-1} versus an increase of 980 IU l^{-1}, $P = 0.06$; γGT, an increase of 23 IU l^{-1} versus increase of 104 IU l^{-1}, $P = 0.004$). MPI livers showed a reduction in measurable levels of haemolysis (haemolysis index) as NMP progressed, in contrast to SPI livers in which the levels of haemolysis rose (MPI, a decrease of 0.04 U versus SPI, an increase of 0.09 U; $P = 0.03$). Bile production was greater in the MPI group (MPI 13.1 ml h^{-1} versus SPI 7.8 ml h^{-1}; $P = 0.03$). Lactate clearance was similar in each group. Post-reperfusion syndrome was less common in the MPI group (MPI 0% (0 out of 28) versus SPI 24% (6 out of 25); $P = 0.007$). One NMP liver with perfusate transaminases in excess of 20,000 IU l^{-1} was transplanted successfully.

Adverse events

The proportion of patients for whom adverse events were reported (Extended Data Tables 5a–c, 6) was similar in the two arms (55.4% NMP, 95% confidence interval 46.1–64.4% versus 57.4% SCS, 95% confidence interval, 47.2–67.2%) with a larger total number of events reported for SCS livers (128 NMP versus 164 SCS). Of these, a greater proportion of the serious adverse events (Clavien–Dindo grade ≥IIIb) were in the SCS than NMP arm (16.4% NMP (21 out of 128) versus 22% SCS (36 out of 164)). No statistical tests were applied to these data.

Discussion

To our knowledge, this is the first randomized controlled trial to compare any type of machine perfusion technology with conventional static cold storage in human liver transplantation.

The trial demonstrated significant reductions in peak AST and EAD rates in NMP livers; this is of clinical relevance as both are clinically accepted biomarkers for long-term graft and patient survival[12,13]. These benefits are consistent with previous animal work[10] and the phase-I clinical study[9] that preceded this trial, both of which showed post-transplant AST reductions in NMP livers. No differences were seen in graft or patient survival: a much larger trial is required to test this outcome. It is notable that these reductions in peak AST and EAD rates were achieved in the context of improved organ utilization and longer preservation times, both of which have implications in terms of addressing the donor shortage and logistical barriers that currently limit liver transplants.

DCD donors represent a largely untapped source of organs, comprising 42% of UK deceased donors, but only 21% of transplanted livers[14]. Utilization of DCD livers is limited by poorer outcomes (PNF and ischaemic cholangiopathy) compared with DBD livers. Allowing the limitations of small group analyses, in this study NMP DCD liver primary outcome data were superior to those of both DCD and DBD livers preserved using SCS. In fact, the primary outcome of DCD NMP livers was superior to that of DBD livers preserved by NMP: this was possibly owing to a selection bias, both of donors (lower threshold to decline DCD donors) and recipients (fitter patients selected for higher-risk organs). The AST differences are in the context of longer functional warm ischaemia times, longer preservation times and fewer organ discards in the NMP arm, suggesting that NMP may be achieving the desired objective of increasing organ utilization without compromising outcome. If these findings were translated into clinical practice, the increase in organ utilization would have substantial implications for waiting list mortality, which is currently approximately one in five patients[1].

The longer preservation times in the NMP group were not planned, but were all within the maximum perfusion time defined in the protocol. There was no stipulation in the trial protocol that the preservation times should be matched. As clinicians gained experience, it appeared that some centres had started to organize their operating schedule according to the preservation method, although no overall difference between arms was seen in the proportion of transplants occurring in daylight hours. If, as appears to be the case, NMP can safely extend preservation times without compromising outcomes, this will have implications for operating department planning as well as organ utilization.

There were over 50% fewer discarded organs in the NMP group, resulting in 20% more transplanted livers (121 NMP versus 101 SCS). The SCS discard rate of 23.7% was higher than the 17% reported in UK registry data[14], and may reflect the high proportion of DCD livers enrolled in the trial; the discard rate of retrieved DCD livers in the UK is 30%[14]. This reported difference in organ utilization is likely to be an underestimation of the full potential impact that NMP could have on transplant numbers. The trial stipulated that only livers considered transplantable according to standard practice could be enrolled. For the full extent of improved organ utilization to be measured, livers would need to be randomized to NMP or SCS before being offered for transplant; this should form the basis of a future study. An increase of 20% or more in the number of transplantable donor livers would have a transformative effect on the mortality on liver transplant waiting lists around the world.

The haemodynamic characteristics of the NMP recipients following reperfusion were measurably superior to those of SCS recipients, in line with previously reported findings[15]. This did not translate into a difference in ICU stay, hospital stay or need for renal replacement therapy between the two groups, despite previous reports showing a correlation between peak AST and renal replacement therapy[16]. The magnitude of the reperfusion syndrome is a factor in determining the eligibility of the sickest patients for high-risk organs, due to the limited capacity of such patients to tolerate cardiovascular instability; NMP might therefore increase the options for the most urgent patients.

Perhaps the greatest limitation to more widespread utilization of DCD livers is the high rate of clinically important non-anastomotic biliary strictures (NAS) which lead to a high rate of graft failure; this is believed to develop due to the vulnerability of the biliary tree to prolonged warm ischaemia. The rate of NAS in the NMP DCD group (11.1%) was lower than in SCS (26.3%) livers, despite longer functional warm ischaemia times. This did not reach statistical significance, which may be a function of sample size; the trial was not powered for this outcome. Reported rates of NAS in DCD transplants vary from 10 to 30%[17,18], but these are in patients with symptoms suggesting biliary pathology, rather than those only apparent on imaging; biliary

investigations are usually only performed for clinical indications (typically deranged liver function). Prior to this study, the radiological incidence of both anastomotic and non-anastomotic strictures in asymptomatic patients was unknown; in particular, there is no real benchmark against which to compare the rate of NAS seen in the DCD SCS group. Apart from the two patients retransplanted for ischaemic cholangiopathy, almost all of the remaining patients with radiological evidence of NAS had normal liver function at one year; this questions the clinical relevance of a protocol MRCP at six months. The longer-term follow-up of these patients will shed light on the importance of a radiological diagnosis of biliary stricturing in patients with normal graft function, and the role of MRCP as an endpoint in future trials.

As well as demonstrating improved graft preservation, this trial tested the feasibility, usability and safety of NMP, a vital component of the evaluation of any new technology. It showed that the logistical challenges of NMP can be met successfully within clinical practice. Over 120 NMP livers were transplanted in seven transplant centres across four European countries. Nonetheless, adoption of this technology into clinical practice may necessitate changes in the organ retrieval process, particularly with respect to technical support and transport arrangements. It remains to be seen whether NMP is required for the full duration of an organ's preservation or can equally well be applied after a short period of SCS when the organ reaches the transplanting centre—this would simplify the logistics but may not be suitable for the most marginal organs[19]. A phase-II study to test this has recently completed enrolment in the UK (NCT03176433).

For this new technology to be supported by healthcare funders, a health–economic case is needed. The results of this study suggest that benefits will accrue not only from improved early graft function and transplantation logistics, but also from improved utilization. Secondary economic benefits will accrue from logistic changes, enabling transplants to be moved predominantly into daytime operating, with reduction in staffing costs and likely improvements in outcome. More timely intervention will also bring economic benefits—earlier transplantation is associated with lower morbidity and cost.

The effects of NMP demonstrated in this study are unequivocal with respect to the primary endpoint, implying a benefit in livers currently used for transplantation. However, the greatest benefit may be realized by applying this technology to livers outside current acceptance criteria, in order to transplant organs currently deemed untransplantable. Algorithms to assess organ viability, based on data obtained during NMP, will be essential if this potential is to be realized[10]. This study sheds some light on which perfusion parameters may be used to assess organ quality: bile production, acid–base stability, lactate clearance, perfusate transaminase levels, falling measurable haemolysis—all correlate with the degree of preservation injury evident after transplant. However, all but one of the livers transplanted in the NMP group functioned postoperatively, including one NMP liver with perfusate transaminases in excess of 20,000 IU l^{-1} and 18 livers with minimal bile production. Data from much larger numbers of NMP transplants (typically from a registry) would be required to determine specific markers of viability.

The importance of bile production during NMP is unclear. Preliminary evidence from our group[20] suggests that preservation injury causes impaired hepatocellular uptake of bile salts. We have shown evidence of progressive accumulation of bile salts in the perfusate of livers with high post-transplant transaminase levels; something that also correlates with poor bile production during NMP. The extent and nature of the injury required to produce this effect is not clear but does appear to reflect organ quality rather than viability.

High-risk organs (for example, those with steatosis) may benefit from therapeutic interventions delivered during NMP: several groups are exploring potential strategies, including stem cell treatments, de-fatting agents and immunological modification of the organ. Future trials may be needed to formally test the size of the effect of NMP on organ utilization; for this it will be necessary to randomize livers at the time of organ offering rather than the time of retrieval. Organ utilization, or organ utilization with 12-month graft survival (functional utilization) would be a logical primary endpoint for a study of this sort.

This study describes the formal clinical evaluation of a novel technology in liver transplantation, and could herald the start of a new era of intervention during organ preservation. It represents a first, necessary step in demonstrating that NMP is feasible, safe and effective in clinical practice; the fact that the study has definitively met its primary endpoint should now enable the exploration of the technology's wider potential.

Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0047-9.

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Competing interests P.J.F. is a co-founder, chief medical officer and consultant to OrganOx Limited and also holds shares in the company. C.C.C. is a co-founder, chief technical officer and consultant to OrganOx Limited and also holds shares in the company. Neither P.J.F. nor C.C.C. were involved in the selection, recruitment or transplantation of patients in this study.

Additional information
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METHODS

Study design. This investigator-led, multinational, open-label, two-arm, parallel randomized controlled trial included seven liver transplant centres from the UK (Addenbrooke's Hospital, Cambridge; King's College Hospital, London; Queen Elizabeth Hospital, Birmingham; Royal Free Hospital, London), Belgium (Universitaire Ziekenhuizen, Leuven), Spain (Hospital Clínic de Barcelona, Barcelona) and Germany (Universitatsklinikum, Essen), and was part of the EU-funded Consortium for Organ Preservation in Europe (COPE, http://www.cope-eu.org/). Approval was obtained from national research ethics committees and medical device regulatory bodies in each trial region, in particular the London–Dulwich National Research Ethics Committee (NREC) and the Medicines and Healthcare Regulatory Agency (MHRA) in the UK; the Federaal Agentschap voor Geneesmiddelen en Gezondheidsproducten (FAGG) and the Commissie Medische Ethiek of Universitaire Ziekenhuizen, Leuven, Belgium; the Comité Ético de Investigación Clínica of the Hospital Clínic de Barcelona; the Deutsche Ärztekammer and the ethics committee of University Hospital Essen, Germany. The trial protocol was registered before recruitment (ISRCTN 39731134). All relevant ethical regulations relating to the conduct of this study were followed at each trial site. The trial is reported in accordance with the CONSORT statement[21].

No major amendments were made to the trial design after the start of recruitment.

Eligibility and consent. Inclusion criteria for donors and recipients were deliberately broad to represent the full spectrum of clinical practice. Whole livers from DBD and DCD (Maastricht category III[22]) donors at least 16 years of age were eligible. Specific donor consent was not required for trial inclusion. No organs were procured from prisoners. Recipients were eligible provided they were at least 18 years old and listed for a liver-only transplant, excluding those with fulminant liver failure, owing to the poor prognosis of this group regardless of organ quality. Potential participants were consented while on the waiting list; consent was affirmed on the day of transplantation. The consent included the recording of anonymized data for trial purposes and the collection of biological samples for storage in the trial biobank (see 'Sample collection'). No patient identifiable data were collected.

Randomization. Once an eligible donor organ was allocated to a consented recipient and the availability of the NMP device and team was confirmed, the liver was randomized. All clinical decisions thereafter, including graft suitability and procedure scheduling, were made independently of the trial team.

Using an online randomization tool, livers were assigned to NMP or SCS with 1:1 allocation ratio as per a computer-generated randomization schedule, using variable block size, stratified by transplant centre and donor type (DBD/DCD). The unit of randomization was donor livers rather than recipients, but analysis is reported for the transplant recipients.

Static cold storage group. Livers randomized to SCS were retrieved, preserved, transported and transplanted according to local standard practice.

Normothermic machine perfusion group. The OrganOx metra normothermic liver perfusion device was used (Extended Data Fig. 5a), which enables automated organ preservation for up to 24 h. Following randomization to NMP, the device and accompanying researcher were transported to the donor hospital. The device was set-up during the retrieval procedure, as has been previously described[9]. A sterile disposable set was installed on to the device and primed with 500 ml gelofusine (B. Braun Ltd) and three units of donor-matched packed red blood cells. Antibiotics were given at the outset and heparin, insulin, prostacyclin, bile salts and fat-free parenteral nutrition were infused during the perfusion (Extended Data Fig. 5b).

Following retrieval of the donor organ, and while still at the donor hospital, the liver back-table operation was performed[23], followed by cannulation of the hepatic artery, portal vein, inferior vena cava and bile duct. The liver was connected to the NMP device and perfusion commenced (Fig. 1). During the early part of the perfusion sodium bicarbonate was added incrementally to achieve a physiological pH. The OrganOx metra perfusion device incorporates online blood gas measurement (Terumo CD1-500) together with software-controlled algorithms to control P_{O_2} and P_{CO_2} (within physiological limits), temperature (37 °C), mean arterial pressure (65–75 mm Hg) and), inferior vena cava pressure (0–2 mm Hg). Typical blood flows of 200–400 ml min^{-1} (artery) and 1,000-1,200 ml min^{-1} (portal vein) were obtained. Glucose was measured manually and the value entered into the device. If glucose fell below 10 mmol l^{-1} this automatically triggered the infusion of a fat-free TPN mixture (Nutriflex Special, B. Braun Ltd) into the perfusate.

NMP continued throughout the duration of transport and storage until the transplanting team were ready to implant the liver. The minimum protocol-stipulated NMP duration was 4 h, the time needed for ATP repletion in animal studies[11]. The maximum allowed NMP duration was 24 h in line with the experience in the phase-I study and the regulatory approval for the device[9].

Sample collection. Tissue biopsies (donor liver and bile duct), recipient blood and urine were collected at pre-specified time points from every liver/transplanted patient in the study. In addition to these, samples of perfusate fluid and bile were collected from every NMP liver. These were stored in a central biobank established by the COPE Consortium for use in ongoing mechanistic studies. Each sample was allocated a unique bar code, which the biobank coordinator was able to match to a specific trial identification number. No patient identifiable data were associated with each sample.

Study end points. The primary endpoint was defined as the difference between the two treatment arms in the peak level of serum AST within seven days after transplant. This is a clinically accepted biomarker, predictive of primary non-function as well as graft and patient survival[12,24] and is also associated with histological evidence of moderate to severe perfusion injury[25,26].

A surrogate marker of graft survival was used in this trial for two reasons: (1) the relatively high survival rates in liver transplantation (> 90%) and (2) the multifactorial causes of graft loss. A trial based directly on graft or patient survival would have had to be unfeasibly large.

In order to ensure consistency and to minimise the hypothetical AST 'wash-out' effect in the NMP-treated organs, the first post-transplantation value was measured between 12 and 24 h after reperfusion.

Secondary end points included: (1) organ discard rate (after retrieval); (2) post-reperfusion syndrome[27]: > 30% drop in mean arterial pressure persisting for > 1 min within five minutes of reperfusion; (3) primary non-function: irreversible graft dysfunction, for non-technical and non-immunological causes, leading to death or emergency liver replacement during the first 10 days after liver transplantation; (4) early allograft dysfunction[13] as indicated by any one of the following clinical indicators: (i) bilirubin > 170 μmol l^{-1} on day 7 after transplant; (ii) INR > 1.6 on day 7 after transplant; (iii) peak-AST > 2,000 IU l^{-1} during the first 7 days; (4) length of hospital and ICU stays; (5) need for renal replacement therapy; (6) evidence of cholangiopathy on MRCP at six months; (7) graft and patient survival at one year.

Full details of all secondary outcomes are available in the trial protocol[28].

Six-month MRCP. An MRCP scan was performed six months (range 5–7 months) after transplant to evaluate the biliary tree for features of cholangiopathy evident by biliary strictures. All scans were reviewed by two independent radiologists blinded to the method of organ preservation with disparities adjudicated by a third radiologist. Owing to the lack of any existing grading system for biliary strictures, a system was agreed to in advance by consensus among the radiologists to allow definitive categorization of the presence and site of strictures. The findings were reported as follows: (1) normal biliary tree; (2) anastomotic stricture (> 70% of luminal diameter); (3) unequivocal evidence of non-anastomotic stricture anywhere in the biliary tree; (4) both anastomotic and non-anastomotic biliary strictures.

Statistical analysis. Previous data from Universitaetsklinikum Essen, Germany (A.Pau. & S.R.K., unpublished observations), demonstrated the geometric mean of peak AST to be 608.59 IU l^{-1} in patients transplanted following SCS. The present study was powered to detect a (clinically relevant) 33% reduction in peak AST with 90% power at a 5% significance level, requiring 220 transplanted livers (110 per arm).

Results are reported as a modified intention-to-treat analysis. A per-protocol sensitivity analysis was also performed excluding livers that received machine perfusion outside the protocol specified range (4–24 h) and comparing the groups according to the treatment actually received. Livers randomized but not retrieved were excluded from the analysis.

Primary outcome was analysed using ANOVA with adjustment for stratification factors. The peak AST was calculated for each recipient with at least two values available. Missing AST values were not imputed. Binary outcomes were assessed using test for proportions or logistic regression to adjust for potential confounders and report odds ratios. Continuous outcomes were compared using a Student's *t*-test, if normally distributed, or by Mann–Whitney *U*-test otherwise. Time-to-event outcomes were analysed using Kaplan–Meier estimates and log-rank tests. Outcomes are reported with 95% confidence intervals and *P* values to three decimal places. $P < 0.05$ was regarded as statistically significant.

Pre-specified subgroup analyses were performed for donor type (DCD versus DBD), donor risk index (ET-DRI) and MELD score using tests for interaction and reported using forest plots. Interaction methods were used to look for consistency of treatment effect across the different subgroups and reported using forest plots. The study was not powered to detect differences in the subgroups; these results should only be regarded as hypothesis-generating.

Analyses were conducted using Stata version 14.2 (StataCorp).

No formal interim analyses of end points were carried out. At regular intervals, an independent Data Monitoring Committee reviewed confidential reports covering recruitment, safety parameters and primary end point data.

Full details of the statistical methodology are available in the Supplementary Information.

Machine perfusion parameters. During NMP continuous displays of pressures, flows, metabolic (pH) and synthetic (bile production) liver function were available

to the operator. In addition, lactate measurements were carried out using external blood gas analysis. The trial protocol did not stipulate the manner in which these parameters should be interpreted.

Once trial recruitment was complete, an ad hoc analysis was performed in which NMP organs were categorized according to those which, following transplantation, displayed minimal preservation injury (MPI; peak AST < 250 IU l^{-1}) and those with severe preservation injury (SPI; peak AST $> 1{,}000$ IU l^{-1}). Groups were compared for differences in donor and recipient characteristics, perfusate biochemistry, bile production and evidence of post-reperfusion syndrome.

Adverse events. Reporting of adverse events was in accordance with the European Commission MEDDEV guidelines[29]. Following trial completion, these were reviewed by two independent clinicians blinded to the treatment arm. Adverse events with a Clavien-Dindo[30] grading greater than IIIa were considered serious adverse events. Rates of adverse events are reported with 95% confidence intervals. No statistical tests were applied to these data.

Full details of the trial methodology are available in the clinical trial protocol[28].

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. The full trial protocol, statistical analysis plan and final statistical report are available in the Supplementary Information.

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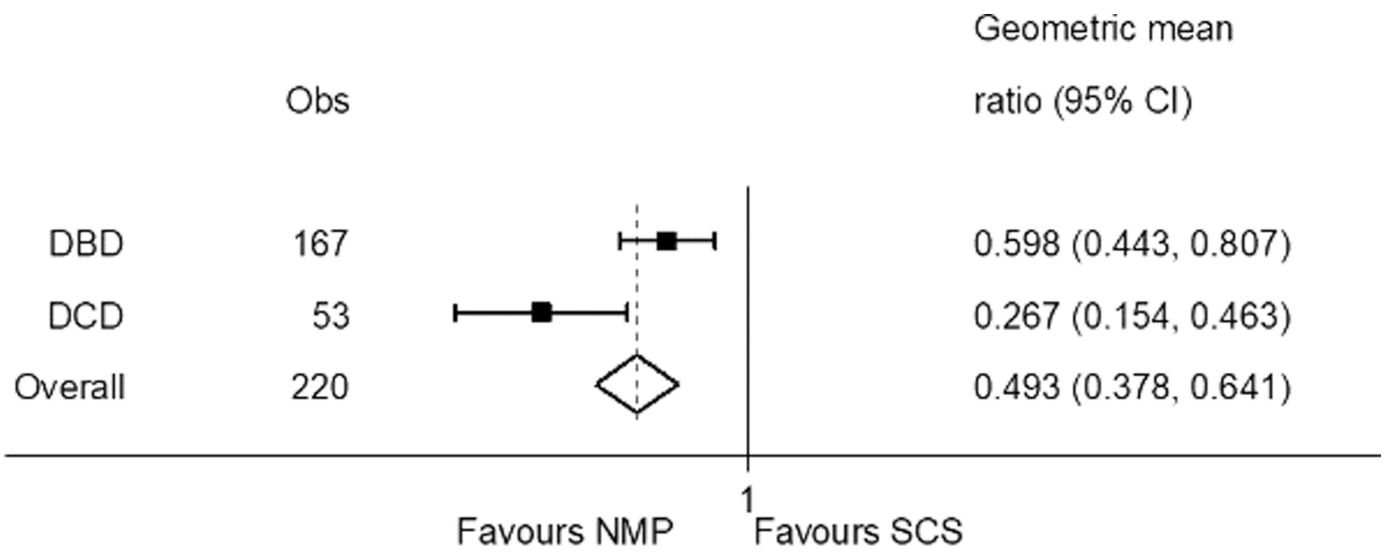
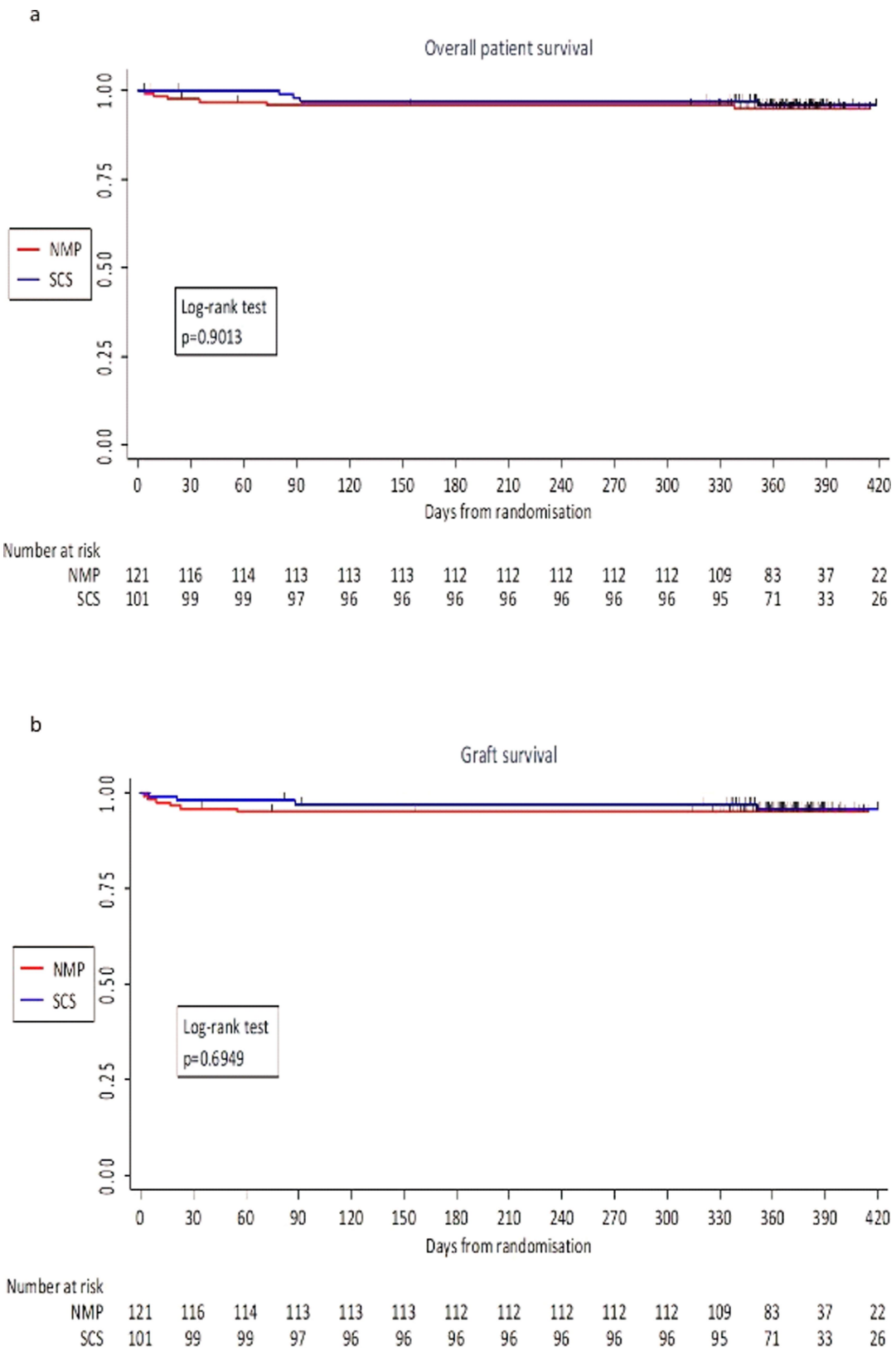
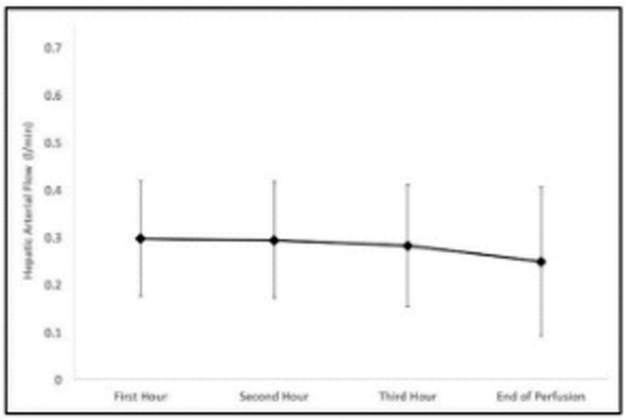


Extended Data Fig. 1 | Forest plot for subgroup analysis of peak AST by donor type. Geometric mean ratio and 95% confidence interval are reported for each subgroup and overall for all groups. DBD group, $n = 87$ NMP, $n = 80$ SCS; DCD group, $n = 33$ NMP, $n = 20$ SCS.



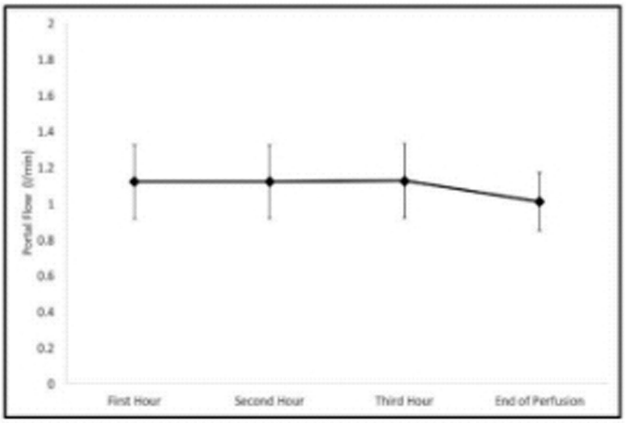
Extended Data Fig. 2 | Post-reperfusion syndrome. a, Kaplan–Meier plot for one-year survival of patients with two-sided log-rank test. **b**, Kaplan–Meier plot for one-year graft survival with two-sided log-rank test.

a



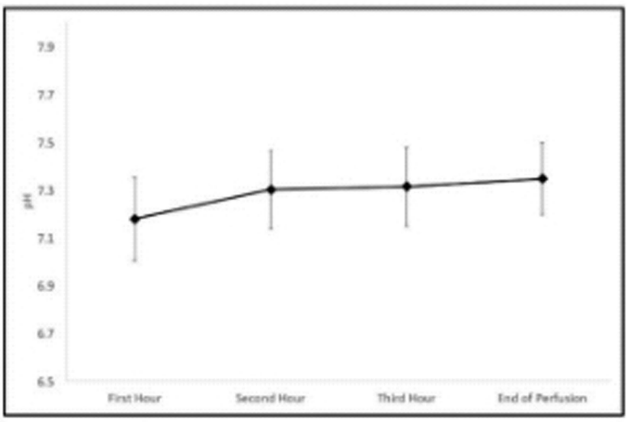
Time Point	Mean flow (litres/min)	Standard Deviation
First hour	0.296	0.121
Second hour	0.294	0.122
Third hour	0.282	0.128
End of perfusion	0.248	0.157

b



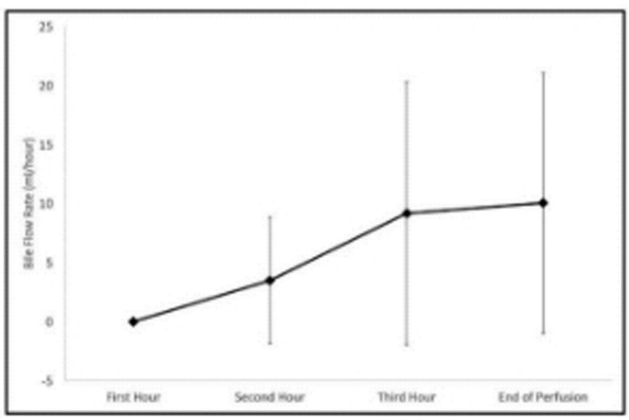
Time Point	Mean flow (litres/min)	Standard Deviation
First hour	1.118	0.205
Second hour	1.118	0.202
Third hour	1.124	0.206
End of perfusion	1.008	0.160

c



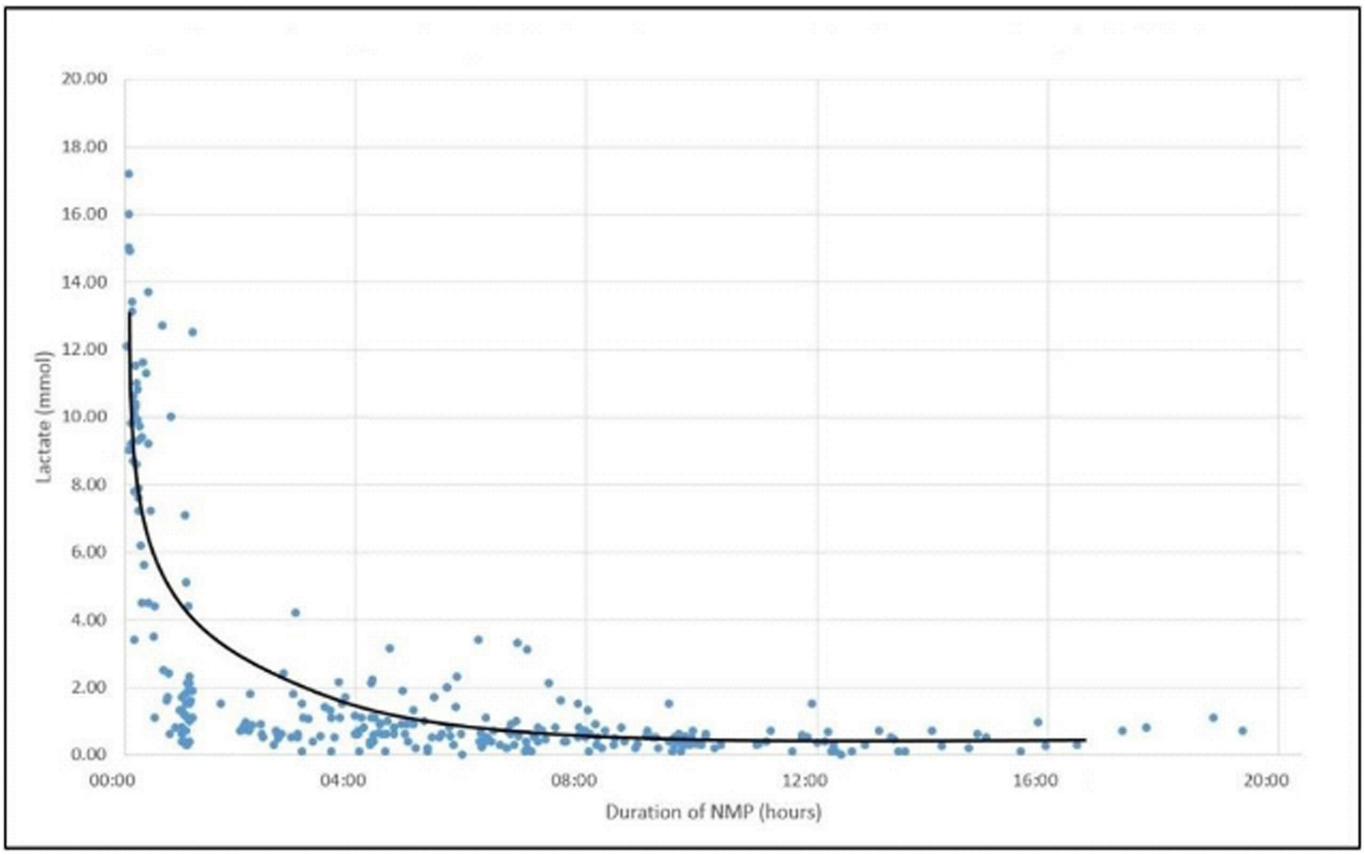
Time Point	Mean pH	Standard Deviation
First hour	7.178	0.174
Second hour	7.301	0.165
Third hour	7.313	0.166
End of perfusion	7.347	0.150

d

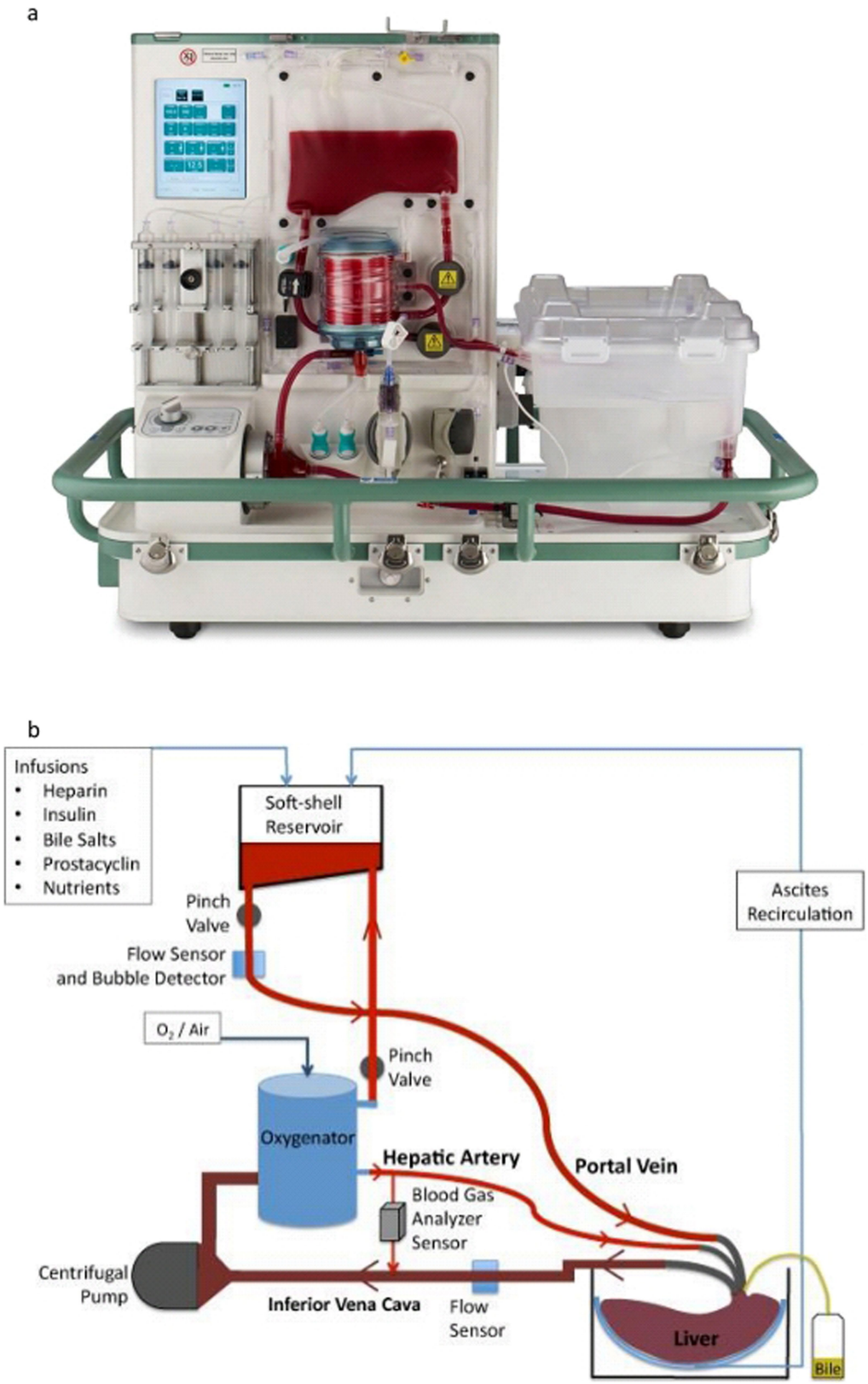


Time Point	Mean flow (ml/hour)	Standard Deviation
First hour	0	0
Second hour	3.486	5.358
Third hour	9.166	11.160
End of perfusion	10.050	11.036

Extended Data Fig. 3 | Machine perfusion parameters during NMP. **a**, Hepatic artery flow during NMP. **b**, Portal vein flow during NMP. **c**, Perfusate pH during NMP. **d**, Bile production during NMP. **a**–**d**, Data are mean ± s.d. of each time point. Actual values are shown in the table. $n = 87$.



Extended Data Fig. 4 | Perfusate lactate levels during NMP. Scatter graph with trend line showing perfusate lactate levels at different time points during NMP for all transplanted livers. $n = 94$.



Extended Data Fig. 5 | NMP device and circuit. a, OrganOx metra (generation 1). The NMP device used in the trial. **b**, OrganOx metra NMP circuit. The liver is perfused via the hepatic artery and portal vein. It drains via the inferior vena cava to a centrifugal pump through which the perfusate passes, via a heat exchanger/oxygenator, to a reservoir or directly into the hepatic artery. The perfusate in the reservoir drains under gravity into the portal vein.

Extended Data Table 1 | Detailed breakdown of reasons for discard of NMP livers

Discarded Liver No	Device Error	Device User Error	Poor Perfusion Parameters	Donor Malignancy	Door Cirrhosis	Poor In-situ Perfusion	Prolonged Donor Warm Ischaemia	Liver Size	Steatosis	Further Details
1	N	Y	N	N	N	N	N	N	Y	Poor perfusion due to IVC cannula positioning. Safely converted to cold storage and discarded due to steatosis.
2	N	N	N	N	Y	N	N	N	Y	Appearances consistent with cirrhosis in donor with known hepatitis C.
3	N	N	Y	N	N	N	N	N	N	Poor hepatic artery flow during NMP with increasing lactate.
4	N	N	N	Y	N	N	N	N	N	Incidental lung tumour found at retrieval
5	N	N	N	N	N	N	Y	N	Y	Warm ischaemia time greater than 30 minutes in DCD donor.
6	N	N	N	N	N	Y	Y	N	Y	Warm ischaemia time greater than 30 minutes in DCD donor. Poor in situ cold perfusion.
7	N	N	Y	N	N	N	N	N	Y	Persistently raised lactate >6mmol after 6 hours NMP.
8	N	N	N	Y	N	N	N	N	Y	Colonic tumour found at retrieval.
9	N	N	N	N	N	N	N	N	Y	60% steatosis on biopsy.
10	N	N	Y	N	N	N	N	N	N	Persistent acidosis with lactate >6mmol after 8hours NMP.
11	N	N	N	N	N	N	N	Y	Y	Large steatotic liver, no size-matched recipient found.
12	N	N	Y	N	N	N	N	N	Y	Persistently raised lactate >3mmol with acidosis.
13	N	N	N	N	N	N	N	N	Y	Moderate steatosis on biopsy, surgeon decision to discard.
14	N	N	Y	N	N	N	N	N	Y	Poor hepatic artery and portal vein flow during NMP in a steatotic liver.
15	N	Y	N	N	N	N	N	N	Y	Excessive bleeding during NMP from phrenic veins and hepatic artery in a steatotic liver. Safely converted to cold storage and declined due to steatosis.
16	Y	N	N	N	N	N	N	N	Y	See Supplementary Information for narrative description of device error.

Extended Data Table 2 | Post-reperfusion syndrome analysis

a

Post-reperfusion syndrome	NMP (n=121)	SCS (n=101)
No	106 (87.6%)	65 (67.0%)
Yes	15 (12.4%)	32 (33.0%)
Total	121	97
Difference = -20.6% (95% C.I. -31.6%, -9.5%) p = 0.000		

b

Post-reperfusion lactate	NMP (n=81)	SCS (n=51)	p-value
Median (IQR)	3.6 (2.6, 4.2)	4.1 (3.2, 5.0)	0.018

c

	NMP (n=121)	SCS (n=101)
Requiring pre-reperfusion vasopressor infusion	92 (76.0%)	82 (81.2%)
missing	*2 (1.7%)*	*6 (5.9%)*
Post-reperfusion vasopressor bolus	41 (33.9%)	60 (59.4%)
(missing)	*4 (3.3%)*	*9 (8.9%)*
Requiring post-reperfusion vasopressor infusion	65 (53.7%)	80 (79.2%)
(missing)	*1 (0.8%)*	*9 (8.9%)*

a, Post-reperfusion syndrome by treatment group. Frequencies and column percentages are reported. Difference in proportions was tested using a Fisher's test for proportions. **b**, Difference in post-reperfusion lactate in the recipient in each treatment arm. This relates to the first lactate measurement recorded by the anaesthetist after liver reperfusion and occurred within 30 min of reperfusion. Analysis using non-parametric Mann–Witney *U*-test. IQR, inter-quartile range. **c**, Difference in use of vasopressor medications before, during and after liver reperfusion in the recipient. Percentage of total events are reported in brackets. Details of the specific vasopressors that were used were not recorded.

Extended Data Table 3 | Extended primary outcome analysis

a

	NMP N=120	SCS N=100	Difference / Mean ratio^ [% reduction]
Mean ln Peak AST (95% C.I)	6.191 (6.013, 6.368)	6.872 (6.678, 7.066)	-0.681 (-0.946, -0.417)
Geometric Mean Peak AST (95% C.I.)	488.142 (408.856, 582.804)	964.934 (794.471, 1171, 972)	0.506 (0.388, 0.659) [49.4% (34.1%, 61.2%)]

b

Donor Type	Obs	Effect (geometric mean ratio)	[95% Conf. Interval]	p-value
DBD	167	0.598	(0.443, 0.807)	0.001
DCD	53	0.267	(0.154, 0.463)	0.000

a, Primary outcome results from the unadjusted analysis. Sample size for analysis of primary outcome is $n = 220$. A Student's *t*-test was used. ^First cell in this column refers to the mean difference in natural logarithm of peak AST (variable used to run the analysis models). The second cell in this column refers to the geometric mean ratio of the peak AST, used to look at the reduction in the original measurement. **b**, Treatment effect on peak AST for donor type subgroups. Sample size for subgroup analysis is $n = 220$ (DBD group, $n = 87$ NMP, $n = 80$ SCS; DCD group, $n = 33$ NMP, $n = 20$ SCS). A Student's *t*-test was used. No power calculation or adjustment was made for subgroup analysis.

Extended Data Table 4 | Characteristics and perfusate analysis of livers included in NMP liver quality model development

a

	MPI	SPI	p-value
NMP duration (mins)	592.8 ± 51.10	633.7 ± 52.49	0.579
Donor Type (DBD/DCD)	19/9	20/5	0.365
Donor Age (years)	56.2 ± 3.42	51.0 ± 3.02	0.260
Donor Sex	9M; 19F	18M; 7 F	0.006
ET-DRI	1.83 ± 0.22	1.76 ± 0.16	0.598
Recipient Age	52.2 ± 2.11	56.0 ± 2.77	0.276
Recipient Sex	17M; 11F	19M; 6F	0.257
MELD	15.0 ± 1.09	13.5 ± 1.28	0.392

b

Time (mins)	15 mins			60 mins			End (range 240-1440 mins)			Difference (End – 15mins)		
	MPI	SPI	p-value	MPI	SPI	p-value	MPI	SPI	P value	Change MPI	Change SPI	p-value
Haemolysis Index	0.25	0.19	**0.727**	0.18	0.21	0.960	0.11	0.38	**0.072**	-0.04	0.09	**0.0278**
Urea	3.4 ± 0.19	3.4 ± 0.23	**0.994**	5.0 ± 0.34	5.0 ± 0.34	0.997	18.9 ± 1.99	18.0 ± 1.43	**0.708**	15.5 ± 1.93	14.5 ± 1.37	**0.6880**
Bilirubin	2 (2)	2 (2-10)	**0.034**	2 (2-5)	2 (2-28)	0.026	2 (2-108)	4.5 (2-121)	**0.102**	0	2.5	**0.1443**
ALT	170.5 (64-1811)	669 (58-4390)	**0.005**	193.5(78-2306)	570 (78-4809)	0.006	268.5 (106-4107)	1334 (266-16772)	**<0.001**	56	461	**0.0000**
Alkaline Phosphatase	7 (5-21)	7 (5-32)	**>0.999**	7 (5-45)	10 (5-105)	0.113	40 (5-394)	71.5 (8-322)	**0.051**	32	61.5	**0.0682**
GGT	4 (4-30)	4.5 (4-92)	**0.262**	5 (4-103)	12 (4-110)	0.132	35.5 (4-190)	108 (8-759)	**0.008**	23 (0-183)	104 (4-667)	**0.0039**
LDH	1073 ± 180	1838 ± 245	**0.015**	1491 ± 256.6	1884 ± 207.8	0.257	1479 ± 157.2	2610 ± 187.6	**<0.001**	482.8 ± 110.6	980.1 ± 278.7	**0.0591**
CRP	8.3 ± 0.87	8.6 ± 1.47	**0.857**	22.1 ± 2.83	23.0 ± 3.63	0.853	147.4 ± 24.39	191.5 ± 36.07	**0.300**	131.9 ± 27.28	189.2 ± 42.74	**0.2445**
Lactate	10.5 ± 0.61	10.7 ± 0.78	**0.852**	1.7 ± 0.39	3.0 ± 0.78	0.106	0.5± 0.09	0.9 ± 0.29	**0.146**	-8.6 ± 0.64	-7.7 ± 0.83	**0.3672**

a, Demographic data for organ quality model development livers. Demographic data for minimal preservation injury (MPI; peak AST < 250 IU l^{-1}; $n = 28$) and significant preservation injury (SPI; peak AST > 1,000 IU l^{-1}; $n = 25$) groups. Continuous variables were analysed using an unpaired Student's *t*-test and categorical variables using Fisher's exact test. Data are mean ± s.e.m. **b**, Comparison of NMP perfusate analyses between MPI (peak AST < 250 IU l^{-1}; $n = 28$) and SPI (peak AST > 1,000 IU l^{-1}; $n = 25$) groups. A D'Agostino–Pearson normality test was performed to assess data distribution. Parametric data were analysed using an unpaired Student's *t*-test and non-parametric data were analysed using a Mann–Whitney *U*-test. Parametric data are presented as mean ± s.e.m. and non-parametric data are presented as median and range.

Extended Data Table 5 | Adverse events analysis

a

Patients with	NMP (N=121)	SCS (N=101)	Total
No events reported	54 (44.6%)	43 (42.6%)	97 (43.7%)
Adverse events (95% C.I.)	67 (55.4%) (46.1%, 64.4%)	58 (57.4%) (47.2%, 67.2%)	125 (56.3%)

b

Clavien-Dindo grading	NMP	SCS	Total
I	15 (11.7%)	30 (18.3%)	45 (15.4%)
II	64 (50.0%)	72 (43.9%)	136 (46.6%)
IIIa	28 (21.9%)	26 (15.9%)	54 (18.5%)
IIIb	8 (6.3%)	9 (5.5%)	17 (5.8%)
IVa	5 (3.9%)	15 (9.2%)	20 (6.9%)
IVb	3 (2.3%)	9 (5.5%)	12 (4.1%)
V	5 (3.9%)	3 (1.8%)	8 (2.7%)
Total	128	164	292

c

Classification	NMP	SCS	Total
AE	107 (83.6%)	128 (78.1%)	235 (80.5%)
SAE	21 (16.4%)	36 (22.0%)	57 (19.5%)
Total	128	164	292

a, Number of patients with any adverse events reported in each trial arm. The percentage of total events is reported in brackets. No statistical tests have been applied. **b**, Adverse events were categorized by Clavien–Dindo grade. Breakdown of adverse events in each trial arm according to Clavien–Dindo grading. The percentage of total events is reported in brackets. Adverse events with Clavien–Dindo grading ≥IIIb were categorized as serious adverse events. No statistical tests have been applied. **c**, Breakdown of adverse events and serious adverse events in each trial arm. The percentage of total events is reported in brackets. Adverse events with Clavien–Dindo grading ≥IIIb were categorized as serious adverse events. No statistical tests have been applied.

Extended Data Table 6 | Detailed breakdown of adverse events in each trial arm

Event Category	NMP	SCS	Total
Infection	25 (19.5%)	17 (10.4%)	42 (14.4%)
Chest	1	1	2
Blood	10	3	13
Biliary	6	0	6
Abdominal	2	3	5
Gastrointestinal	4	5	9
Other	2	5	7
Hepatic	44 (34.4%)	48 (29.3%)	92 (31.5%)
Bile leak	2	1	3
Biliary stricture (anastomotic)	9	11	20
Ischaemic cholangiopathy	1	3	4
Biliary other	1	0	1
Drainage of ascites	0	1	1
Hepatic artery aneurysm	0	1	1
Hepatic artery thrombosis	2	4	6
Hepatic artery stenosis	5	3	8
Hepatic artery other	0	2	2
Hepatic vein thrombosis	1	0	1
Portal vein thrombosis	2	0	2
Portal vein stenosis	2	0	2
Portal vein other	1	0	1
Graft dysfunction	3	2	5
Rejection	12	13	25
Other	3	7	10
Cardiovascular	5 (3.9%)	5 (3.1%)	10 (3.4%)
Congestive heart failure	1	0	1
Myocardial infarction	2	3	5
Other	2	2	4
Dermatologic	1 (0.8%)	0 (0.0%)	1 (0.3%)
Seroma	1	0	1
Gastrointestinal	5 (3.9%)	6 (3.7%)	11 (3.8%)
Colitis	0	1	1
Diarrhea	3	2	5
Other	2	3	5
Genitourinary	8 (6.3%)	17 (10.4%)	25 (8.6%)
Renal insufficiency	6	13	19
UTI	2	3	5
Other	0	1	1
Respiratory	4 (3.1%)	9 (5.5%)	13 (4.5%)
Cold/flu	0	1	1
Pneumonia	4	6	10
Shortness of breath	0	1	1
Other	0	1	1
Bleeding complications	9 (7.0%)	6 (3.7%)	15 (5.1%)
Bleeding – no transfusion required	0	2	2
Hemorrage (Bleeding requiring transfusion)	3	0	3
Bleeding from hepatic artery	1	1	2
Bleeding from liver parenchyma	2	0	2
Other	3	3	6
Fluid Collection	7 (5.5%)	18 (11.0%)	25 (8.6%)
Abdominal	5	10	15
Pleural	2	7	9
Other	0	1	1
Device error	1 (0.8%)	-	1 (0.3%)
Device user error	2 (1.6%)	-	2 (0.7%)
Other systemic diseases	17 (13.3%)	38 (23.2%)	55 (18.8%)
Total	128	164	292

The percentage of total events is reported in brackets. No statistical tests have been applied.

nature research

Corresponding author(s): David Nasralla, Constantin C Coussios, Peter J Friend

☐ Initial submission ☐ Revised version ☒ Final submission

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Experimental design

1. Sample size

Describe how sample size was determined.

Data from 416 liver transplant recipients from University Hospital Essen demonstrated the geometric mean of peak AST to be 608.59 IU/L (the geometric mean is used as peak AST is non-normally distributed). 220 transplants (110 per arm) would have 90% power at 5% significance level to detect a 33% reduction (to 401.67 IU/L) in the geometric mean of peak AST. Thus the trial required a sample size of 220 livers to be transplanted and reach the primary endpoint.

Once trial recruitment was complete, an ad hoc analysis was performed in which NMP organs were categorised according to those which, following transplant, displayed minimal preservation injury (MPI; peak AST <250 IU/litre; n=28) and those with more severe preservation injury (SPI; peak AST >1000 IU/litre; n=25).

2. Data exclusions

Describe any data exclusions.

Inclusion criteria for donors and recipients were deliberately broad to represent the full spectrum of clinical practice. Whole livers from brainstem death (DBD) and circulatory death (DCD) donors aged at least 16 years were eligible.
Recipients were eligible provided they were at least 18 years old and listed for a liver-only transplant, excluding those with fulminant liver failure, due to their poor prognosis regardless of organ quality.

3. Replication

Describe whether the experimental findings were reliably reproduced.

This is the first phase 3 randomised controlled trial to compare any form of machine perfusion technology with static cold storage in human liver transplantation. This findings have broadly confirmed those demonstrated in small scale animal studies and in small retrospectively matched case series.

This was a clinical trial not involving experiments that need replication.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Once an eligible donor organ was allocated to a consented recipient and the availability of the normothermic liver perfusion (NMP) team was confirmed, the liver was randomised. All clinical decisions thereafter, including graft suitability and procedure scheduling, were made independently of the trial team.
Using an online randomisation tool, livers were assigned to NMP or static cold storage with 1:1 allocation ratio as per a computer generated randomisation schedule using variable block size, stratified by transplant centre and donor type (DBD/DCD). The unit of randomisation was donor livers rather than recipients, but analysis is reported for the transplant recipients.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

This was an open label study. Due to the nature of the intervention (large machine perfusion device), it was not possible to blind investigators as to which arm the liver had been randomised. .

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Analyses were conducted with the use of Stata version 14.2 (StataCorp, College Station, TX).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The normothermic machine perfusion device used in this study is now CE marked and can be used for clinical practice, but is only available through the manufacturing company, OrganOx Ltd. There are no restrictions on the other materials used in this study.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No eukaryotic cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used in the study

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Livers were obtained from deceased donors aged at least 16 years who's family members had provided appropriate consent for the donation of their organs. Recipients were eligible provided they were at least 18 years old and listed for a liver-only transplant, excluding those with fulminant liver failure, due to their poor prognosis regardless of organ quality.

Donors in both arms of the study were well matched in the following respects: Donor type (DBD, DCD); age; sex; ethnicity; cause of death; BMI; eurotransplant donor risk index.
Recipients in both arms of the study were well matched in the following respects: Age; sex; cause of liver failure; BMI; retransplants; Model of end-stage liver disease (MELD) score; estimated glomerular filtration rate (eGFR).

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