Transient cold storage prior to normothermic liver perfusion may facilitate adoption of a novel technology

Carlo DL Ceresa¹, David Nasralla¹, Christopher JE Watson², Andrew J Butler², Constantin C Coussios³, Keziah Crick², Leanne Hodson⁴, Charles Imber⁵, Wayel Jassem⁶, Simon R Knight¹, Hynek Mergental⁷, Rutger J Ploeg¹, Joerg M Pollok⁵, Alberto Quaglia⁶, AM James Shapiro⁸, Annemarie Weissenbacher¹, Peter J Friend¹

1. Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK
2. University of Cambridge Department of Surgery, Addenbrooke's Hospital, Cambridge, UK
3. Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, Oxford, UK
4. Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK
5. Department of Hepatopancreatobiliary and Liver Transplant Surgery, Royal Free Hospital, London, UK
6. Institute of Liver Studies, King's College Hospital, London, UK
7. Queen Elizabeth Hospital Birmingham, Birmingham, UK
8. Department of Surgery, University of Alberta, Edmonton, Canada

Key words: preservation, utilisation, ischaemia-reperfusion injury

Abbreviations:

AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
DBD	Donor after brain stem death
DCD	Donor after circulatory death
DLI	Donor liver index
EAD	Early allograft dysfunction
HAT	Hepatic artery thrombosis
H&E	Haematoxylin & eosin
MAP	Mean arterial pressure
MELD	Model for end-stage liver disease
NMP	Normothermic machine perfusion
PAS	Periodic acid Schiff
PNF	Primary non-function
PRS	Post reperfusion syndrome
pSCS-NMP	Post-static cold storage normothermic machine perfusion
RRT	Renal replacement therapy
SCS	Static cold storage
UW	University of Wisconsin

Grants and Financial Support

Carlo DL Ceresa was supported by a Medical Research Council (MRC) clinical research training fellowship. Consumables for the study were provided by OrganOx Ltd.

Conflicts of Interest

Carlo DL Ceresa has received income from OrganOx Ltd. for teaching and training and for carrying out normothermic organ preservations out of hours.

Simon Knight has previously received consultancy fees from Organox Ltd. for assisting with the design of clinical trials.

Peter J. Friend and Constantin C. Coussios are full-time academics at the University of Oxford and also chief medical officer and chief technical officer, respectively, and shareholders in OrganOx Ltd, a spin-out company from the University of Oxford.

Corresponding Authors

Carlo DL Ceresa

Peter J Friend

Nuffield Department of Surgical Sciences

Nuffield Department of Surgical Sciences

Oxford Transplant Centre

Oxford Transplant Centre

Churchill Hospital

Churchill Hospital

Oxford

Oxford

OX3 7LE

OX3 7LE

carlo.ceresa@nds.ox.ac.uk

peter.friend@nds.ox.ac.uk

Abstract

Introduction

Clinical adoption of normothermic machine perfusion (NMP) may be facilitated by simplifying logistics and reducing costs. This can be achieved by cold storage of livers for transportation to recipient centres before commencing NMP. The purpose of this study was to assess the safety and feasibility of post-static cold storage NMP (pSCS-NMP) in liver transplantation.

Materials and Methods

In this multi-centre prospective study, 31 livers were transplanted. The primary endpoint was 30-day graft survival. Secondary endpoints included: peak post-transplant aspartate aminotransferase (AST), early allograft dysfunction (EAD), post-reperfusion syndrome (PRS), adverse events, critical care and hospital stay, biliary complications and 12-month graft survival.

Results

Thirty-day graft survival was 94%. Livers were preserved for a total of 14 h 10 min $\pm$ 4 h 46 min; this included 6 h 1 min $\pm$ 1 h 19 min of SCS before 8 h 24 min $\pm$ 4 h 4 min of NMP. Median peak serum AST in the first 7 days postoperatively was 457 U/L (92 – 8669 U/L) and 4 livers (13%) developed EAD. PRS was observed in 3 (10%) livers. The median duration of initial critical care stay was 3 (1 – 20) days and median hospital stay was 13 (7 – 31) days. Seven patients (23%) developed complications of grade IIIb severity or above and 2 (6%) patients developed biliary complications; one bile leak and one anastomotic stricture with no cases of ischaemic cholangiopathy. Twelve-month overall graft survival (including death with a functioning graft) was 84%.

Conclusion

This study demonstrates that pSCS-NMP was feasible and safe which may facilitate clinical adoption.

Introduction

Normothermic machine perfusion (NMP) is a novel preservation method which is rapidly generating clinical interest in the field of liver transplantation. NMP maintains the liver *ex situ* at 37°C in a functioning state through the delivery of oxygen and nutrition. This permits the objective assessment of organ function during preservation through lactate clearance, glucose metabolism, pH maintenance and bile production and quality (1). A primary objective of this technology is to minimise graft injury during preservation and to improve organ utilisation through the use of higher-risk organs without compromising outcomes. This is of paramount importance in the context of the current donor organ shortage, which leads to the death of up to 20% of patients on the waiting list before receiving a transplant (2, 3). The paucity of donor organs is exacerbated by the poor quality of many livers offered for transplantation. Organ donation increasingly involves older donors with co-morbidities and the global obesity epidemic results in more livers with significant steatosis. These high-risk, or “extended criteria”, livers have a lower tolerance of ischaemia, which is associated with a more severe reperfusion injury. The resilience of an extended criteria liver to recover from the physiological and metabolic traumas of brain death, organ procurement, cold preservation and implantation is diminished. Furthermore, along with primary non-function (PNF), extended criteria livers are at increased risk of acute and chronic rejection and biliary complications including ischaemic cholangiopathy, particularly in organs retrieved after circulatory determination of death (DCD) (4, 5).

Pilot studies have demonstrated the safety and feasibility of NMP in the clinical setting (6-8), followed by a multicentre phase III randomised controlled trial which demonstrated the superiority of NMP compared to standard static cold storage (SCS) in terms of biochemical markers of early graft injury and improved organ utilisation (9). In this, the only randomised trial of NMP so far reported, perfusion was implemented for the entire preservation period,

from retrieval to transplantation, including transportation (continuous NMP). The logistics of continuous NMP, whilst achievable, can be challenging, particularly in the context of organs procured a long distance from the transplanting centre which may need to be transported by air. The clinical implementation of NMP may be facilitated by the use of NMP after a period of cold storage (10), allowing transport of organs in an ice-box, thereby simplifying logistics and reducing costs.

However, there is experimental evidence suggesting that rapid re-warming after a period of cooling can induce cellular injury (11, 12). Work in a porcine model comparing continuous NMP with a post-SCS approach (pSCS-NMP), demonstrated livers that were subjected to pSCS-NMP had increased markers of hepatocellular injury, sinusoidal endothelial cell dysfunction and Kupffer Cell injury (13). Conversely, two clinical studies investigating NMP of discarded or high-risk human livers have demonstrated evidence that pSCS-NMP can be implemented without any apparent detriment to the graft (when using physiological oxygen tensions) (1, 14, 15) and the end-ischaemic approach has been used successfully in hypothermic machine perfusion studies (16, 17). However, it remains unclear whether the deleterious effects of cooling the graft can be mitigated by subsequent NMP, resulting in comparable outcomes to grafts preserved by continuous NMP.

The aim of this study was to formally assess the safety and feasibility of pSCS-NMP and to provide some insight into how this might compare to continuous NMP.

Materials and Methods

Study Design and Ethical Approval

In this prospective clinical study, livers were recruited by three UK transplant centres until 30 recipients were transplanted after the intervention. In order to test safety and feasibility, 30-day graft survival was selected as the primary outcome. The study was sponsored by the University of Oxford and approved by the London-Dulwich research ethics committee in accordance with the Helsinki Declaration (16/LO/2196) and registered prior to commencing recruitment (NCT03176433).

Eligibility and Consent

Whole livers from donors after brainstem death (DBD) and Maastricht category III DCD (18), aged at least 16 years were eligible. For DCD livers, functional warm ischaemia time (fWIT) commenced when either systolic blood pressure dropped below 50 mmHg or oxygen saturation below 70% until cold *in situ* perfusion. Recipients were suitable provided they were at least 18 years old and listed for an elective liver-only transplant. All recipients provided formal informed consent prior to recruitment. This included the recording the use of anonymized data for trial purposes and the collection of biological samples for further analysis. No patient identifiable data were collected.

The study protocol stipulated that livers should be cold-stored for 3 to 8 hours before being placed on the NMP circuit. These time limits were established in order to ensure the intervention was tested consistently and to reflect the UK average cold ischaemia times (3).

pSCS-NMP Livers

Livers were retrieved and transported in University of Wisconsin (UW) solution at 4°C according to standard practice. On arrival of the organ at the transplanting centre a standard liver back-table was performed (19). The coeliac artery, portal vein, inferior vena cava and bile

duct were then cannulated and the liver flushed with 500ml Gelofusine (B Braun Ltd). The liver was then connected to the OrganOx *metra*[®] normothermic device as previously described (6-9).

The minimum NMP duration was set at 4 hours, the time taken for adenosine triphosphate (ATP) repletion in an animal DCD model (20) and to provide time for functional assessment. Livers could be perfused for a maximum of 24 hours, as per the regulatory approval of the device, governed by local operating room logistics. The duration of perfusion was at the discretion of the transplanting surgeon. Blood gas analysis, sampled from an outlet via the hepatic artery line, was performed after 15 minutes and one hour from the beginning of perfusion and then at least 4 hourly until the end of perfusion. Functional parameters, including: lactate clearance, glucose metabolism, pH maintenance, bile production, perfusate transaminase levels and flow rates, were available to the transplanting surgeon, as previously described (1, 14, 15). The final decision to transplant the liver was made by the transplanting surgeon. Specific functional criteria were not pre-defined as optimal functional parameters are yet to be validated.

Study End-Points

The primary endpoint was 30-day graft survival, in order to test the safety and feasibility of the pSCS-NMP approach.

Secondary end-points included:

- Peak serum aspartate aminotransferase (AST) in the first 7-days post-transplant. This is a validated surrogate marker, predictive of PNF as well as graft and patient survival (21, 22). It is also associated with histological evidence of moderate to severe reperfusion injury (23, 24).

- Early allograft dysfunction (EAD) (25): any one of: (i) bilirubin > 172 μ mol/L on day 7 post-transplant; (ii) INR > 1.6 on day 7 post-transplant; (iii) peak-AST > 2000 U/L during the first 7 days.
- Model for Early Allograft Dysfunction (MEAF) score (26): a validated continuous scoring model graded 0-10 comprising the highest serum alanine aminotransferase (ALT) and INR in the first 3 post-operative days and bilirubin value on day 3 following liver-transplantation. The MEAF score has been proposed as a superior predictor of transplant survival than EAD (27).
- PNF: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes.
- Post-reperfusion syndrome (PRS): 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 (28, 29).
- Need for renal replacement therapy (RRT).
- Duration of initial postoperative critical care and hospital stays.
- Adverse event rates and severity, graded according to the Clavien-Dindo classification (30).
- Biliary complications at 12-months: bile leak, anastomotic strictures and ischaemic cholangiopathy.
- Graft histology
- 12-month graft and patient survival

Post-SCS NMP Liver Histology

For the 30 transplanted pSCS-NMP livers, core biopsies were obtained at the end of SCS and at the end of NMP and fixed in formalin. Sequential biopsies in individual livers were taken

from the same part of the liver. Sections were stained with haematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS) and scored by a consultant histopathologist, blinded to the time at which the biopsy was taken. H&E sections were scored for small and large droplet macrovesicular steatosis (none/mild/moderate/severe), necrosis, fibrosis, sinusoidal leukocytosis and PAS sections scored for glycogen depletion (none/focal/scattered/confluent).

Comparison with continuous NMP

In order to contextualize the results from this study, we compared preservation parameters and early biochemical outcomes with livers preserved via continuous NMP. This comparator group comprises all of the NMP livers transplanted in the UK (n = 104) as part of a randomized trial comparing the efficacy of NMP compared to SCS (9). Livers in this study were transplanted at the same UK centres with identical inclusion and exclusion criteria. We included the whole cohort (rather than formal matching) to eliminate any risk of selection bias.

Statistical Analysis

The present study was primarily designed to investigate the safety and feasibility of the pSCS-NMP approach to preservation. As such a formal sample size calculation to assess efficacy was not performed.

All analyses were performed using GraphPad Prism version 7 for Mac OS X (GraphPad Software, La Jolla California USA). A Student's *t*-test was used to analyse parametric, continuous variables and the Mann-Whitney U test used for non-parametric continuous variables. Fisher's exact test was used for categorical variables. Pearson r test was used to test for correlation. Continuous variables are presented as mean $\pm$ standard deviation (SD) or median with range. A p value <0.05 is considered statistically significant.

Results

Recruitment

Between 4th May and 18th July 2017, 30 liver transplants were performed using donor organs subjected to pSCS-NMP during preservation. One further liver could not be perfused due to a leak from the NMP disposable circuit during priming resulting in the liver remaining cold stored. This liver is included in the results as part of an intention-to-treat analysis. In total, 51 livers were recruited to the study with 20 being excluded for the following reasons: non-proceeding DCD (n = 12); suboptimal appearance at retrieval (n = 4); poor function during NMP (n = 3); change to a non-eligible super-urgent recipient (n = 1).

Donor, Recipient and Preservation Characteristics

The indications for transplantation were: primary sclerosing cholangitis (n = 6), non-alcoholic steatohepatitis (n = 6), hepatocellular carcinoma (n = 5), alcoholic liver disease (n = 5), hepatitis C cirrhosis (n = 4), autoimmune hepatitis (n = 2), primary biliary cirrhosis (n = 1), polycystic liver disease (n = 1) and Budd-Chiari (n = 1).

Twenty-three (74%) DBD and 8 (26%) DCD livers were transplanted from donors with a median age of 58 (17 – 78). The median donor risk index (DRI) was 1.87 (1.06 – 3.20) and median donor liver index (DLI), 1.33 (0.6 – 3.38). The median recipient age was 58 (25 – 73) with a median MELD score of 14 (17 – 24). For the DCD livers, the median fWIT was 16 min (12 – 28 min) and the median UK-DCD risk score (31) was 7 (4 – 11).

The mean total preservation time of livers was 14 h 10 min $\pm$ 4 h 46 min; this included 6 h 1 min $\pm$ 1 h 19 min of SCS before 8 h 24 min $\pm$ 4 h 4 min of NMP. The longest total preservation time was 25 h 39 min. During NMP, the mean hepatic arterial flow rate was 0.44 $\pm$ 0.15 L/min and portal venous flow rate was 1.08 $\pm$ 0.12 L/min. After an initial bolus of sodium bicarbonate (10 – 40 ml in the first 2 h of perfusion, according to pH), perfusate pH increased from 6.99 $\pm$

0.11 to 7.33 ± 0.06 ($p < 0.0001$). A significant reduction in perfusate lactate from 6.8 ± 2.3 mmol/L to 0.9 ± 0.5 mmol/L ($p < 0.0001$) and glucose from 24 mmol/L (13 – 39 mmol/L) to 10 mmol/L (2 – 23 mmol/L) ($p < 0.0001$) was observed over the course of NMP. Perfusate AST remained relatively stable from a median of 847 U/L (83 U/L – 3379 U/L) at the beginning of NMP to 1070 U/L (327 U/L – 4122 U/L) at the end of NMP ($p = 0.29$). There was no correlation between perfusate AST and peak serum AST ($r = 0.04$, $p = 0.89$). Median bile production was 11 ml/h (0 – 50 ml/h).

Clinical Outcomes

30-day Graft Survival (primary outcome)

Thirty-day graft survival was 94% in the study group. The two graft losses were due to acute hepatic artery thrombosis (HAT); both patients were successfully re-transplanted. The donor and preservation data for these livers are shown in Table 1.

There were no cases of PNF and the median duration of initial inpatient stay was 13 (7 – 31) days.

Adverse Events

Seven patients (23%) developed complications of grade IIIb severity (Clavien-Dindo classification (30)) or above. These included surgical intervention (re-transplantation ($n = 2$), bleeding ($n = 1$), corrective vascular intervention ($n = 1$) and sepsis control ($n = 1$)) and RRT alone ($n = 2$). Eleven patients (35%) developed more minor complications (2 (6%) grade I, 7 (23%) grade II and 2 (6%) grade IIIa). Thirteen (42%) patients had an uncomplicated post-operative course.

Early Biochemical Function

The median peak serum AST in the first 7 days postoperatively was 457 U/L (92 – 8669 U/L).

There was no relationship between the duration of cold storage ($r = 0.16$, $p = 0.38$) or total preservation time ($r = 0.1$, $p = 0.58$) and peak serum AST.

Four of the 31 livers (13%) developed EAD; 2 due to elevated peak AST only, one due to both elevated peak AST and elevated day 7 bilirubin and one due to elevated day 7 bilirubin only.

Recipient serum AST, bilirubin and INR in the first 6 months post-transplantation are shown in Figure 1A-C. The median MEAF score was 2.99 (0.74 – 8.85).

Physiological Response to Preservation Injury

Three patients (10%) in the study experienced PRS immediately following the liver reperfusion through the portal vein. This was in the context of modest vasopressor use, with 21 patients (68%) requiring a pre-reperfusion vasopressor infusion, 16 patients (52%) requiring a post-reperfusion vasopressor bolus and 19 patients (61%) requiring a post-reperfusion vasopressor infusion. The median post-reperfusion lactate level was 3.95 mmol/L (2.6 – 8.3 mmol/L). Five patients (16%) required RRT in the first 30-days post-transplantation and the median duration of initial critical care stay was 3 (1 – 20) days.

Biliary Complications

At 12-months follow-up, 2 patients experienced biliary complications; one with a biliary anastomotic leak and the other, an anastomotic stricture successfully managed by endoscopic retrograde cholangiopancreatography and stent insertion. No cases of ischaemic cholangiopathy were observed in the 31 patients.

12-month Patient and Graft Survival

Twelve-month overall graft survival (including one death with a functioning graft) was 84% and patient survival was 90%. Donor, preservation details and causes of all death-censored graft losses are shown in Table 1.

Histology

Histological assessment of the pSCS-NMP livers did not show any evidence of deterioration during NMP in terms of necrosis or fibrosis. Following the initial period of SCS, sinusoidal leukocytosis was observed in 50% of livers. This fell to 17% of livers after NMP ($p = 0.03$) (Figure 2A + B). In 56% of livers, replenishment of glycogen stores was observed by at least one category (none, focal, scattered, confluent) after NMP (Figure 2C + D). Six livers displayed moderate/severe macrovesicular steatosis (predominantly large droplet) and the degree of steatosis did not appear to change during NMP.

Livers Declined after NMP

Three livers in the pSCS-NMP group were discarded following a period of NMP. The justifications for discard are shown in Table 2.

Comparison with continuous NMP

The pSCS-NMP study group and continuous NMP comparator group displayed similar donor and recipient characteristics (enrollment criteria and follow-up protocols were the same for the two studies) (Table 3). The addition of a period of cold storage prior to NMP in the pSCS-NMP group significantly prolonged the total preservation time compared to continuous NMP (Table 4). Furthermore, some interesting differences were observed in the perfusion parameters between the groups with significantly higher hepatic arterial flow rates and significantly lower

perfusate lactate levels at the beginning of preservation observed in the pSCS-NMP study group compared to continuous NMP comparator group (Table 4). Clinical outcomes appeared similar between the groups (Table 5).

Discussion

In this group of livers, we have demonstrated that pSCS-NMP is safe and feasible with 94% 30-day graft survival. The 6% incidence of acute HAT observed in this study is in keeping with other published series' where an incidence of up to 9% is quoted (32-34). Neither case of hepatic artery thrombosis appeared to be related to the perfusion process and are discussed later.

Applying NMP after SCS has evident logistical and cost benefits. Removing the need to transport the device to and from a donor hospital reduces costs and avoids prolonging the donation procedure. In order to deploy NMP at the donor hospital, it is necessary to provide alternative transportation to support the extra equipment required and an extra member of the retrieval team may also be necessary. This will undoubtedly increase the cost associated with the organ retrieval process. Notably, Selzner *et al* also acknowledged that initiating NMP at the donor hospital, prolonged the procedure by 2 h (7) which may have implications on local operating lists. Although continuous NMP has been carried out in around 400 cases globally, transporting the liver on the device does add inherent risk; any problem on the device is likely to be much more difficult to resolve if it were to occur in transit. Furthermore, a multi-organ retrieval surgeon may not possess the necessary experience and expertise to perform a vascular reconstruction at the donor hospital prior to the commencement of NMP (7). The logistic benefits of NMP could be considerable; by facilitating prolonged preservation, centres would be able to accept organs simultaneously or indeed plan elective operating more effectively. In the UK, from 2013 - 2016, a total of 163 livers were declined for logistical reasons alone (3). During this study, we observed a shift from out-of-hours operating with 22 (71%) transplants being performed between 8am and 8pm; the start time was often delayed, ensuring the staff were well-rested.

There is an ongoing pressure to increase utilization of higher-risk deceased donor organs; this is driven by increasing numbers of waiting list deaths (2, 35). There is an expanding body of evidence that NMP is of benefit, not only by reducing preservation associated reperfusion injury (9), but also allowing surgeons to assess the viability of an organ before transplantation (1, 14). It is not clear whether the benefit of NMP is restricted to higher-risk organs – certainly the livers in this study had a median DLI within the top quartile of UK donor livers (52% of the transplanted pSCS-NMP livers), implying a 3-fold increase in the risk of graft loss compared to low-risk livers (36). It may not be possible to assess the benefit in low-risk organs until more experience has been gained in this context. Perhaps more importantly, livers in the upper quartile of donor risk (DLI) have a 34% utilisation rate (36) and it is plausible that this technology, by removing the uncertainty surrounding these organs, may give surgeons the confidence to improve this rate of utilisation. This aligns with the observed increase in organ utilisation in the normothermic arm of the recently published randomised study of Nasralla *et al* (9).

Three livers were not transplanted in this study due to concerns over their *ex situ* function. In all of these cases, it was not one parameter in isolation, but a combination of factors which led to the decision to discard (Table 2). One liver was from a young DBD donor, and appeared to have mild steatosis on visual inspection. However, poor function during NMP (Table 2) prompted a biopsy of the graft which demonstrated 80% macrovesicular steatosis. This case illustrates the possible benefit of NMP in successfully discriminating between grafts and identifying those which are likely to have a poor post-transplant outcome (which may otherwise have been transplanted). In other cases, the decision to transplant the liver may not be as straightforward. For example, of four transplanted livers that failed to produce bile, two were ultimately lost (one HAT and one recurrent hepatitis C infection, rejection and cholestasis);

these livers otherwise functioned well during NMP. Increasing evidence suggests that lack of bile production alone should not support discarding the liver (1, 9). Indeed, it is now becoming apparent that the quality of bile in terms of pH, glucose and bicarbonate levels are more predictive of biliary complications than bile volume (1, 37).

The graft losses in this study do not appear to be attributable to the preservation method (Table 1). However, it is notable that the hepatic artery flow during NMP was low in one of the two livers which developed arterial thrombosis. Explant histology from this liver demonstrated marked medial muscular hypertrophy of the right and left hepatic arteries with narrow lumens with a normal common hepatic artery. In the case of sub-optimal hepatic artery flow, a doppler ultrasound scan during perfusion may be indicated to confirm vascular integrity and global organ perfusion. For the other liver that developed acute HAT, there were concerns about a small right hepatic artery upon implantation. The recipient was electively anticoagulated and the graft demonstrated good primary function. A spontaneous bleed from a diaphragmatic artery resulted in prolonged hypotension, thrombosis of the right hepatic artery and infarction of the right lobe of the liver.

This study has also identified some interesting comparisons between pSCS-NMP and continuous NMP. We observed a significantly higher arterial flow rate in the pSCS-NMP livers than in the continuous NMP comparator group during preservation (0.44 ± 0.15 L/min vs. 0.29 ± 0.12 L/min, respectively; $p < 0.0001$). The reasons for this are unclear, although a previous study observed initial vasoconstriction after cold flush followed by vasodilatation during cold storage in a pig renal artery which appeared to be attributable to intracellular sources of calcium (38). If this phenomenon also occurs in the human hepatic artery, it may explain our observation, although this remains unproven.

The perfusate lactate level after 15 min in the pSCS-NMP group (7.3 mmol/L (1.9 - 10.1 mmol/L)) was significantly lower than that of the continuous NMP group (10.1 mmol/L (1.2 - 16.0 mmol/L)) ($p < 0.0001$). It has previously been shown that livers do not reach 4°C until around 90 min of immersion in the ice box (39). It is possible that the continuous NMP livers have more active anaerobic metabolism during the back-table phase, as this takes place before complete cooling. Increased (anaerobic) metabolic activity may manifest as an increased lactate level at the beginning of perfusion. There is no evidence that this affects the liver's functional ability to clear the lactate or post-transplant outcome.

It also appears that early biochemical liver function between the groups is comparable although these results should be interpreted with caution as this study was not specifically designed or adequately powered to test this hypothesis.

In this study there is no evidence that prolonged NMP preservation is detrimental to the graft. There was no correlation observed between the duration of preservation and peak postoperative serum AST and histological analysis showed no new injury after NMP. There was a reduction in the clinical phenomenon of preservation injury after transplantation, as well as a reduction in sinusoidal leukocytosis after NMP, suggesting that NMP may reduce the inflammatory load within the liver. Recently, Jassem *et al* provided a mechanistic insight into how NMP may attenuate reperfusion injury by demonstrating an altered gene expression profile in NMP compared to cold-stored livers from pro-inflammation to pro-healing and regeneration (40). There may also be a benefit in a period of hypothermic machine perfusion prior to NMP, or “controlled re-warming” as was shown by Boteon *et al* where livers which had been cold stored for prolonged periods benefited from 2 hours of hypothermic oxygenated machine perfusion (HOPE) before NMP (12). Although these livers were not transplanted, HOPE + NMP livers

achieved lower tissue expression markers of oxidative injury and inflammation as well as enhanced metabolic recovery compared to livers subjected directly to NMP (12).

This study is not without limitations. The number of recruited livers was small and the study is purely observational in nature. The study is not powered to detect any small differences that may be present between the pSCS-NMP and continuous NMP cohorts. To test equivalence to continuous NMP an appropriately powered randomised trial would be needed. The small sample size also precludes subgroup analyses, and it is possible that continuous NMP (as opposed to pSCS-NMP) may still be beneficial in the highest risk livers, in particular those with severe steatosis, where the pronounced adverse effects of cooling are well documented (41, 42). Use of a retrospective comparison with historical cohorts is a recognized limitation, although use of the same inclusion criteria and comparison with the entire UK contemporary cohort from the previous RCT does limit the risk of selection bias.

In summary, we have demonstrated that NMP after a period of SCS is both feasible and safe. This may have significant logistical and cost benefits that are likely to facilitate the uptake of NMP in routine clinical practice.

Acknowledgements

The following organizations, groups and individuals made substantial contributions without which this trial could not have been completed successfully: NHS Blood and Transplant; the Clinical Trials and Research Governance Unit, University of Oxford; the Liver Transplant Coordinators, anaesthetists and liver unit physicians at the Royal Free Hospital, London Addenbrooke's Hospital, Cambridge, and King's College Hospital, London; D. Sharma, B. Davidson, M. Malago, R Ravikumar, R. Praseedom, P. Gibbs, A. Jah, N. Russell, S. Harper, J. Richards, L. Selves, Y. Puri, A. Zamalloa. Of course, thanks are also due to all of the donors' families and to the participating recipients.

Figure Legends

Figure 1

Figure 1. Trend in recipient serum AST (A), bilirubin (B) and INR (C) in first 6-months post-transplantation. Data presented as median with IQR.

Figure 2

Figure 2. H&E stained liver section demonstrating extensive sinusoidal leukocytosis after 6 h 42 min of SCS (as highlighted) (A) with resolution after 11 h 58 min NMP (B). PAS stained liver section demonstrating scattered glycogen depletion after 6 h 9 min of SCS (C) and complete replenishment of glycogen stores after 8 h 33 min of NMP (D).

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