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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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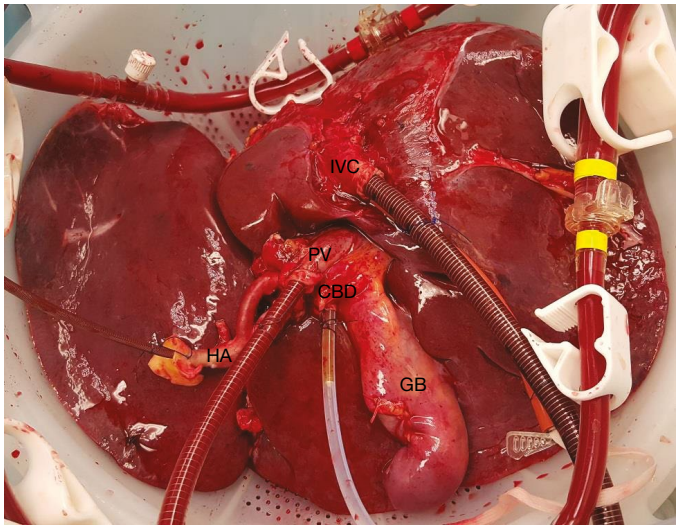

Fig. 1 | Image of liver during normothermic machine perfusion. The hepatic artery (HA), portal vein (PV), inferior vena cava (IVC) and common bile duct (CBD) are all cannulated. The gallbladder (GB) is also present although this was often removed during the retrieval process before NMP. This image has 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.

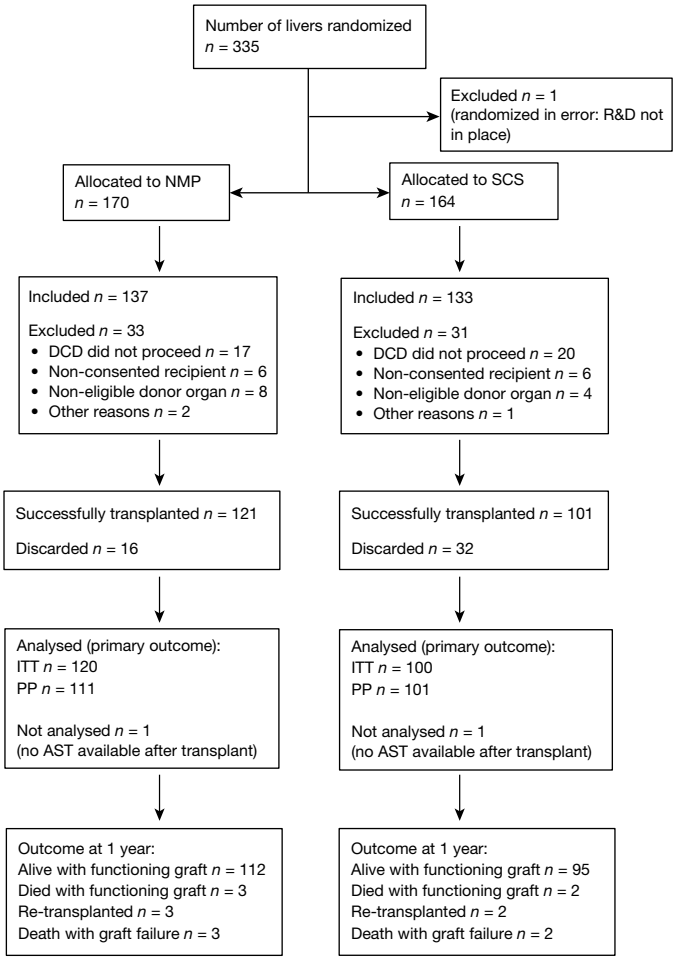

Fig. 2 | CONSORT diagram. CONSORT diagram depicting the outcome for all donor livers enrolled in the trial. ITT, intention to treat; PP, per protocol; R&D, research and development.

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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Correspondence and requests for materials should be addressed to D.N. or C.C.C. or P.J.F.

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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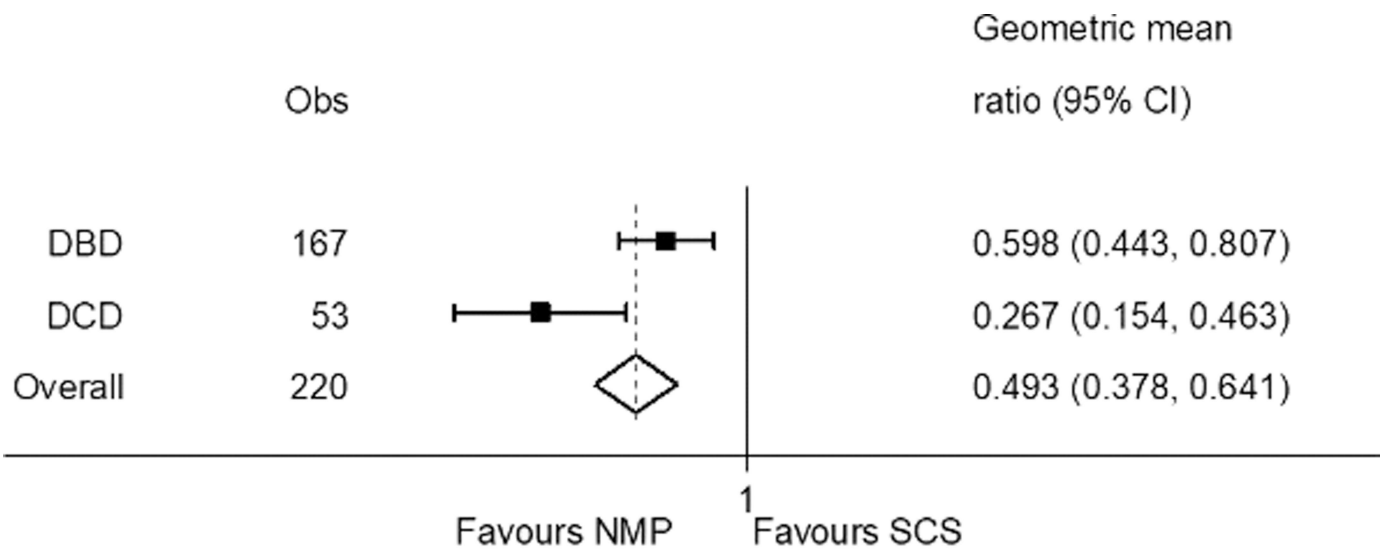
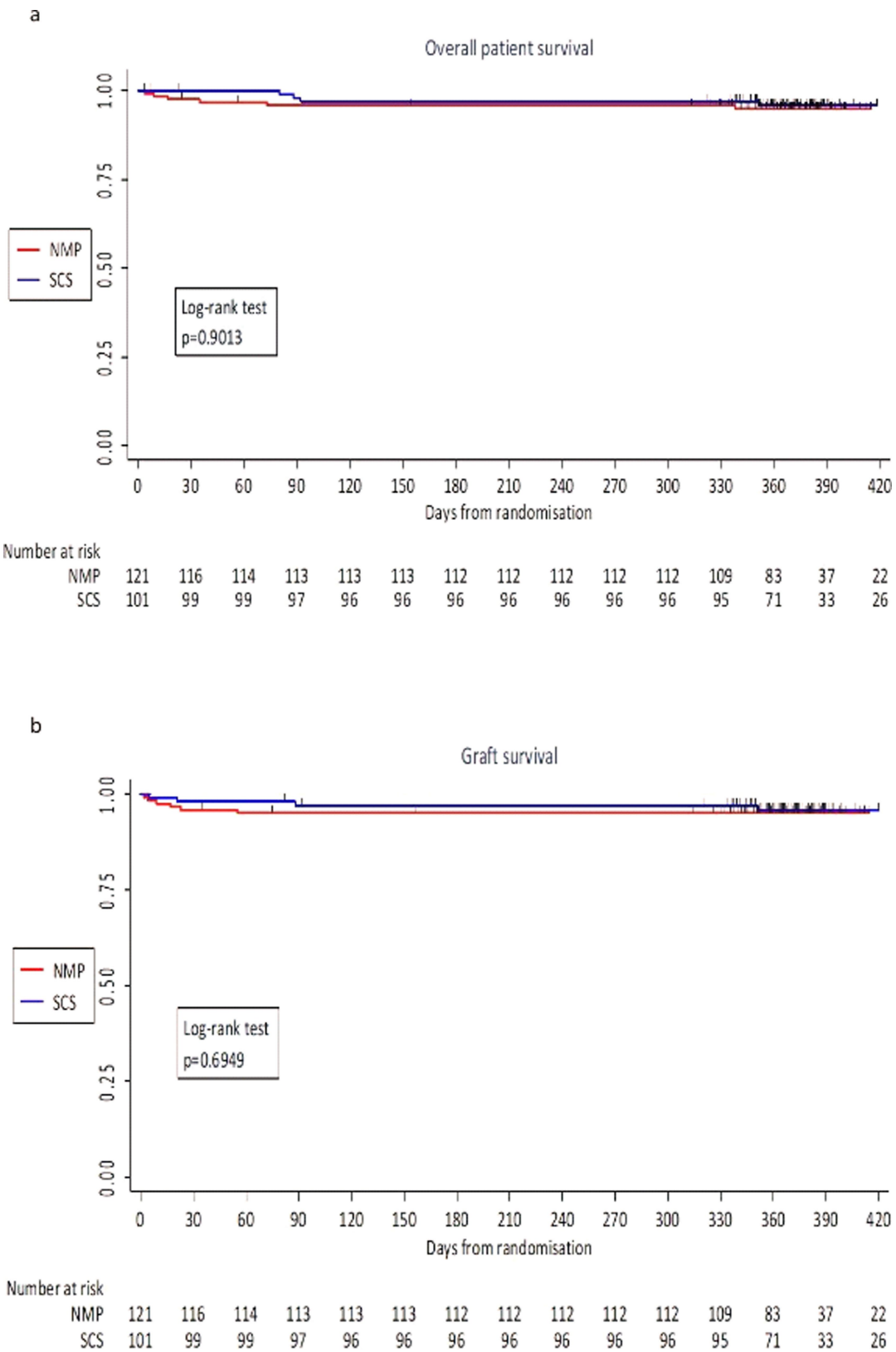
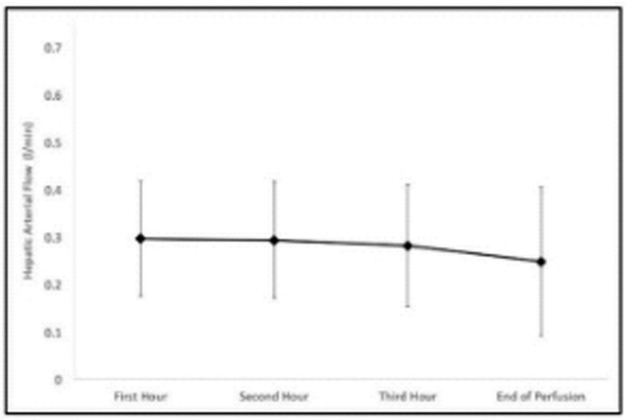


Extended Data Fig. 1 | Forest plot for subgroup analysis of peak AST by donor type. Geometric mean ratio and 95% confidence interval are reported for each subgroup and overall for all groups. DBD group, $n = 87$ NMP, $n = 80$ SCS; DCD group, $n = 33$ NMP, $n = 20$ SCS.



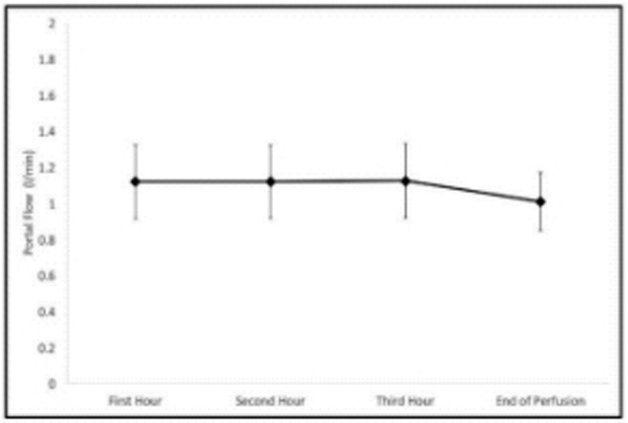
Extended Data Fig. 2 | Post-reperfusion syndrome. a, Kaplan–Meier plot for one-year survival of patients with two-sided log-rank test. **b**, Kaplan–Meier plot for one-year graft survival with two-sided log-rank test.

a



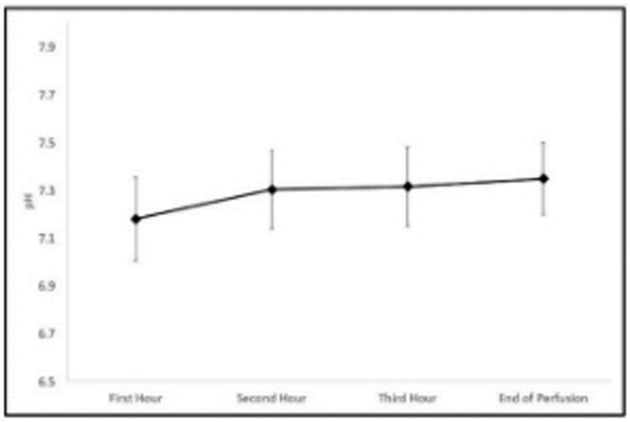
Time Point	Mean flow (litres/min)	Standard Deviation
First hour	0.296	0.121
Second hour	0.294	0.122
Third hour	0.282	0.128
End of perfusion	0.248	0.157

b



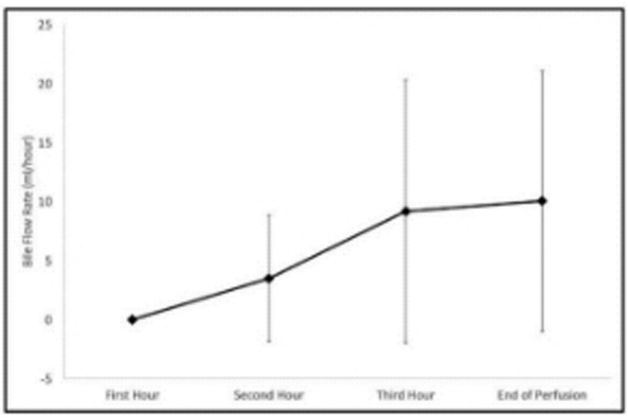
Time Point	Mean flow (litres/min)	Standard Deviation
First hour	1.118	0.205
Second hour	1.118	0.202
Third hour	1.124	0.206
End of perfusion	1.008	0.160

c



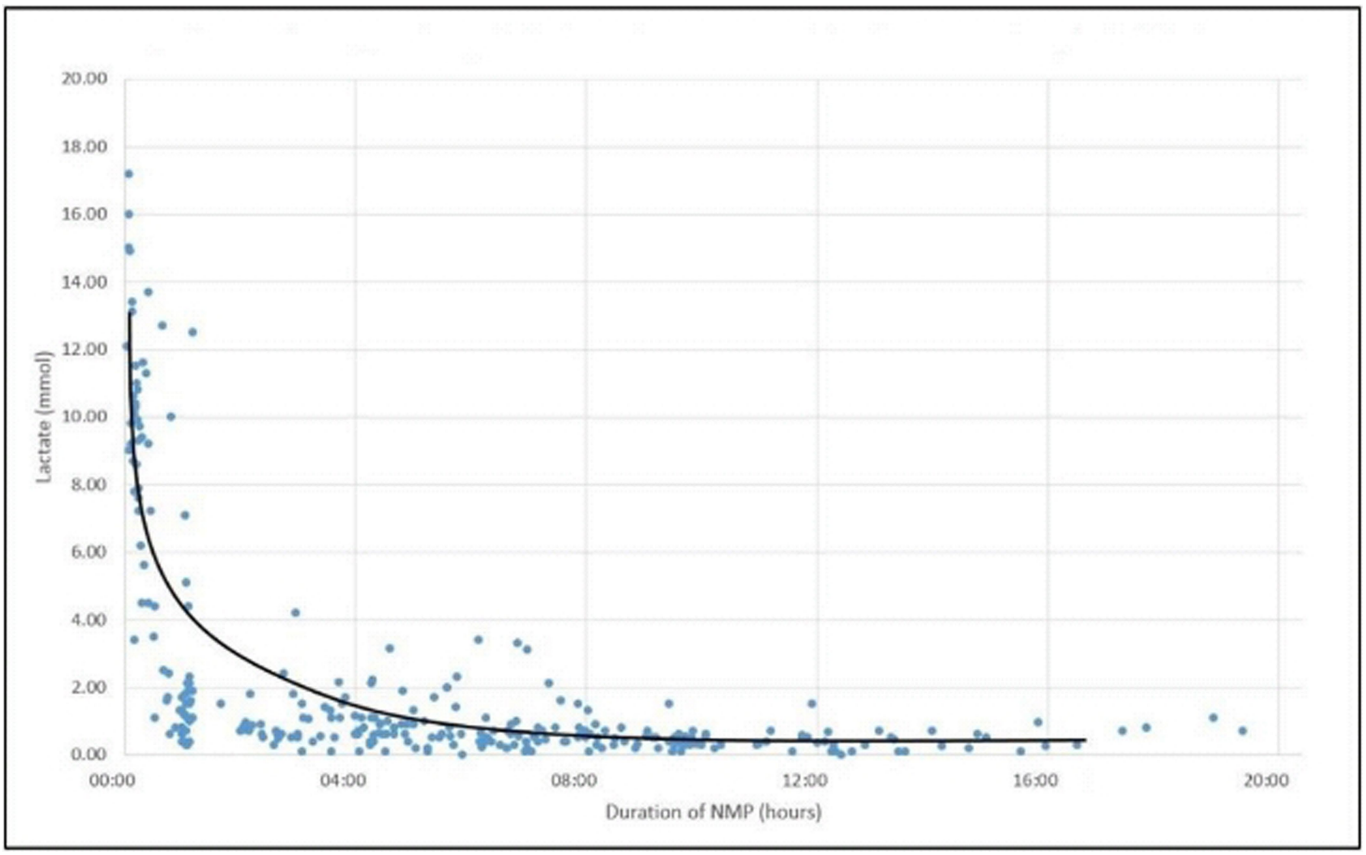
Time Point	Mean pH	Standard Deviation
First hour	7.178	0.174
Second hour	7.301	0.165
Third hour	7.313	0.166
End of perfusion	7.347	0.150

d

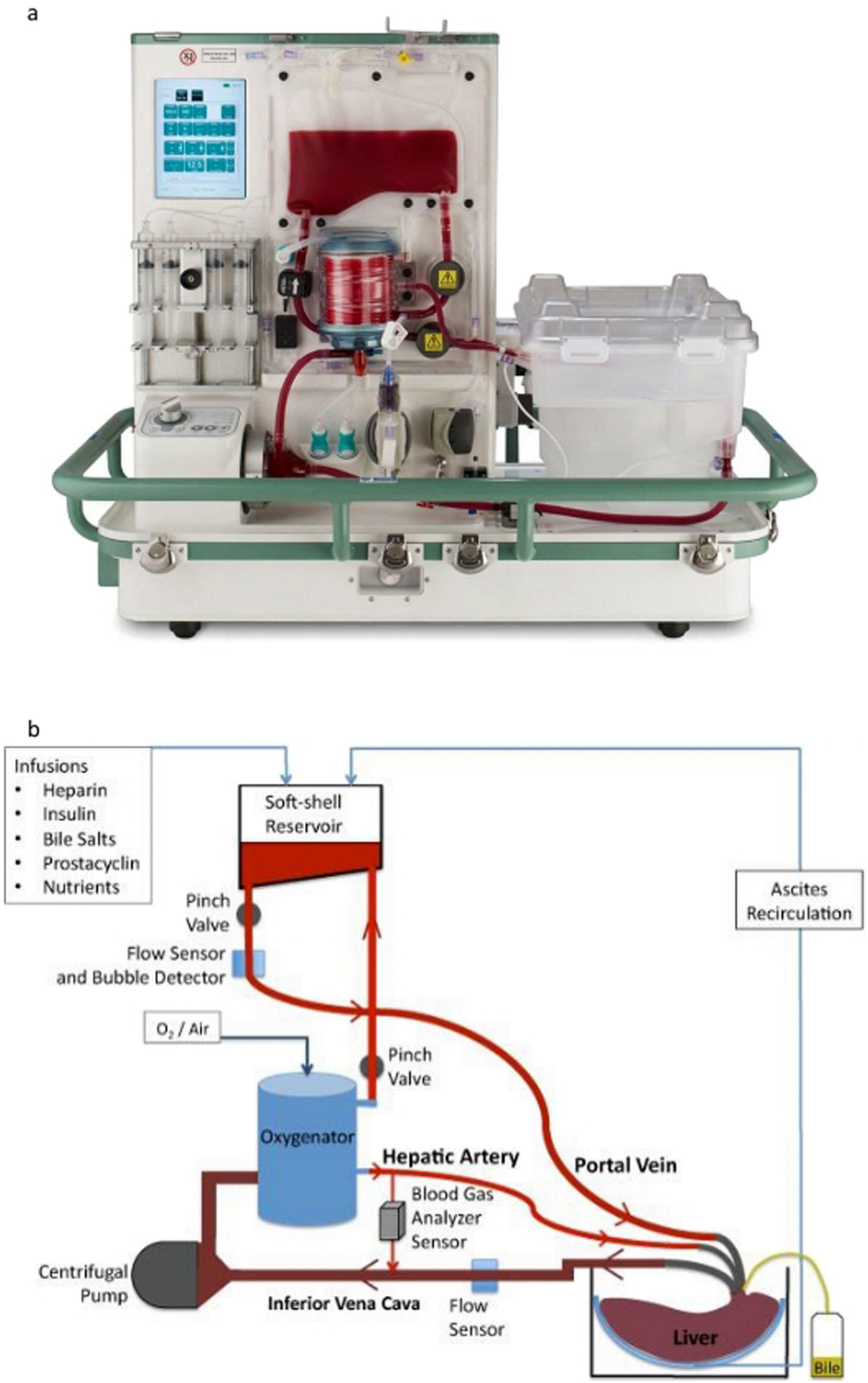


Time Point	Mean flow (ml/hour)	Standard Deviation
First hour	0	0
Second hour	3.486	5.358
Third hour	9.166	11.160
End of perfusion	10.050	11.036

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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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

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).