Consortium for Organ Preservation in Europe

# WP2

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

## Statistical Report

Version 2.0_07Jun2017

Based on Protocol version 3.0 11Feb2016
Based on Statistical Analysis Plan version 3.0 25Oct2016

| Name | Title | Role | Signature | Date |
|---|---|---|---|---|
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| David Nasralla | Central Investigator | Reviewer | | 8th June 2017 |
| Susan Dutton | Senior Statistician | Approver | Susan Dutton | 07Jun2017 |
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# Contents

# 1. INTRODUCTION

This document details the analysis for the main paper(s) reporting results from ***the European Union Seventh Framework Programme (FP7) funded multicentre randomised controlled trial to compare the efficacy of ex-vivo normothermic machine perfusion with static cold storage in human liver transplantation (COPE - WP2)***. The results reported in these papers follow the strategy set out in the Statistical Analysis Plan (WP2 SAP_V3.0_Oct2016). Exploratory analyses not pre-specified in the protocol and/or SAP will be expected to follow the broad principles laid down in the SAP and will be reported as post-hoc analyses in this report.

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

Any deviations from the SAP will be described and justified in this report.

## 1.1 Key Personnel

*Key people involved in the drafting, reviewing and approving this statistical report, together with their role in the trial and their contact details.*

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## 2. CHANGES FROM PREVIOUS VERSIONS OF THE STATISTICAL REPORT

| Version number Issue date | Author of this issue | Protocol Version & Issue date | Significant changes from previous version together with reasons |
|---|---|---|---|
| V1.0_12Apr2017 | Virginia Chiocchia | Protocol_V3.0_11Feb2016 | Not applicable as this is the 1st issue |
| V2.0_May2017 | Virginia Chiocchia | Protocol_V3.0_11Feb2016 | 1 year outcomes (patient and graft survival, biochemical liver function) added in relevant results sections. |

# 3. STUDY METHODS

## 3.1 Software employed

Analyses were undertaken using Stata version 14.2 (StataCorp, College Station, TX)

## 3.2 Data

*Data manipulations*

Bilirubin and Creatinine are measured in mg/dl in the European centres and in µmol/L in UK centres. All values were converted to mg/dl for analysis.

Initially, only the Eurotransplant Donor Risk Index (ET-DRI) was recorded in the study but, during one the DMC meeting, it was suggested to collect the UK Donor Risk Index (UK-DRI), a new index that had just been developed and submitted for publication at that time. However, as reported in the Baseline Characteristics section, neither of the two indices was complete, particularly the UK-DRI which presented a high amount of missing data mostly in the European livers. Therefore, for the purpose of the analysis the ET-DRI was used as it had less missing values between the two.

Date of retransplant was imputed retrospectively for one recipient and the functioning status of the graft for two recipients were changed due to their deaths being due to graft failure. These changes were instructed by the central investigator.

Age of donors and recipients was calculated as time from their date of birth to date of randomisation.

Duration of warm ischaemia was calculated only for DCD livers as time from its onset to cross clamp. Duration of cold ischaemia was calculated only for livers preserved on the machine as time from cross clamp to initiation of NMP. Total preservation time was calculated as the duration in minutes from time of cross clamp to time of arterial or portal reperfusion (whichever occurred first). Anhepatic time was calculated as duration in minutes from time of liver retrieval to time of arterial or portal reperfusion (whichever occurred first). Anastomosis time was calculated as duration in minutes from time of removing the liver from ice or machine to time of arterial or portal reperfusion (whichever occurred first).

*Primary Outcome*

There was a very high data completeness overall. Some AST values were missing in the first 7 days post-transplant particularly in centres that do not collect this routinely but as the primary outcome is the peak of these values and it is expected to occur in the first 36 hours post-transplant, this was assessed as long as at least two AST values were available (i.e. AST values available for at least two days post-transplant). No missing data were imputed.

The completeness of AST values is reported by treatment arm and by centre in the following figure and tables.

*Table 1: Number and percentages of daily AST values available by treatment group*

| Arm Randomised | AST Day 1 | AST Day 2 | AST Day 3 | AST Day 4 | AST Day 5 | AST Day 6 | AST Day 7 | Total included |
|---|---|---|---|---|---|---|---|---|
| **NMP** | 109 (90.1%) | 113 (93.4%) | 114 (94.2%) | 113 (93.4%) | 108 (89.3%) | 105 (86.8%) | 106 (87.6%) | 121 |
| **SCS** | 99 (98.0%) | 95 (94.1%) | 95 (94.1%) | 93 (92.1%) | 92 (91.1%) | 88 (87.1%) | 88 (87.1%) | 101 |
| **Total** | 208 (93.7%) | 208 (93.7%) | 209 (94.1%) | 206 (92.8%) | 200 (90.1%) | 193 (86.9%) | 194 (87.4%) | 222 |

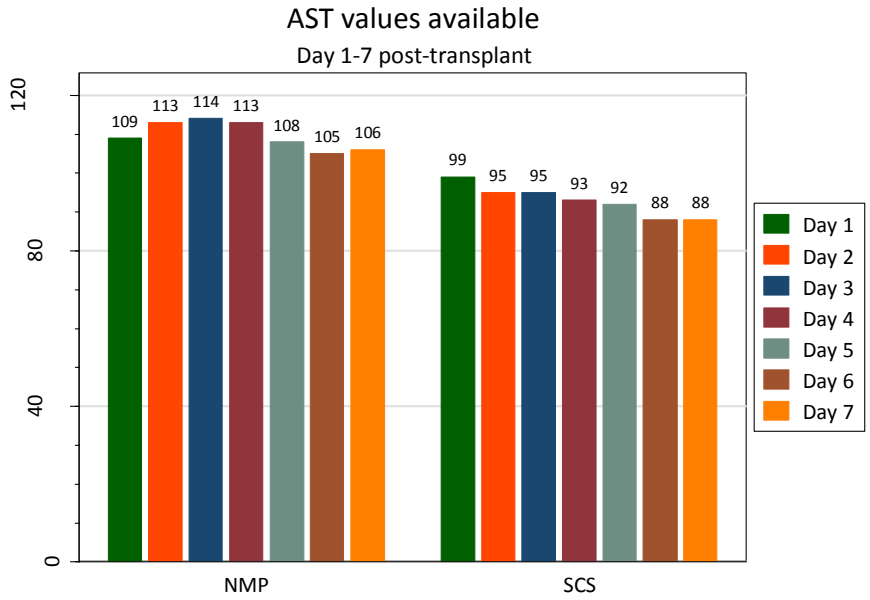



*Figure 1: Number of daily AST values available by treatment group*

*Table 2: Daily AST values available at each trial centre*

| Centre | AST Day 1 | AST Day 2 | AST Day 3 | AST Day 4 | AST Day 5 | AST Day 6 | AST Day 7 | Total randomised |
|---|---|---|---|---|---|---|---|---|
| **Addenbrooke's Hospital, Cambridge, UK** | 16 | 16 | 19 | 18 | 17 | 15 | 15 | 24 |
| **Kings College Hospital, London, UK** | 25 | 24 | 25 | 25 | 25 | 25 | 25 | 25 |
| **Queen Elizabeth Hospital, Birmingham, UK** | 98 | 100 | 97 | 95 | 92 | 91 | 89 | 104 |
| **Royal Free Hospital, London, UK** | 35 | 34 | 34 | 34 | 33 | 34 | 34 | 35 |
| **University Hospital, Essen, Germany** | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| **University of Barcelona, Barcelona, Spain** | 16 | 16 | 16 | 16 | 15 | 10 | 14 | 16 |
| **University Hospital, Leuven, Belgium** | 11 | 11 | 11 | 11 | 11 | 11 | 10 | 11 |
| **Total** | 208 | 208 | 209 | 206 | 200 | 193 | 194 | 222 |

The tables below give an overview of the timing of peak AST and the number of occurrence for each day. In all centres majority of the peak AST occurred on Day 1 with 94.1% of the patients overall having their highest value in the first 48 hours post-transplant.

*Table 3: Day of experiencing peak AST by treatment group*

| Day of Peak AST | NMP | SCS | Total |
|---|---|---|---|
| **1** | 97 (80.8%) | 93 (93.0%) | 190 (86.4%) |
| **2** | 13 (10.8%) | 4 (4.0%) | 17 (7.7%) |
| **3** | 4 (3.3%) | 1 (1.0%) | 5 (2.3%) |
| **4** | 0 (0.0%) | 1 (1.0%) | 1 (0.5%) |
| **5** | 1 (0.8%) | 1 (1.0%) | 2 (0.9%) |

| | | | |
|---|---|---|---|
| **6** | 2 (1.7%) | 0 (0.0%) | 2 (0.9%) |
| **7** | 2 (1.7%) | 0 (0.0%) | 3 (1.4%) |
| **Total** | 120 | 100 | 220 |

*Table 4: Day of experiencing peak AST by trial centre*

| Centre | Day of Peak AST | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | |
| **Addenbrooke's Hospital, Cambridge, UK** | 15 (68.2%) | 3 (13.6%) | 2 (9.1%) | 0 (0.0%) | 0 (0.0%) | 1 (4.6%) | 1 (4.6%) | 22 |
| **Kings College Hospital, London, UK** | 23 (92.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (4.0%) | 1 (4.0%) | 25 |
| **Queen Elizabeth Hospital, Birmingham, UK** | 89 (85.6%) | 11 (10.6%) | 2 (1.9%) | 0 (0.0%) | 1 (1.0%) | 0 (0.0%) | 1 (1.0%) | 104 |
| **Royal Free Hospital, London, UK** | 34 (97.1%) | 0 (0.0%) | 1 (2.9%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 35 |
| **University Hospital, Essen, Germany** | 7 (100.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 7 |
| **University of Barcelona, Barcelona, Spain** | 14 (87.5%) | 0 (0.0%) | 0 (0.0%) | 1 (6.3%) | 1 (6.3%) | 0 (0.0%) | 0 (0.0%) | 16 |
| **University Hospital, Leuven, Belgium** | 8 (72.7%) | 3 (27.3%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 11 |
| **Total** | 190 (86.4%) | 17 (7.7%) | 5 (2.3%) | 1 (0.5%) | 2 (0.9%) | 2 (0.9%) | 3 (1.4%) | 220 |

Subgroup analyses were performed for MELD Score and ET-DRI. As reported in the SAP, the MELD Score was divided into 5 score subgroups based on the UNOS standard reference ranges: 0-9; 10-19; 20-29; 30-39; 40 and over. However, no patients had a MELD Score of 40 and over and only 2 fell in the 30-39 subgroup so it was decided to merge this category with the previous one leaving, therefore, only 3 subgroups: 0-9; 10-19; 20 and over. The ET-DRI was divided into "low", "medium" and "high" subgroups based on the 33$^{rd}$ percentiles. The intervals were, respectively: 1.53 and under; 1.53-1.91; 1.91 and over.

*Secondary Outcomes*

Four cases of severe graft dysfunction occurred during the first 10 days after liver transplantation, which were flagged as Primary Non-function (PNF) in the database. However, only one of these occurred in the absence of technical or immunological causes. Therefore, only one was considered as a true PNF (according to the trial protocol definition) in the secondary outcome analysis.

The area under the curve (AUC) for the serum biochemical tests was calculated for each patient who had at least 5 daily measurements available and then compared between groups using the Mann-Whitney test due to the non-normal distribution of the variable. The AUC was not calculated for the Lactate as this is only measured whilst the recipient is in ITU/HDU care which means there were not many cases where the Lactate values were available for 5-7 days. Comparing the average values over the 7 days is, therefore, a more appropriate assessment for the Lactate.

As reported in the SAP, early allograft dysfunction (EAD) has been defined as the presence of at least one of the following postoperative laboratory tests:

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

In case of missing data in any of the three tests above, EAD will be considered as present if any of the available values satisfies the specified criteria. In case of Bilirubin and/or INR missing due to the patient being discharged before Day 7 post-transplant, this will be considered as absence of EAD.

Length of initial ITU stay was imputed for those who died and did not have it recorded after instruction from the central investigator. For those with missing length of hospital stay, this was calculated from their date of transplant until their date of discharge, date of retransplant or date of death, as appropriate.

After the final datalock the serious adverse events were independently reviewed by two clinicians blinded to the treatment group and assigned an appropriate clinical events category which was needed to produce safety analysis summaries.

### 3.3 Interim Analysis

The DMC met approximately 6 monthly during the trial to assess safety of the participants and conduct of the trial. No formal interim analysis of outcomes were undertaken during the trial. Only descriptive summaries were produced for the DMC meetings. The DMC recommendations did not result in any major amendments.

### 3.4 Changes to original randomisation

Randomisation to NMP or SCS was based on a 1:1 allocation as per a computer generated randomisation schedule using variable block size (4, 6, 8 in a ratio of 1:2:1 for the DBD randomisation schedules; 2, 4 in a ratio of 1:1 for the DCD schedules) and the following stratification factors: participating (recipient) centre and donor type (DBD or DCD).

No changes to the original randomisation plan were required. When all original codes for one of the strata were used, additional codes were created following the same schedule.

### 3.5 Deviations from the original planned statistical analysis plan

Original analysis was planned to be adjusted for stratification factors including centre. As some centres included as little as 3.2% of the total livers transplanted with more than 40% of the transplanted livers randomised by a single centre, this factor was not included as a covariate in regression models for the secondary outcome analysis as planned and described in the SAP.

Survivals will also be reported at 30 days, as stated in the protocol. This was not described in the SAP due to an oversight.

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

Apart from the pre-specified subgroup analysis (Donor Type, Meld Score and ET-DRI), no adjustment for other prognostic factors was performed in the primary outcome analysis.

Duration of renal replacement therapy at 7 days was compared between groups using Mann-Whitney non-parametric test as the variable is ordinal and not continuous as described in the SAP.

Discard rate was reported as difference in incidence and 95% confidence interval as described in the SAP but also using test for proportions.

The pre-specified exploratory analyses described in the SAP were not performed. The formula reported in the paper by Pareja et al. (Liver Transpl, 2015) describing the development of the MEAF is slightly different from the one included in the Excel spreadsheet the authors provided in the online supporting information. Due to this mismatch the MEAF was not calculated and subsequently analysed. As mentioned above, Cox proportional

hazards regression was not performed so the relationship between AST on day 3 and graft and patient survival could not be explored.

### 3.6 Suggested Statistical Methods Section for Publication

Previous data from 416 liver transplant recipients at the University Hospital Essen demonstrated the geometric mean of peak AST to be 608.59 IU/L. The present study was powered to detect a (clinically significant) 33% reduction (to 401.67 IU/L) with 90% power at a 5% significance level, requiring 220 transplants (110 per arm). 2011/12 NHSBT data suggest that 12% of livers retrieved are not transplanted. Therefore, assuming losses at 15%, randomisation of 260 livers into the trial were required to achieve adequate power.

Results are reported as an intention-to-treat analysis. A sensitivity analysis was also performed using a per-protocol approach. This excluded livers that received machine perfusion for a period of time outside the range specified in the protocol (4-24 hours) and the groups compared included livers according to the treatment they actually received. Livers that were randomised but then not retrieved for any reason were withdrawn from the trial and not included in later analysis.

The intervention (NMP) was compared against control (SCS) for all primary and secondary outcomes. Primary outcome, difference in peak serum (AST) level, was analysed using ANOVA with adjustment for stratification factors (centre and donor type). Binary outcomes were assessed using test for proportions and logistic regression to adjust for potential confounders and report odds ratios. Continuous outcomes were compared using the T-test if normally distributed, or by the Mann-Whitney U test otherwise. Time-to-event outcomes were analysed using survival analysis methods, including Kaplan-Meier and log-rank test. Outcomes are reported as treatment effect with 95% confidence intervals. A p-value of less than 0.05 was regarded as statistically significant.
Pre-specified subgroup analyses were performed for donor type (DCD vs DBD), donor risk index (ET-DRI) and MELD Score. 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 and the results should only be regarded as hypothesis-generating.

Analyses were conducted with the use of Stata version 14.2 (StataCorp, College Station, TX).

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

## 4. RESULTS

### 4.1 Study participants

The original plan was to randomise 260 donor livers in order to end up with 220 transplanted livers. As randomisation had to occur before the donor liver was retrieved there was a higher rate of discarded or excluded livers than expected. In order for 220 successfully transplanted livers to be included in the trial, 335 randomisations took place with 170 livers allocated to NMP and 164 allocated to the control arm. One randomisation occurred in error before the R&D approval was in place, so that liver was excluded. Sixty-three livers (33 in the NMP and 26 in the SCS arm) were excluded after randomisation and 50 livers (16 in the NMP and 34 in the SCS arm) discarded before transplantation, leaving 222 successfully transplanted livers.

Section 4.3.1 details the different analysis populations.

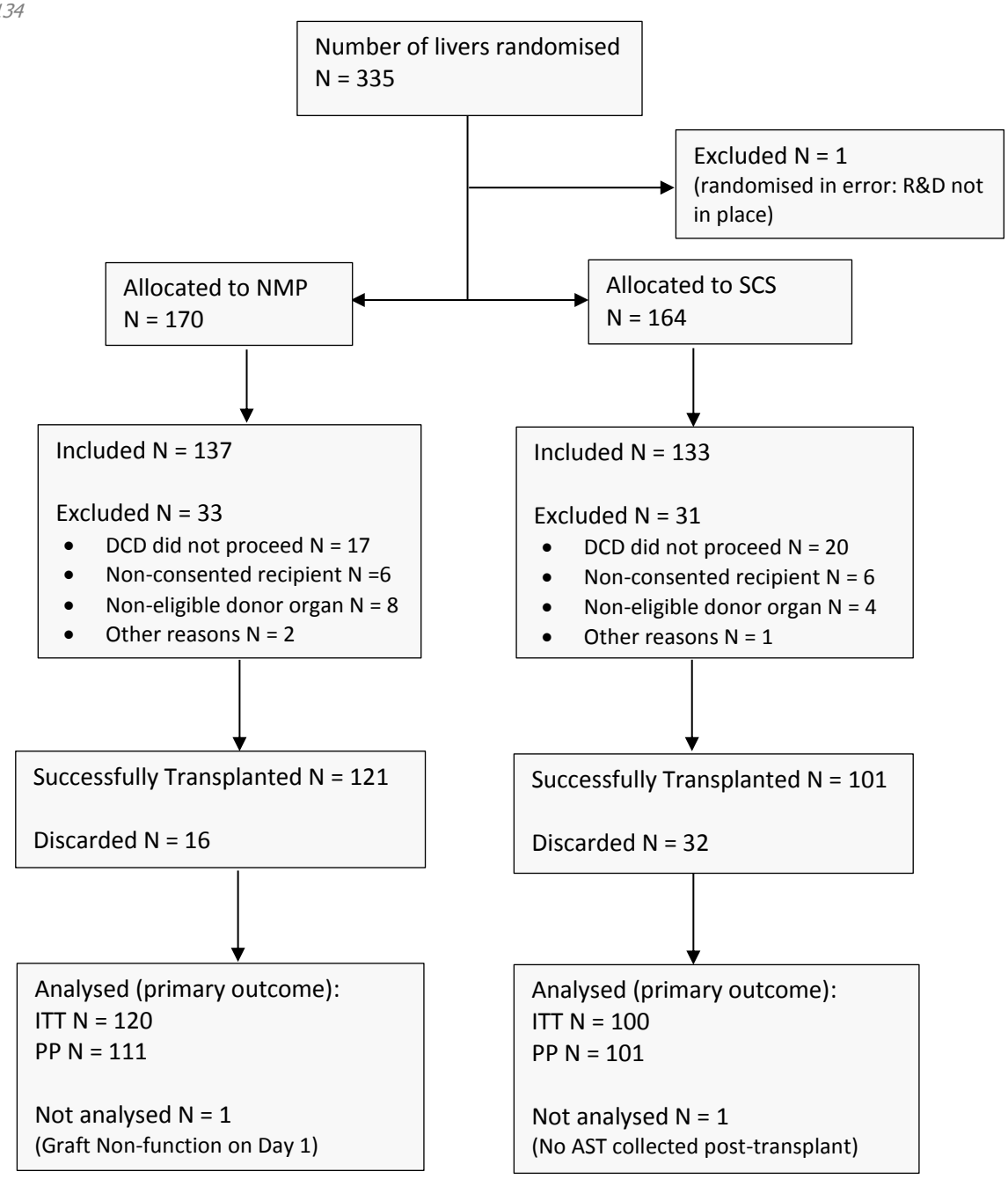



*Figure 2: Flowchart of livers throughout the trial*

## 4.2 Recruitment

Unit of randomisation was livers that entered the study once an eligible recipient was identified and before donor organ retrieval. Data was collected from both donors and recipients. 335 livers were randomised from 7 centres, between 26th June 2014 and 8th March 2016 leading to 222 (121 NMP and 101 SCS) successfully transplanted livers.

## 4.3 Baseline Characteristics

Baseline characteristics are reported with different totals according to whether they refer to donors or recipients. Transplant centre and donor type (Donation after Brain Death, DBD, or Donation after Cardiac Death, DCD) are reported for all randomised livers as these are the stratification factors. However, these will also be reported for the transplanted livers to check balance is maintained. Donor characteristics are reported only for livers that were not withdrawn from the study. The two donor risk indices are also reported for the transplanted livers as these are important characteristics that can be included in the analysis. Recipient characteristics are reported for all transplanted livers.

As normally patients are randomised, significance testing of baseline characteristics is not performed as this would only check if randomisation has worked. However, in this study livers are randomised before donor organ retrieval and some get declined before transplantation. Therefore, recipient baseline characteristics were compared to check the groups remained balanced using Mann-Whitney non-parametric test for continuous variables and Chi-square test for categorical variables. Table 5 and 6 show that the livers were similar in the two groups in terms of both donor and recipients characteristics.

*Table 5: Stratification factors and donor characteristics*

| **Stratification factors** *(all randomised livers)* | **NMP (N = 170)** | **SCS (N = 164)** | **Total (N = 334)** |
|---|---|---|---|
| **Centre*** | | | |
| Addenbrooke's Hospital, Cambridge, UK | 19 (11.2%) | 18 (11.0%) | 37 (11.1%) |
| King's College Hospital, London, UK | 25 (14.7%) | 25 (15.2%) | 50 (15.0%) |
| Queen Elizabeth Hospital, Birmingham, UK | 71 (41.8%) | 69 (42.1%) | 140 (41.9%) |
| Royal Free Hospital, London, UK | 31 (18.2%) | 30 (18.3%) | 61 (18.3%) |
| University Hospital, Essen, Germany | 5 (2.9%) | 6 (3.7%) | 11 (3.3%) |
| University of Barcelona, Spain | 11 (6.5%) | 9 (5.5%) | 20 (6.0%) |
| University Hospital, Leuven, Belgium | 8 (4.7%) | 7 (4.3%) | 15 (4.5%) |
| **Donor type*** | | | |
| DBD | 107 (62.9%) | 104 (63.4%) | 211 (63.2%) |
| DCD | 63 (37.1%) | 60 (36.6%) | 123 (36.8%) |
| **Donor demographics** *(after exclusions)* | **NMP (N = 137)** | **SCS (N = 133)** | **Total (N = 270)** |
| **Gender*** | | | |
| Female | 54 (39.4%) | 57 (42.9%) | 111 (41.1%) |
| Male | 81 (59.1%) | 76 (57.1%) | 157 (58.2%) |
| *(missing)* | *2 (1.5%)* | *0 (0.0%)* | *2 (0.7%)* |
| **Age^** | 56 (45, 67) (16, 84) | 56 (47, 66) (20, 86) | 56 (46, 66) (16, 86) |
| **Ethnicity*** | | | |
| African-Caribbean | 3 (2.2%) | 1 (0.8%) | 4 (1.5%) |
| Caucasian | 131 (95.6%) | 128 (96.2%) | 259 (95.9%) |
| Other | 1 (0.7%) | 4 (3.0%) | 5 (1.9%) |
| *(missing)* | *2 (1.5%)* | *0 (0.0%)* | *2 (0.7%)* |
| **Cause of death** | | | |
| CVA | 74 (54.0%) | 74 (55.6%) | 148 (54.8%) |

| | | | |
|---|---|---|---|
| Hypoxia | 30 (21.9%) | 32 (24.1%) | 62 (23.0%) |
| Trauma | 17 (12.4%) | 16 (12.0%) | 33 (12.2%) |
| Other | 14 (10.2%) | 11 (8.3%) | 25 (9.3%) |
| *(missing)* | *2 (1.5%)* | *0 (0.0%)* | *2 (0.7%)* |
| **BMI^** | 26.26 (23.66, 30.52) (16.42, 46.65) | 27.01 (23.74, 30.56) (17.24, 49.96) | 26.51 (23.69, 30.54) (16.42, 49.96) |
| *(missing)* | *2 (1.5%)* | *0 (0.0%)* | *2 (0.7%)* |
| **UK-Donor risk index^** | 1.53 (1.19, 2.63) (0.78, 6.35) | 1.49 (1.22, 2.44) (0.77, 4.58) | 1.50 (1.21, 2.49) (0.77, 6.35) |
| *(missing)* | *41 (29.9%)* | *53 (39.8%)* | *94 (34.8%)* |
| **ET-Donor risk index^** | 1.72 (1.47, 2.09) (0.98, 4.31) | 1.72 (1.50, 2.10) (1.06, 3.49) | 1.72 (1.48, 2.10) (0.98, 4.31) |
| *(missing)* | *16 (11.7%)* | *19 (14.3%)* | *35 (13.0%)* |

^Median, IQR and full range reported.
*Frequency and column percentages reported.

*Table 6: Recipient baseline characteristics*

| **Recipient demographics** *(transplanted livers)* | **NMP (N = 121)** | **SCS (N = 101)** | **Total (N = 222)** | **p-value** |
|---|---|---|---|---|
| **Transplant centre*‡** | | | | 0.785 |
| Addenbrooke's Hospital, Cambridge, UK | 13 (10.7%) | 11 (10.9%) | 24 (10.8%) | |
| King's College Hospital, London, UK | 14 (11.6%) | 11 (10.9%) | 25 (11.3%) | |
| Queen Elizabeth Hospital, Birmingham, UK | 59 (48.8%) | 46 (45.5%) | 105 (47.3%) | |
| Royal Free Hospital, London, UK | 19 (15.7%) | 15 (14.9%) | 34 (15.3%) | |
| University Hospital, Essen, Germany | 5 (4.1%) | 2 (2.0%) | 7 (3.2%) | |
| University of Barcelona, Spain | 7 (5.8%) | 9 (8.9%) | 16 (7.2%) | |
| University Hospitals, Leuven, Belgium | 4 (3.3%) | 7 (6.9%) | 11 (5.0%) | |
| **Donor type*** | | | | 0.209 |
| DBD | 87 (71.9%) | 80 (79.2 %) | 167 (75.2%) | |
| DCD | 34 (28.1%) | 21 (20.8%) | 55 (24.8%) | |
| **Gender*** | | | | 0.717 |
| Female | 35 (28.9%) | 27 (26.7%) | 62 (27.9%) | |
| Male | 86 (71.1%) | 74 (73.3%) | 160 (72.1%) | |
| **Age^** | 55 (48, 62) (20, 72) | 55 (48,62) (22, 70) | 55 (48, 62) (20, 72) | 0.713 |
| **Cause of Liver Failure*** | | | | 0.782 |
| Alcoholic | 36 (29.8%) | 29 (28.7%) | 65 (29.3%) | |
| Auto-Immune Hepatitis | 2 (1.7%) | 5 (5.0%) | 7 (3.2%) | |
| Drug Induced | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | |
| Hepatitis B | 3 (2.5%) | 2 (2.0%) | 5 (2.3%) | |
| Hepatitis C | 4 (3.3%) | 4 (4.0%) | 8 (3.6%) | |
| Hepatocellular Carcinoma on background of Cirrhosis | 15 (12.4%) | 16 (15.8%) | 31 (14.0%) | |
| Hepatocellular Carcinoma without Cirrhosis | 4 (3.3%) | 2 (2.0%) | 6 (2.7%) | |
| Metabolic | 1 (0.8%) | 0 (0.0%) | 1 (0.5%) | |
| Non-Alcoholic Fatty Liver Disease | 2 (1.7%) | 3 (3.0%) | 5 (2.3%) | |
| Non-Alcoholic Steato-Hepatitis | 9 (7.4%) | 8 (7.9%) | 17 (7.7%) | |
| Other Cancers | 1 (0.8%) | 0 (0.0%) | 1 (0.5%) | |
| Primary Biliary Cirrhosis | 10 (8.3%) | 3 (3.0%) | 13 (5.9%) | |
| Primary Sclerosis Cholangitis | 18 (14.9%) | 13 (12.9%) | 31 (14.0%) | |

| | | | | |
|---|---|---|---|---|
| Other | 16 (13.2%) | 16 (15.8%) | 32 (14.4%) | |
| **BMI^** | 26.18 (23.12, 32.39) (18.02, 50.99) | 26.94 (24.36, 30.42) (18.91, 42.95) | 26.47 (23.72, 31.64) (18.02, 50.99) | 0.626 |
| *(missing)* | *0 (0.0%)* | *1 (1.0%)* | *1 (0.4%)* | |
| **Retransplant*** | 12 (9.9%) | 8 (7.9%) | 20 (9.0%) | 0.605 |
| **MELD score^** | 13 (10, 18) (6, 35) | 14 (9, 18) (6, 29) | 14 (10, 18) (6, 35) | 0.970 |
| UK | 13 (10, 17) (6, 33) | 14 (9, 18) (6, 28) | 13 (9, 18) (6, 33) | |
| Essen, Germany | 17 (14, 19) (13, 23) | 15.5 (14, 17) (14, 17) | 17 (14, 19) (13, 23) | |
| Barcelona, Spain | 16 (8, 26) (8, 35) | 14 (9, 16) (8, 29) | 14.5 (8.5, 22) (8, 35) | |
| Leuven, Belgium | 19 (13.5, 25) (13, 26) | 16 (16, 20) (9, 27) | 16 (14, 24) (9, 27) | |
| **eGFR^** | 87.36 (69.61, 107.66) (33.45, 156.43) | 92.22 (69.72, 104.24) (30.19, 155.04) | 90.46 (69.61, 106.11) (30.19, 156.43) | 0.928 |
| *(missing)* | *4 (3.3%)* | *3 (3.0%)* | *7 (3.2%)* | |
| **UK-Donor risk index^** | 1.45 (1.17, 2.55) (0.78, 6.35) | 1.43 (1.20, 2.19) (0.77, 3.42) | 1.44 (1.19, 2.39) (0.77, 6.35) | 0.456 |
| *(missing)* | *37 (30.6%)* | *34 (33.7%)* | *71 (32.0%)* | |
| **ET-Donor risk index^** | 1.70 (1.47, 2.07) (0.98, 4.31) | 1.71 (1.50, 2.01) (1.06 3.49) | 1.70 (1.48, 2.04) (0.98, 4.31) | 0.610 |
| *(missing)* | *13 (10.7%)* | *13 (12.9%)* | *26 (11.8%)* | |

^Median, IQR and full range reported.

*Frequency and column percentages reported.

‡Transplant centre refers to the recipient centre were the liver was actually transplanted. In 4 UK livers this differs from the centre reported in the stratification factors table.

### 4.3.1 Numbers analysed

The primary analysis was intention-to-treat and involved all livers who were successfully transplanted by their assigned randomised arm. These were in total 222, 101 in the SCS and 121 in the NMP arm; however, for the intention-to-treat analysis of the primary outcome two livers (one in each arm) were excluded due to no AST values being available post-transplant so 100 and 120 were analysed respectively in the SCS and NMP groups.

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

## 4.4 Compliance

### 4.4.1 Withdrawals (exclusions) & Discarded livers

Livers were excluded after randomisation if they were later found to be ineligible for the trial. The possible reasons are listed in the table below.

*Table 7: Reasons of withdrawal*

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

^DCD donor did not proceed to asystole within the required timescale

Other reasons include:

- Donor died before retrieval team arrived (n=1)
- DCD donor became DBD donor/ randomised in error (n=1)
- Logistically not possible for NMP due to multiple staff absence not able to send out machine (n=1)

Discarded livers are those that did not proceed to transplant due to the implanting surgeon's decision. These are reported below followed by the reasons for discards.

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

*Table 8: Discarded livers by treatment arm and discard rate*

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

*Table 9: Reasons for discarding/ declining livers*

| Reason | NMP | SCS | Total |
|---|---|---|---|
| **Steatosis** | 13 | 24 | 37 |
| **Prolonged Warm Ischaemic Time** | 2 | 6 | 8 |
| **Poor Perfusion Parameters** | 5 | 0 | 5 |
| **Device User Error** | 4 | 0 | 4 |
| **Donor Problem e.g. malignancy** | 2 | 2 | 4 |
| **Abnormal Lesion** | 0 | 3 | 3 |
| **Fibrosis** | 1 | 1 | 2 |
| **Poor In-situ Perfusion** | 1 | 2 | 3 |
| **Capsular Damage** | 1 | 1 | 2 |
| **Device Error** | 1 | 0 | 1 |
| **Injury to Hepatic Artery** | 0 | 1 | 1 |
| **Parenchymal Damage** | 1 | 0 | 1 |
| **Other** | 1 | 1 | 2 |

Note that some livers may have been declined for more than one reason.

Other reasons include:

- Liver too large for recipient and no other appropriate AB recipients

- 3.5kg liver therefore too large for recipient despite good machine perfusion

### 4.4.2 Treatment compliance

The following tables give a summary of the treatment compliance as well as preservation and transplant procedure details.

The total preservation time is clinically considered an important factor that may affect the outcomes. Therefore, a non-parametric test was used to compare it between the two groups. The NMP preservation time appeared to be significantly longer (p<0.001) than the SCS preservation time.

*Table 10: Treatment received by randomised treatment*

| | Randomised treatment | | |
|---|---|---|---|
| **Treatment received** | **NMP (N = 121)** | **SCS (N = 101)** | **Total (N = 222)** |
| **NMP** | 120 | 0 | 120 |
| **SCS** | 1^ | 101 | 102 |

^The crossover was due to Accessory left hepatic artery from Aorta.

*Table 11: Preservation details*

| | **NMP (N = 121)** | **SCS (N = 101)** | **Total (N = 222)** |
|---|---|---|---|
| **Total Preservation time (minutes)^** | 714 (542, 876) (258, 1527) | 465 (375, 575) (223, 967) | 581.5 (445, 747) (223, 1527) |
| **Machine perfusion time (minutes)^** (N = 120) | 547.5 (372.5, 710.5) (85, 1388) | - | 547.5 (372.5, 710.5) (85, 1388) |
| **Steatosis assessed pre-preservation*** | | | |
| None | 36 (29.8%) | 44 (43.6%) | 80 (36.0%) |
| Mild | 55 (45.5%) | 45 (44.6%) | 100 (45.1%) |
| Moderate | 18 (14.9%) | 10 (9.9%) | 28 (12.6%) |
| Severe | 11 (9.1%) | 2 (2.0%) | 13 (5.9%) |
| *(missing)* | *1 (0.8%)* | | *1 (0.5%)* |
| **Quality of perfusion*** | | | |
| Good | 106 (87.6%) | 83 (82.2%) | 189 (85.1%) |
| Moderate | 11 (9.1%) | 7 (6.9%) | 18 (8.1%) |
| Poor | 3 (2.5%) | 1 (1.0%) | 4 (1.8%) |
| *(missing)* | *1 (0.8%)* | *10 (9.9%)* | *11 (5.0%)* |
| **Warm ischaemia time (minutes)^‡** (N = 55, 34 NMP, 21 SCS) | 21 (17, 25) (9, 93) | 16 (10, 20) (2, 32) | 20 (15, 23) (2, 93) |
| **Cold ischaemia time (minutes)^** (N = 120) | 126 (106.5, 143) (49, 218) | - | 126 (106.5, 143) (49, 218) |

^Median, IQR and full range reported.
*Frequency and column percentages reported.
‡Warm ischaemia time is only calculated for DCD livers.

*Table 12: Transplant intervention details*

| | **NMP (N = 121)** | **SCS (N = 101)** | **Total (N = 222)** |
|---|---|---|---|
| **Anhepatic time (minutes)^** | 50 (38, 66) (23, 287) | 50 (40, 65) (23, 239) | 50 (39, 66) (23, 287) |
| *(missing)* | | *1 (1.0%)* | *1 (0.5%)* |
| **Anastomosis time (minutes)^** | 35 (25, 51) (5, 163) | 36 (30, 45) (10, 112) | 35 (28, 47) (5, 163) |
| **Total operative time (minutes)^** | 333 (265, 405) (150, 925) | 345 (280, 424) (155, 862) | 338.5 (271, 415) (150, 925) |
| **Technical problems*** | 20 (16.5%) | 21 (21.0%) | 41 (18.6%) |

| | | | |
|---|---|---|---|
| *(missing)* | | *1 (1.0%)* | *1 (0.5%)* |
| **Pre-reperfusion vasopressor infusion*** | 92 (76.0%) | 82 (81.2%) | 174 (78.4%) |
| *(missing)* | *2 (1.7%)* | *6 (5.9%)* | *8 (3.6%)* |
| **Post-reperfusion bolus*** | 41 (33.9%) | 60 (59.4%) | 101 (45.5%) |
| *(missing)* | *4 (3.3%)* | *9 (8.9%)* | *13 (5.9%)* |
| **Post-reperfusion vasopressor infusion*** | 65 (53.7%) | 80 (79.2%) | 145 (65.3%) |
| *(missing)* | *1 (0.8%)* | *9 (8.9%)* | *10 (4.5%)* |

^Median, IQR and full range reported.
*Frequency and column percentages reported.

### 4.4.3 Blinding

Not applicable as open-label trial.

## 4.5 Results

### 4.5.1 Primary outcome – peak AST

The primary outcome is the peak AST value in the first 7 days post-transplant.

As expected, the Peak AST is not normally distributed so a log-transformation was performed in order to carry out the parametric analysis. This transformation proved successful. Details of the statistics related to this transformation are in the Appendix.

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

*Table 13: ANOVA table for (ln) Peak AST*

| Source | Partial SS | df | MS | F | Prob>F |
|---|---|---|---|---|---|
| **Model** | 39.952 | 8 | 4.994 | 5.21 | 0.000 |
| **Treatment arm** | 24.700 | 1 | 24.700 | 25.76 | 0.000 |
| **Centre (as Randomised)** | 13.355 | 6 | 2.226 | 2.32 | 0.034 |
| **Donor Type** | 0.206 | 1 | 0.206 | 0.21 | 0.644 |
| **Total** | 242.305 | 219 | 1.106 | | |

The treatment effect on the peak AST is highly statistically significant ($p<0.001$). Centre also showed a statistically significant effect, although not highly significant. Also, as stated in section 3.5, some centres included as little as 3.2% of the total livers transplanted with more than 40% of the transplanted livers randomised by a single centre. It would, therefore, be inappropriate to draw conclusion based on this result.

This study was powered to detect a 33% reduction in the peak AST (in terms of geometric means). After back-transforming the means of the natural logarithm (ln) Peak AST to the geometric means, the geometric mean ratio is then obtained as shown in the table below. We can reject the null hypothesis that there is no difference between NMP and SCS, with NMP reducing the Peak AST by 49.4% (95%CI 34.1% to 61.2%).

*Table 14: Primary outcome results from the adjusted analysis (ANOVA model)*

| | NMP | SCS | 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%)] |

^First cell in this column refers to the mean difference in natural logarithm 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.

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

*Table 15: Primary outcome results from the unadjusted analysis (t-test)*

| | NMP | SCS | Difference / Mean ratio^ [% reduction] |
|---|---|---|---|
| **Mean ln Peak AST (95% C.I)** | 6.183 (6.007, 6.359) | 6.881 (6.679, 7.084) | -0.698 (-0.963, -0.433) |
| **Geometric Mean Peak AST (95% C.I.)** | 484.477 (406.361, 577.610) | 973.700 (795.194, 1192.277) | 0.498 (0.382, 0.649) [50.2% (35.1%, 61.8%)] |

^First cell in this column refers to the mean difference in natural logarithm 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.

*Subgroup analysis by donor type, MELD Score and ET-DRI*

The subgroup analysis was performed by including the interaction between the treatment and donor type in the ANOVA model. This interaction proved to be statistically significant (p-value=0.012) as shown in the table below.

*Table 16: ANOVA table for the (ln) Peak AST including interaction between treatment and donor type*

| Source | Partial SS | df | MS | F | Prob>F |
|---|---|---|---|---|---|
| **Model** | 32.808 | 3 | 10.936 | 11.28 | 0.000 |
| **Treatment arm** | 32.248 | 1 | 32.248 | 33.25 | 0.000 |
| **Donor Type** | 0.401 | 1 | 0.401 | 0.41 | 0.521 |
| **Treatment*Donor Type** | 6.210 | 1 | 6.210 | 6.40 | 0.012 |
| **Total** | 242.305 | 219 | 1.106 | | |

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

*Table 17: Treatment effect on Peak AST for donor type subgroups.*

| Donor Type | Obs | NMP | SCS | Effect (geometric mean ratio) | [95% Conf. Interval] | p-value |
|---|---|---|---|---|---|---|
| **DBD** | 167 | 526.157 (427.302, 647.882) | 880.209 (708.491, 1093.547) | 0.598 | (0.443, 0.807) | 0.001 |
| **DCD** | 53 | 389.746 (277.992, 546.426) | 1458.081 (944.666, 2250.532) | 0.267 | (0.154, 0.463) | <0.001 |

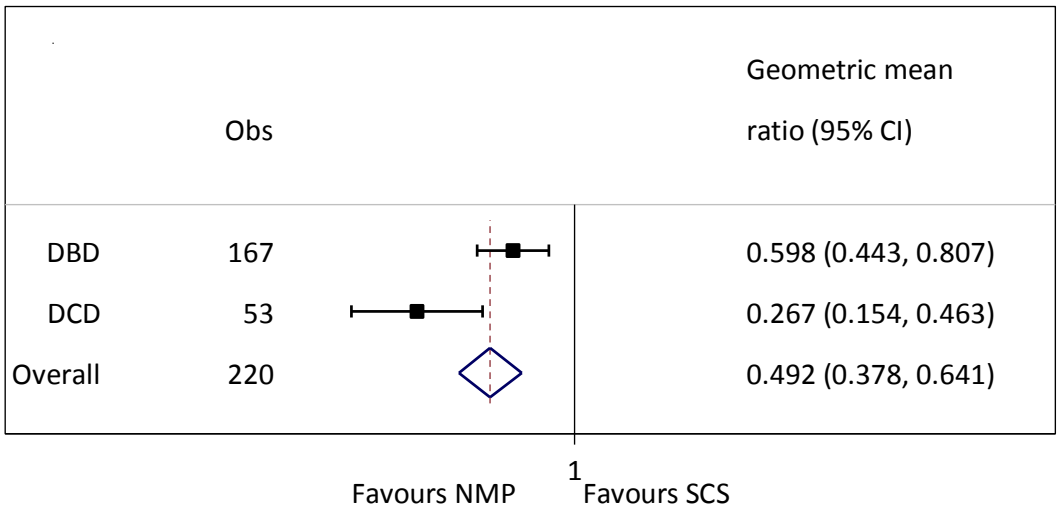



*Figure 3: Forest plot for subgroup analysis of Peak AST by donor type*

Subgroup analysis were also performed for the MELD Score and Eurotransplant Donor Risk Index (ET-DRI).

When tested as interactions there were no statistically significant differences within these subgroups as shown by the ANOVA results below. Note the study is not powered to detect any interactions.

*Table 18: ANOVA table for the (ln) Peak AST including interaction between treatment and ET-DRI*

| Source | Partial SS | df | MS | F | Prob>F |
|---|---|---|---|---|---|
| **Model** | 25.433 | 5 | 5.087 | 5.05 | 0.000 |
| **Treatment arm** | 22.121 | 1 | 22.121 | 21.96 | 0.000 |
| **ET-DRI group** | 0.090 | 2 | 0.045 | 0.04 | 0.956 |
| **Treatment*ET-DRI group** | 3.104 | 2 | 1.552 | 1.54 | 0.217 |
| **Total** | 214.795 | 193 | 1.113 | | |

*Table 19: ANOVA table for the (ln) Peak AST including interaction between treatment and MELD Score*

| Source | Partial SS | df | MS | F | Prob>F |
|---|---|---|---|---|---|
| **Model** | 29.573 | 5 | 5.915 | 5.95 | 0.000 |
| **Treatment arm** | 21.077 | 1 | 21.077 | 21.20 | 0.000 |
| **MELD group** | 2.981 | 2 | 1.490 | 1.50 | 0.226 |
| **Treatment*MELD group** | 0.0179 | 2 | 0.009 | 0.01 | 0.991 |
| **Total** | 242.305 | 219 | 1.106 | | |

*Treatment by centre interaction*

No formal test was carried out to assess consistency of effect across the centres. This is only shown graphically in the forest plot below and no conclusion should be drawn given the small number of patients recruited in some of the centres.

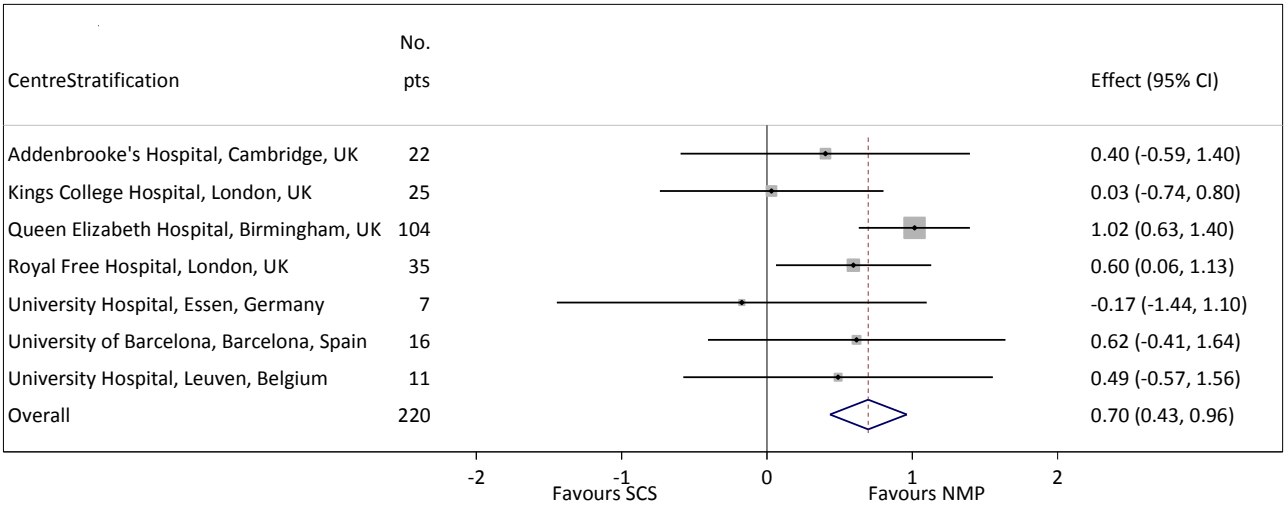



*Figure 4: Forest plot of (ln) Peak AST by centre*

*Sensitivity analysis – per-protocol population*

The primary analysis for the log peak AST was repeated in the per-protocol population (refer to section 4.3.1). In this analysis the livers were analysed according to the treatment actually received and to the centre where they were eventually transplanted rather than those that randomised them (note some livers were rejected for the original recipient but transplanted into other consented recipients, at a different trial centre).

*Table 20: ANOVA table for the (ln) Peak AST – Per Protocol population*

| Source | Partial SS | df | MS | F | Prob>F |
|---|---|---|---|---|---|
| **Model** | 35.247 | 8 | 4.406 | 4.61 | 0.000 |
| **Treatment received** | 23.750 | 1 | 23.750 | 24.87 | 0.000 |
| **Centre (as transplanted)** | 10.577 | 6 | 1.763 | 1.85 | 0.092 |
| **Donor Type** | 0.363 | 1 | 0.363 | 0.38 | 0.538 |
| **Total** | 229.120 | 211 | 1.086 | | |

The results from the sensitivity analysis are reported in the ANOVA table above. The treatment effect remained significant in the per-protocol population and the reduction only changed slightly (reduction 49.3% (95% CI 33.7% to 61.2%)) as show in the table below.

*Table 21: Primary outcome results from the adjusted analysis (ANOVA model) – Per Protocol population*

| | NMP | SCS | Difference / Mean ratio^ [% reduction] |
|---|---|---|---|
| **Mean ln Peak AST (95% C.I)** | 6.212 (6.028, 6.396) | 6.891 (6.698, 7.084) | -0.679 (-0.947, -0.410) |
| **Geometric Mean Peak AST (95% C.I.)** | 498.646 (414.842, 599.380) | 982.944 (810.413, 1192.206) | 0.507 (0.388, 0.663) [49.3% (33.7%, 61.2%)] |

^First cell in this column refers to the mean difference in natural logarithm 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.

The unadjusted analysis showed the treatment effect magnitude and confidence interval also do not seem to differentiate much from those in the primary analysis, as shown in the table below.

*Table 22: Primary outcome results from the unadjusted analysis (t-test) – Per Protocol population*

| | NMP | SCS | Difference / Mean ratio^ [% reduction] |
|---|---|---|---|
| **Mean ln Peak AST (95% C.I)** | 6.210 (6.031, 6.389) | 6.893 (6.691, 7.095) | -0.683 (-0.950, -0.416) |
| **Geometric Mean Peak AST (95% C.I.)** | 497.615 (415.939, 595.329) | 985.183 (805.131, 1205.5) | 0.505 (0.387, 0.660) [49.5% (34.0%, 61.3%)] |

^First cell in this column refers to the mean difference in natural logarithm 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.

### 4.5.2 Secondary outcome – Primary Non Function

Due to the small number of events and no PNF occurring in one of the treatment arms, it was not possible to analyse this outcome using the methods described in the analysis plan.

*Table 23: Primary Non-Function*

| PNF | NMP | SCS | Total |
|---|---|---|---|
| **No** | 120 (99.2 %) | 101 (100.0%) | 221 (99.6 %) |
| **Yes** | 1 (0.8 %) | 0 (0.0%) | 1 (0.5%) |
| **Total** | 121 | 101 | 222 |

### 4.5.3 Secondary outcome – Patient and graft survival

At the time of 1 year follow-up data lock 11 recipients had died. Ten of these deaths occurred during the 1 year follow-up showing a survival of 0.949 (95% C.I 0.890 to 0.977) in the NMP group and of 0.958 (95% C.I 0.902 to 0.982) in the SCS group.
Eight deaths occurred during the 6 month follow-up showing a survival of 0.958 (95% C.I 0.902 to 0.982) in the NMP group and of 0.970 (95% C.I 0.909 to 0.990) in the SCS group.
At the 30 days follow-up 3 deaths occurred, all in the NMP group showing a survival of 0.975% (95% C.I 0.925 to 0.992). Since no deaths occurred in the SCS group in the first 30 days post-transplant, the survival rate is shown as 1.000 and no 95% C.I. is calculated.

There were in total 10 graft failures, all occurring in the 6 months post-transplant. The graft survival at 6 months is 0.950 (95% C.I 0.893 to 0.977) in the NMP group and 0.960 (95% C.I 0.897 to 0.985) in the SCS group.

No further graft failure occurred after the 6 months follow-up, showing 1 year survival rates similar to the 6 months ones.

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

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

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

*Table 24: Recipient deaths by treatment group*

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

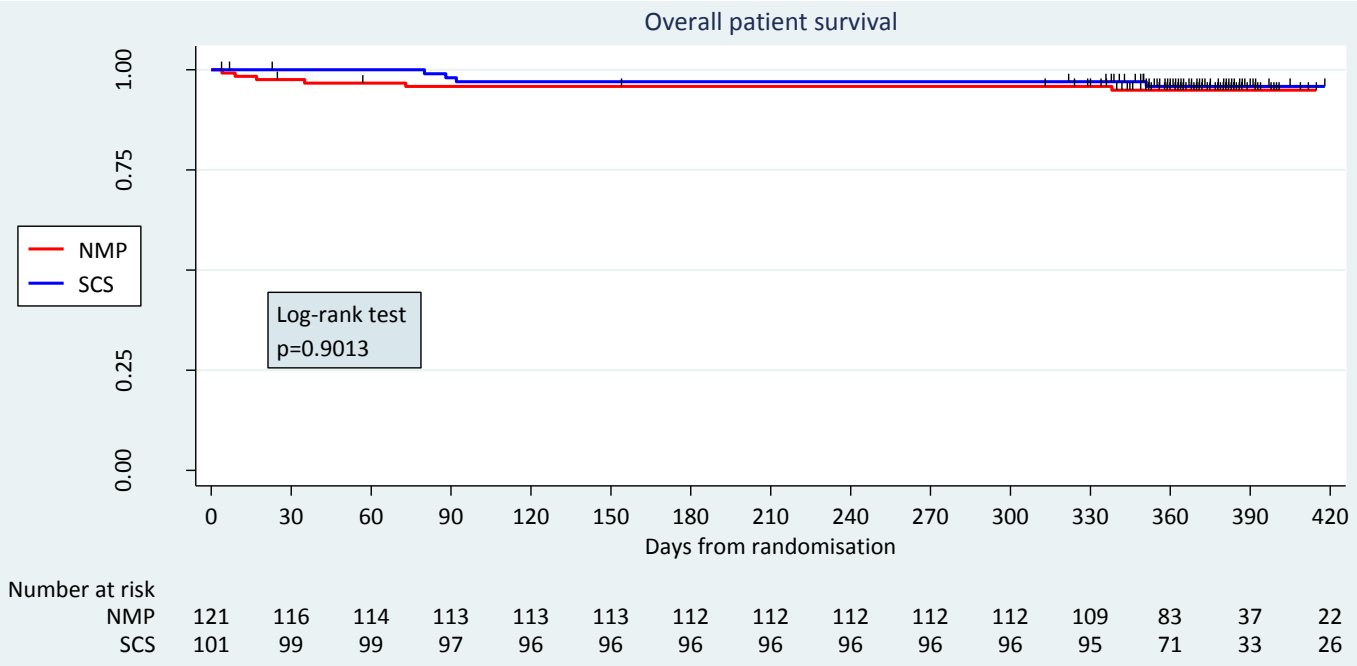



*Figure 5: Kaplan-Meier plot for patient survival with log-rank test*

*Table 25: Graft failure overall at 6 months data lock by treatment group*

| Treatment group | Graft failures at 30 days | Graft failure overall |
|---|---|---|
| **NMP** | 5 | 6 |
| **SCS** | 2 | 4 |
| **Total** | 7 | 10 |

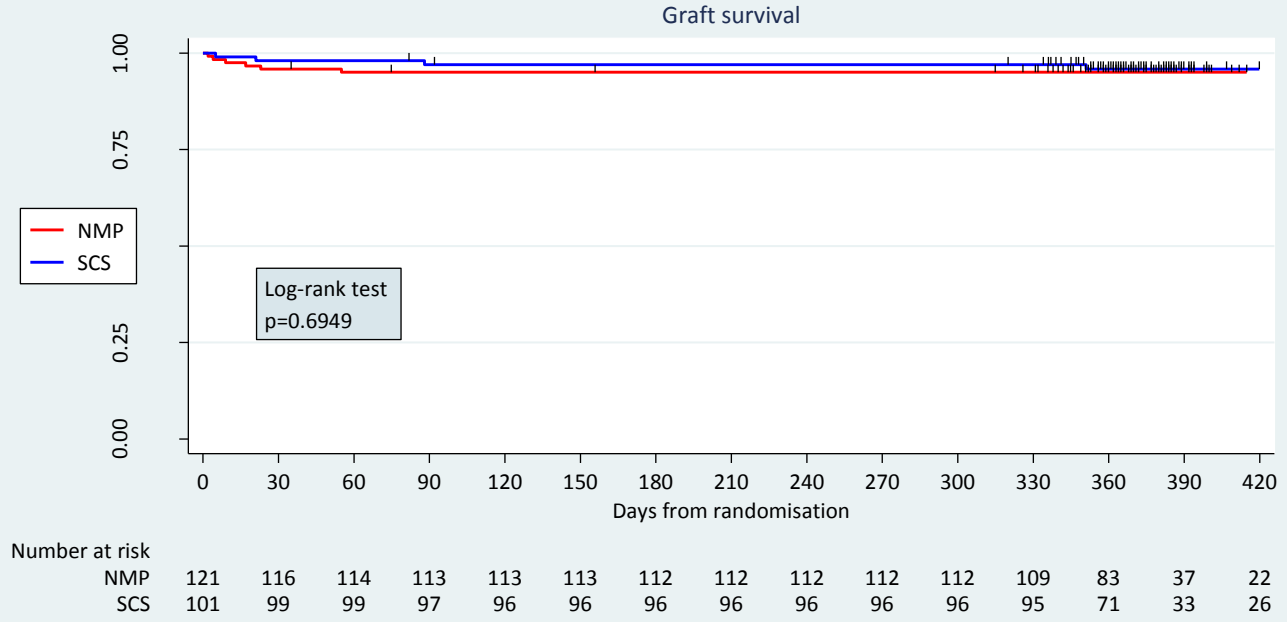



*Figure 6: Kaplan-Meier plot for graft survival and log-rank test*

### 4.5.4 Secondary outcome – Biochemical liver function

*Blood serum biochemical components*

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

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

*Table 26: Results for AUC of biochemical tests by treatment groups*

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

| | | | |
|---|---|---|---|
| ***n*** | *119* | *101* | |

Median and interquartile range displayed for each treatment group

The average value over the 7 days was calculated for each patient irrespective of any missing value/measurement. The Mann-Whitney test was then used to compare them between treatment arms as the mean measurement was not normally distributed. Since Lactate levels were only measured while in ITU, P-values were not reported for the Lactate difference at 30 days and 6 months as very few values were collected.

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

*Table 27: Results for average value of biochemical tests by treatment groups*

| Biochemical test^ | NMP | SCS | p-value |
|---|---|---|---|
| **Bilirubin** | | | |
| **Day 1-7** | 2.25 (1.23, 4.28) | 2.87 (1.52, 5.00) | 0.029 |
| ***n*** | *120* | *101* | |
| **30 days** | 0.76 (0.47, 1.29) | 0.76 (0.53, 1.23) | 0.479 |
| ***n*** | *115* | *99* | |
| **6 months** | 0.53 (0.35, 0.88) | 0.53 (0.35, 0.76) | 0.671 |
| ***n*** | *110* | *95* | |
| **12 months** | 0.59 (0.4, 0.88) | 0.53 (0.35, 0.76) | 0.338 |
| ***n*** | *102* | *88* | |
| **AST** | | | |
| **Day 1-7** | 167.5 (98, 320.7) | 318.5 (152, 611.5) | 0.000 |
| ***n*** | *120* | *100* | |
| **30 days** | 20 (14, 35) | 22 (15, 40) | 0.707 |
| ***n*** | *109* | *89* | |
| **6 months** | 23 (18, 33) | 23 (18, 37) | 0.931 |
| ***n*** | *101* | *83* | |
| **12 months** | 23 (20, 30) | 21 (17, 30.5) | 0.133 |
| ***n*** | *81* | *72* | |
| **GGT** | | | |
| **Day 1-7** | 268.1 (156.3, 408.3) | 301 (201.1, 443.9) | 0.157 |
| ***n*** | *99* | *83* | |
| **30 days** | 178 (109.5, 410) | 200 (96, 397.5) | 0.949 |
| ***n*** | *88* | *68* | |
| **6 months** | 47 (28, 144) | 47 (26, 128) | 0.452 |
| ***n*** | *79* | *69* | |
| **12 months** | 44.5 (25.5, 120) | 60 (28, 112) | 0.892 |
| ***n*** | *64* | *61* | |
| **INR** | | | |
| **Day 1-7** | 1.24 (1.15, 1.38) | 1.24 (1.16, 1.39) | 0.644 |
| ***n*** | *120* | *101* | |
| **30 days** | 1.1 (1, 1.2) | 1.1 (1, 1.2) | 0.735 |
| ***n*** | *109* | *93* | |

| | | | |
|---|---|---|---|
| **6 months**<br>***n*** | 1.1 (1, 1.2)<br>*103* | 1.1 (1, 1.14)<br>*87* | 0.167 |
| **12 months**<br>***n*** | 1.07 (1, 1.18)<br>*80* | 1.1 (1, 1.13)<br>*72* | 0.867 |
| **Creatinine** | | | |
| **Day 1-7**<br>***n*** | 1.05 (0.68, 1.37)<br>*120* | 1.1 (0.76, 1.62)<br>*101* | 0.139 |
| **30 days**<br>***n*** | 0.93 (0.75, 1.18)<br>*116* | 1.02 (0.82, 1.37)<br>*99* | 0.019 |
| **6 months**<br>***n*** | 1.13 (0.92, 1.33)<br>*110* | 1.13 (0.94, 1.52)<br>*95* | 0.265 |
| **12 months**<br>***n*** | 1.15 (0.92, 1.34)<br>*102* | 1.18 (0.95, 1.46)<br>*88* | 0.328 |
| **Lactate** | | | |
| **Day 1-7**<br>***n*** | 1.3 (1, 1.7)<br>*101* | 1.1 (0.9, 1.6)<br>*82* | 0.130 |
| **30 days***<br>***n*** | 4.2 (1.1, 7.3)<br>*2* | 1.75 (0.7, 2.8)<br>*2* | - |
| **6 months***<br>***n*** | - | 0.9<br>*1* | - |

^Median and interquartile range displayed for each treatment group.
*Test not performed due to few Lactate values reported.

*Early Allograft Dysfunction*

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

*Table 28: EAD by treatment group*

| EAD | NMP | SCS | Total |
|---|---|---|---|
| **No** | 107<br>(89.9%) | 68<br>(70.1%) | 175<br>(81.0%) |
| **Yes** | 12<br>(10.1%) | 29<br>(29.9%) | 41<br>(19.0%) |
| **Total** | 119 | 97 | 216 |
| **Difference = -19.8% (95% C.I. -30.4%, -9.2%)** | | | **p-value = 0.000** |
| **Odds Ratio = 0.263 (95% C.I. 0.126, 0.550)** | | | **p-value = 0.000** |

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

*Table 29: Logistic regression table (adjusted analysis) for EAD*

| EAD | Odds Ratio | Std. Err. | z | P>z | [95% Conf. Interval] |
|---|---|---|---|---|---|
| **Treatment Arm** | | | | | |
| **NMP** | 0.276 | 0.112 | -3.17 | 0.002 | (0.124, 0.611) |
| **Donor Type** | | | | | |
| **DCD** | 1.871 | 1.102 | 1.06 | 0.287 | (0.590, 5.932) |
| **MELD Score** | 0.992 | 0.036 | -0.23 | 0.814 | (0.924, 1.064) |
| **Donor Risk Index ET** | 0.816 | 0.422 | -0.39 | 0.694 | (0.296, 2.247) |
| **_cons** | 0.572 | 0.633 | -0.51 | 0.613 | (0.065, 5.003) |

### 4.5.5 Secondary outcome – Physiological response to reperfusion

*Post reperfusion syndrome*

Post reperfusion syndrome was more common in the SCS than in the NMP group and the difference (-20.6% (95% C.I. -31.6% to -9.6%)) is statistically significant (p <0.0001) as shown in the table below.

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

*Table 30: Post reperfusion syndrome by treatment group*

| Post reperfusion syndrome | NMP | SCS | Total |
|---|---|---|---|
| **No** | 106 (87.6%) | 65 (67.0%) | 171 (78.4%) |
| **Yes** | 15 (12.4%) | 32 (33.0%) | 47 (21.6%) |
| **Total** | 121 | 97 | 218 |
| **Difference = -20.6% (95% C.I. -31.6%, -9.5%)** | | | **p-value = 0.000** |

*Post reperfusion lactate*

*Table 31: Post reperfusion lactate by treatment group*

| Post reperfusion lactate | NMP (N=81) | SCS (N=51) | p-value |
|---|---|---|---|
| **Median (IQR)** | 3.6 (2.6, 4.2) | 4.1 (3.2, 5) | 0.018 |

*Need for renal replacement therapy (RRT)*

There is no evidence of a significant difference in the need for renal replacement therapy between the two groups either in the first 7 days post-operative or at the subsequent follow-up time points (p-value = 0.621 Day 1-7 post-operative; p-value = 0.648 at 30 days; p-value = 0.784 at 6 months). The duration of RRT at 7 days also did not show any significant difference between arms (p=0.713) as about 80% of recipients of both groups did not require it.

*Table 32: Need for RRT at 7 days*

| Renal Replacement Therapy required Day 1-7 post-op | NMP | SCS | Total |
|---|---|---|---|
| **No** | 95 (78.5%) | 82 (81.2%) | 177 (79.7%) |
| **Yes** | 26 (21.5%) | 19 (18.8%) | 45 (20.3%) |
| **Total** | 121 | 101 | 222 |
| **Difference = 2.7% (95% C.I. -7.9%, 13.2%)** | | | **p-value = 0.621** |

*Table 33: Need for RRT at 30 days*

| Renal Replacement Therapy required 30 days | NMP | SCS | Total |
|---|---|---|---|
| **No** | 94 (77.7%) | 81 (80.2%) | 175 (78.8%) |
| **Yes** | 27 (22.3%) | 20 (19.8%) | 47 (21.2%) |
| **Total** | 121 | 101 | 222 |
| **Difference = 2.5 (95% C.I. -8.2%, 13.3%)** | | | **p-value = 0.648** |

*Table 34: Need for RRT at 6 months*

| Renal Replacement Therapy required 6 months | NMP | SCS | Total |
|---|---|---|---|
| **No** | 94 (77.7%) | 80 (79.2%) | 174 (78.4%) |
| **Yes** | 27 (22.3%) | 21 (20.8%) | 48 (21.6%) |
| **Total** | 121 | 101 | 222 |
| **Difference = 1.5% (95% C.I. -9.3%, 12.4%)** | | | **p-value = 0.784** |

Among the recipients who required RRT in the first 7 days post-transplant (n=45), the duration of RRT was compared between the two groups using non-parametric test.

*Table 35: Duration of RRT in first 7 days post-transplant*

| Duration of RRT at Day 7 (no. of days) | NMP | SCS | Total |
|---|---|---|---|
| **1** | 4 (15.4%) | 1 (5.3%) | 5 (11.1%) |
| **2** | 3 (11.5%) | 2 (10.5%) | 5 (11.1%) |
| **3** | 3 (11.5%) | 1 (5.3%) | 4 (8.9%) |
| **4** | 6 (23.1%) | 3 (15.8%) | 9 (20.0%) |
| **5** | 2 (7.7%) | 7 (36.8%) | 9 (20.0%) |
| **6** | 4 (15.4%) | 3 (15.8%) | 7 (15.6%) |
| **7** | 4 (15.4%) | 2 (10.5%) | 6 (13.3%) |
| **Total** | 26 | 19 | 45 |
| **Median (IQR)** | 4 (2, 6) | 5 (4, 6) | **p-value = 0.346** |

*Length of hospital stay and length of stay in HDU/ITU*

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

*Table 36: Length of hospital stay and HDU/ITU stay by treatment group*

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

The test above was repeated after excluding patients who had non-functioning grafts (due to either recipient death or graft failure). The results from this sensitivity analysis were similar to those above (p=0.684 for length of hospital stay; p=0.468 for length of ITU/HDU stay; output not reported).

### 4.5.6 Secondary outcome – Reperfusion injury

This analysis is based data from graft biopsies which are not available at the time of drafting the first version of the report.

### 4.5.7 Secondary outcome – Ischaemic cholangiopathy

This analysis is based on data from magnetic resonance cholangiopancreatography (MRCP). At the time of drafting the first version of the report, this is still under assessment by a radiologist blinded to the preservation method.

## 4.6 Safety (Harms)

All adverse events reported were reviewed by two independent clinicians blinded to treatment group in order to remove duplicates, double-check and identify the seriousness of the events also by grading them according to the Clavien-Dindo classification (refer to SAP Appendix for grading). Any event graded IIIb or above is to be considered a serious adverse event (SAE). The totals in Table 38, 39 and 40 refers to the adverse events reported (can be more than one for each recipient).

*Table 37: Clavien-Dindo grading by treatment arm for all adverse events reported*

| Clavien-Dindo grading | NMP | SCS | Total |
|---|---|---|---|
| **I** | 15 (11.7%) | 30 (18.3%) | 45 (15.4%) |
| **II** | 64 (50.0%) | 72 (43.9%) | 136 (46.6%) |
| **IIIa** | 28 (21.9%) | 26 (15.9%) | 54 (18.5%) |
| **IIIb** | 8 (6.3%) | 9 (5.5%) | 17 (5.8%) |
| **IVa** | 5 (3.9%) | 15 (9.2%) | 20 (6.9%) |

| | | | |
|---|---|---|---|
| **IVb** | 3 (2.3%) | 9 (5.5%) | 12 (4.1%) |
| **V** | 5 (3.9%) | 3 (1.8%) | 8 (2.7%) |
| **Total** | 128 | 164 | 292 |

*Table 38: Classification of events by seriousness (events not participants)*

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

*Table 39: Summaries of adverse events by category and by treatment group*

| Event Category | NMP | SCS | Total |
|---|---|---|---|
| **Infection** | 25 (19.5%) | 17 (10.4%) | 42 (14.4%) |
| **Chest** | 1 | 1 | 2 |
| **Blood** | 10 | 3 | 13 |
| **Biliary** | 6 | 0 | 6 |
| **Abdominal** | 2 | 3 | 5 |
| **Gastrointestinal** | 4 | 5 | 9 |
| **Other** | 2 | 5 | 7 |
| **Hepatic** | 44 (34.4%) | 48 (29.3%) | 92 (31.5%) |
| **Bile leak** | 2 | 1 | 3 |
| **Biliary stricture (anastomotic)** | 9 | 11 | 20 |
| **Ischaemic cholangiopathy** | 1 | 3 | 4 |
| **Biliary other** | 1 | 0 | 1 |
| **Drainage of ascites** | 0 | 1 | 1 |
| **Hepatic artery aneurysm** | 0 | 1 | 1 |
| **Hepatic artery thrombosis** | 2 | 4 | 6 |
| **Hepatic artery stenosis** | 5 | 3 | 8 |
| **Hepatic artery other** | 0 | 2 | 2 |
| **Hepatic vein thrombosis** | 1 | 0 | 1 |
| **Portal vein thrombosis** | 2 | 0 | 2 |
| **Portal vein stenosis** | 2 | 0 | 2 |
| **Portal vein other** | 1 | 0 | 1 |
| **Graft dysfunction** | 3 | 2 | 5 |
| **Rejection** | 12 | 13 | 25 |
| **Other** | 3 | 7 | 10 |
| **Cardiovascular** | 5 (3.9%) | 5 (3.1%) | 10 (3.4%) |
| **Congestive heart failure** | 1 | 0 | 1 |
| **Myocardial infarction** | 2 | 3 | 5 |
| **Other** | 2 | 2 | 4 |

| | | | |
|---|---|---|---|
| **Dermatologic** | 1 (0.8%) | 0 (0.0%) | 1 (0.3%) |
| **Seroma** | 1 | 0 | 1 |
| **Gastrointestinal** | 5 (3.9%) | 6 (3.7%) | 11 (3.8%) |
| **Colitis** | 0 | 1 | 1 |
| **Diarrhea** | 3 | 2 | 5 |
| **Other** | 2 | 3 | 5 |
| **Genitourinary** | 8 (6.3%) | 17 (10.4%) | 25 (8.6%) |
| **Renal insufficiency** | 6 | 13 | 19 |
| **UTI** | 2 | 3 | 5 |
| **Other** | 0 | 1 | 1 |
| **Respiratory** | 4 (3.1%) | 9 (5.5%) | 13 (4.5%) |
| **Cold/flu** | 0 | 1 | 1 |
| **Pneumonia** | 4 | 6 | 10 |
| **Shortness of breath** | 0 | 1 | 1 |
| **Other** | 0 | 1 | 1 |
| **Bleeding complications** | 9 (7.0%) | 6 (3.7%) | 15 (5.1%) |
| **Bleeding – no transfusion required** | 0 | 2 | 2 |
| **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 |

Table 41 summaries the number of recipients reporting adverse events with the exclusion of the three device/device user error as these livers were not transplanted (discarded or excluded).
55.4% (95% C.I. 46.1% to 64.4%) of NMP recipients compared to 57.4% (95% C.I. 47.2% to 67.2%) of SCS recipients reported one or more adverse events.

*Table 40: Patients with adverse events reported by treatment group*

| Patients with | NMP (N=121) | SCS (N=101) | Total |
|---|---|---|---|
| **No events reported** | 54 (44.6%) | 43 (42.6%) | 97 (43.7%) |
| **Adverse events** | 67 (55.4%) | 58 (57.4%) | 125 (56.3%) |

## 5. ADDITIONAL ANALYSES NOT SPECIFIED IN THE PROTOCOL OR THE SAP

A sub-population analysis of the NMP group comparing DBD and DCD livers was requested by the investigators after the 6 months data lock and analysis. This is reported separately and only considered informative as the numbers are too small to make any strong conclusions.

## 6. EXECUTIVE SUMMARY

*Table 41: Primary and secondary outcomes*

| | NMP | SCS | Effect (95% C.I)* | p-value |
|---|---|---|---|---|
| **Peak AST** | | | | |
| adjusted | 488.142 (408.856, 582.804) | 964.934 (794.471, 1171, 972) | 0.506 (0.388, 0.659) [49.4% (34.1%, 61.2%)] | <0.001 |
| unadjusted | 484.477 (406.361, 577.610) | 973.700 (795.194, 1192.277) | 0.498 (0.382, 0.649) [50.2% (35.1%, 61.8%)] | <0.001 |
| Donor Type subgroup analysis | | | | |
| DBD | 526.157 (427.302, 647.882) | 880.209 (708.491, 1093.547) | 0.598 (0.443, 0.807) [40.2% (19.3%, 55.7%] | 0.001 |
| DCD | 389.746 (277.992, 546.426) | 1458.081 (944.666, 2250.532) | 0.267 (0.154, 0.463) [73.3% (53.7%, 84.6%)] | <0.001 |
| PP analysis | 498.646 (414.842, 599.380) | 982.944 (810.413, 1192.206) | 0.507 (0.388, 0.663) [49.3% (33.7%, 61.2%)] | <0.001 |
| **PNF^** | 1 (0.8 %) | 0 (0.0%) | - | - |
| **Patient survival (6 months)** | 0.958 (0.902, 0.982) | 0.970 (0.909, 0.990) | | 0.671 |
| **Graft survival (6 months)** | 0.950 (0.893, 0.977) | 0.960 (0.897, 0.985) | | 0.707 |
| **Biochemical liver tests‡** | | | | |
| Bilirubin | | | | |
| Day 1-7 | 2.25 (1.23, 4.28) | 2.87 (1.52, 5.00) | | 0.029 |
| 30 days | 0.76 (0.47, 1.29) | 0.76 (0.53, 1.23) | | 0.479 |
| 6 months | 0.53 (0.35, 0.88) | 0.53 (0.35, 0.76) | | 0.671 |
| AST | | | | |
| Day 1-7 | 167.5 (98, 320.7) | 318.5 (152, 611.5) | | <0.001 |
| 30 days | 20 (14, 35) | 22 (15, 40) | | 0.707 |
| 6 months | 23 (18, 33) | 23 (18, 37) | | 0.931 |
| GammaGT | | | | 0.157 |
| Day 1-7 | 268.1 (156.3, 408.3) | 301 (201.1, 443.9) | | 0.157 |
| 30 days | 178 (109.5, 410) | 200 (96, 397.5) | | 0.949 |
| 6 months | 47 (28, 144) | 47 (26, 128) | | 0.452 |
| INR | | | | |
| Day 1-7 | 1.24 (1.15, 1.38) | 1.24 (1.16, 1.39) | | 0.644 |
| 30 days | 1.1 (1, 1.2) | 1.1 (1, 1.2) | | 0.735 |
| 6 months | 1.1 (1, 1.2) | 1.1 (1, 1.14) | | 0.167 |
| Creatinine | | | | |
| Day 1-7 | 1.05 (0.68, 1.37) | 1.1 (0.76, 1.62) | | 0.139 |
| 30 days | 0.93 (0.75, 1.18) | 1.02 (0.82, 1.37) | | 0.019 |
| 6 months | 1.13 (0.92, 1.33) | 1.13 (0.94, 1.52) | | 0.265 |
| Lactate | | | | |
| Day 1-7 | 1.3 (1, 1.7) | 1.1 (0.9, 1.6) | | 0.130 |
| **EAD** | 12 (10.1%) | 29 (29.9%) | 0.263 (0.126, 0.550) | <0.001 |
| **Post reperfusion syndrome** | 15 (12.4%) | 32 (33.0%) | -20.6% (-31.6%, -9.6%) | <0.001 |
| **Post reperfusion lactate‡** | 3.6 (2.6, 4.2) | 4.1 (3.2, 5) | | 0.018 |
| **Need for RRT** | | | | |
| Day 1-7 post-transplant | 26 (21.5%) | 19 (18.8%) | 2.7% (-7.9%, 13.2%) | 0.621 |
| Day 30 | 27 (22.3%) | 20 (19.8%) | 2.5 (-8.2%, 13.3%) | 0.648 |
| Month 6 | 27 (22.3%) | 21 (20.8%) | 1.5% (-9.3%, 12.4%) | 0.784 |
| **Duration of RRT Day 1-7‡** | 4 (2, 6) | 5 (4, 6) | | 0.346 |

| | | | |
|---|---|---|---|
| **Length of hospital stay**‡ | 15 (10, 24) | 15 (11, 24) | 0.926 |
| **Length of HDU/ITU stay**‡ | 4 (2, 7) | 4 (3, 7) | 0.339 |

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

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

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

This is the first large randomised clinical trial to compare normothermic machine preservation technology with static cold storage in human liver transplantation.

The study showed a substantial reduction in peak AST and EAD rates in NMP livers compared to SCS livers. The effect of NMP in reducing peak AST was shown to be of greater magnitude in DCD organs. However, this may reflect a selection bias – of both donors (there is a lower threshold to decline DCD donors) and recipients (many units select fitter patients for higher-risk organs).

However, as post-transplant AST is not routinely measured in some of the trial centres, some of the values for Day 1-7 were missing, despite every effort and reminders being made by the investigators. It was decided not to impute these missing values for several reasons:

- The primary outcome was the peak of these values so the highest of those non-missing was taken, as long as at least two values were available;
- It was expected that in the vast majority of cases the peak would occur in the first 36 hours and there were more missing values in the "later" post-transplant days than in the early days;
- Some of the missing values at "later" days is due to the patient being discharged before Day 7, indication of the well-being of the recipient and good transplant outcomes.

It may be argued that because of these missing values our results may be biased. However, we did check the proportion of patients with all (or most of the) seven values available and more than 90% of them had their peak in the first 2 days.

The per-protocol analysis confirmed the significant result of the reduction in peak AST found in the ITT analysis.

These results are in spite of much longer preservation times and a lower rate of organ discard in the NMP arm, suggesting that NMP may be achieving the desired objective of increasing organ utilisation without compromising the outcome. The longer preservation times in the NMP group were not planned: indeed, every effort was made to ensure parity between the groups. As clinicians gained experience and confidence in the technology, it was clear that some transplant units started to organise their operating start time according to the preservation method: for NMP livers the transplant more often started during daylight hours or was delayed to enable other urgent cases to be performed first. If, as appears to be the case, NMP can safely extend preservation times without compromising recipient outcomes this will have implications for organ utilisation as well as improving operating department planning.

There were 50% fewer discarded organs in the NMP group. The SCS discard rate of 23.7% was higher than the 17% reported in UK registry data, and may reflect the high proportion of included DCD livers which are associated with discard rates in excess of 30% in many European countries. It may also be the case that some clinicians were accepting donor livers into the trial that they would not normally consider for transplant, in the hope that they were randomised to NMP, then discarding the organs randomised to SCS. Such bias is an inherent risk in an open label trial.

Despite the long median preservation time in the NMP group the haemodynamic characteristics of the recipients following reperfusion was measurably superior. This did not translate into a difference in patient

and graft survivals, ITU stay, hospital stay or need for renal replacement therapy between the two groups, despite previous reports showing a correlation between peak-AST and RRT. However, the study was not powered to detect a difference for these outcomes.

This trial has also shown that the logistical challenges associated with NMP can be met successfully within clinical practice. Over 120 NMP livers were transplanted in seven transplant centres across four European countries, each with different logistical arrangements. Nonetheless, widespread adoption of this technology into clinical practice may involve changes in the infrastructure of 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 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. A Phase 2 study to test this has recently started in the UK.

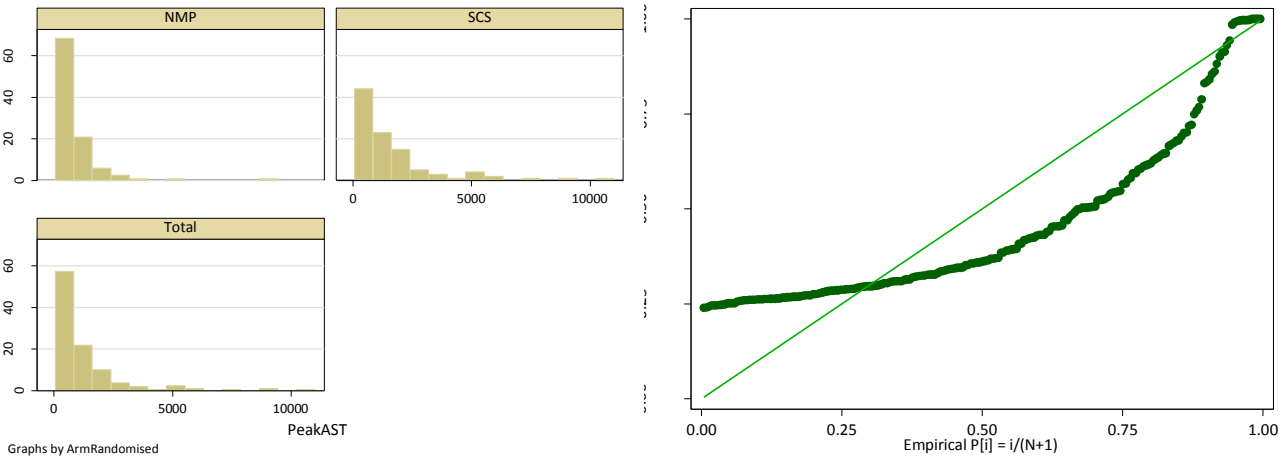
Whilst the effects of NMP demonstrated in this study are unequivocal, this study was not designed to answer every key question. The issue of organ utilisation is of critical importance: the greatest benefit of NMP may be realised by applying this technology to livers outside current acceptance criteria, 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 realised. High-risk organs (e.g. those with high levels of steatosis) may benefit from therapeutic interventions deliverable during NMP: several groups are exploring potential interventions, including stem cell treatments, de-fatting agents and immunological modification of the organ.

This is the first formal evaluation of an exciting new technology in liver transplantation, and perhaps the start of a new era of intervention in organ transplantation. It represents the first necessary step in bringing NMP to clinical practice; the fact that the study has so definitively met its primary endpoint should now open the door to explore the wider potential of this technology.

## 7. APPENDIX

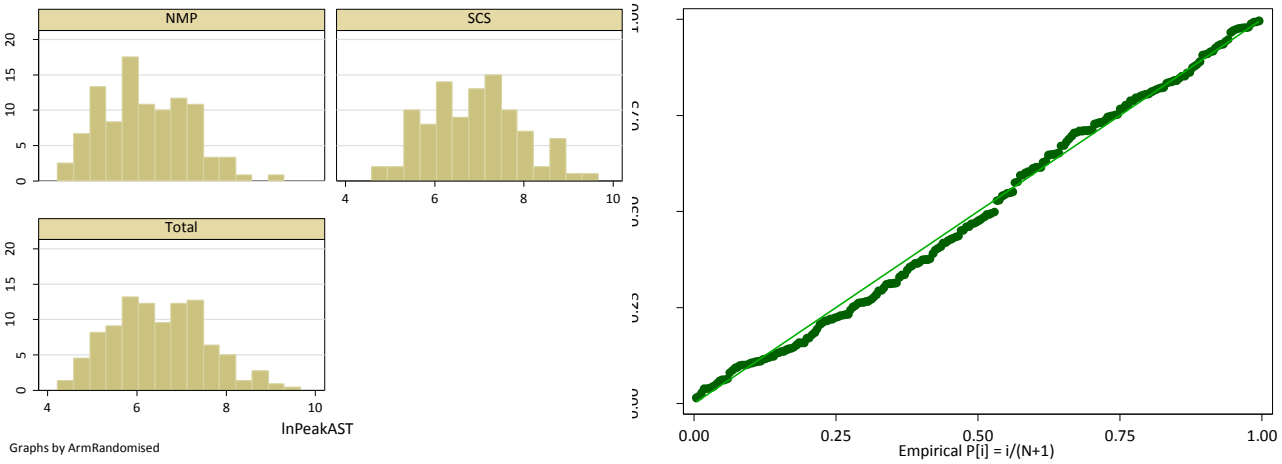
1. Normality distribution graphs and test for Peak AST
2. Statistical Analysis plan

*Normality distribution check of Peak AST and (ln)Peak AST*




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

The p-values of the test above indicates there is enough evidence to reject the null hypothesis the Peak AST follows a Normal distribution.




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

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

Consortium for Organ Preservation in Europe

# WP2

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

## Statistical Analysis Plan
## 3.0 – 25Oct2016

Based on protocol version 3.0 (11st February 2016)

| | Name | Title/Role | Signature | Date |
|---|---|---|---|---|
| Author | Virginia Chiocchia | Trial Statistician | Virginia Chiocchia | 25Oct2016 |
| Reviewer | David Nasralla | WP2 Central Investigator | | 26th Oct 2016 |
| Approver | Peter Friend | Chief Investigator | P Friend | 29/10/16 |
| Approver | Susan Dutton | OCTRU Lead Statistician | | |

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

SITU SURGICAL INTERVENTION TRIALS UNIT | CSM | OCTRU Oxford Clinical Trials Research Unit | University of Oxford

Consortium for Organ Preservation in Europe

# WP2

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| Approver | Susan Dutton | OCTRU Lead Statistician | Susan Dutton | 31Oct2016 |


**Surgical Intervention Trials Unit (SITU)**
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SITU SURGICAL INTERVENTION TRIALS UNIT

CSM

OCTRU Oxford Clinical Trials Research Unit

UNIVERSITY OF OXFORD

# Contents

## GLOSSARY OF ABBREVIATIONS

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

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

# 1 INTRODUCTION

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

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

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

## 1.1 Key personnel

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**DMC Members:**

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

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

Expertise: Clinical trials and Medical statistics

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

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

**WP8 Members:**
**CET:**
Peter Morris – Chair, Director
Simon Knight – Academic Clinical Lecturer
Liset Pengel – Epidemiologist
John O'Callaghan – Research Assistant
**Clinical Trial Unit:**
Susan Dutton – OCTRU Lead Statistician
Allyson Bradley – COPE Central Trials Manager
Virginia Chiocchia – Trial Statistician
Rajeev Kumar – Database Manager

## 2 CHANGES FROM PREVIOUS VERSION OF SAP

| Issue Date | Details |
|---|---|
| 04Feb2015 | First version of SAP based on Protocol version 2.0 14th October 2014. |
| 22Mar2016 | Second version of SAP based on Protocol version 3.0 11th February 2016 including the following changes:<br>– Patrizia Burra removed as DMC member (Section 1.1)<br>– Primary analysis timepoint updated from 6 months to 30 days following new protocol version (Section 3.2)<br>– Mention of Data Management Plan in Section 4 removed as none present for the study<br>– MELD and DRI formulas updated; eGFR equation added (Section 6.2)<br>– Baseline characteristics table updated (Section 6.2)<br>– Patient groups for analysis (Section 7) updated with specific protocol deviations added<br>– Subgroup analysis (section 8.5) updated<br>– Secondary outcomes analysis (Section 9 and subsections) updated and clarified<br>– Exploratory analyses (Section 10.1) added |
| 25Oct2016 | Third version including following changes:<br>– New hepatologist added in DMC memberships (Section 1.1)<br>– Flow diagram refined (Section 6.1)<br>– Baseline characteristics table and section updated with further characteristics and p-value reporting for recipient characteristics (Section 6.2); UK-DRI formula updated and reference added<br>– Summary of intervention details (Section 6.5) updated<br>– Secondary analysis and subgroup analysis of primary outcome updated (Section 8)<br>– Secondary outcomes analysis (Section 9 and subsections) updated and clarified<br>– Exploratory analyses (Section 10.1) updated |

# 3 BACKGROUND INFORMATION

The experimental precursor to the proposed normothermic perfusion system was developed 15 years ago and the method of perfusion of the isolated pig liver with autologous blood has since been extensively tested and refined, although the overall design of the perfusion circuit remains unchanged [1]. The circuit incorporates a centrifugal pump, membrane oxygenator, and heat exchanger. Arterial perfusion is directly pumped and the portal vein is perfused via a soft-shell reservoir using gravitational force. The addition of various substrates to the perfusion solution enables maintenance of metabolic function [2].

The initial preservation experiments were carried out to compare preservation by warm perfusion with conventional cold preservation [3]. Porcine livers were retrieved and stored for a period of 24 hours, either flushed with UW solution and placed in an icebox or attached immediately to the preservation circuit. Both groups of livers were then reperfused on the circuit for 24 hours (as a surrogate for transplantation) and markers of cellular injury and of synthetic and metabolic liver function were measured. These experiments demonstrated significant superiority of normothermic machine perfusion in terms of haemodynamic, biochemical and histological parameters

Subsequent experiments investigated the use of oxygenated, normothermic perfusion in an experimental setting that reflected the clinical situation of DCD donor organ retrieval [4]. Perfusion with normothermic blood was again compared with static cold storage after 60 min of warm ischaemia. Normothermic perfused livers demonstrated recovery of function by synthetic function, substrate utilisation and perfusion hemodynamics. Furthermore these livers displayed less cellular injury as shown by hepatocellular enzymes. In contrast, cold stored livers showed no evidence of viability during reperfusion and massive necrosis on histological examination.

It is recognised that the combination of warm ischaemia and conventional cold preservation leads to a poor outcome in DCD liver transplantation [5]. In the experimental setting, it is possible to institute warm perfusion with minimal exposure of the organ to cooling. However, in contrast, the logistics of clinical multi-organ retrieval in a distant donor hospital are complex and would be simplified by a period of cold preservation prior to normothermic preservation. This would enable the liver to be retrieved in the normal way, transported in an ice box and then attached to the perfusion machine once back at the base hospital. This scenario was simulated in the same experimental model by inserting a period of cold preservation prior to normothermic preservation [6]. Porcine livers were subjected to 60 minutes of warm ischaemia and then assigned to either normothermic preservation for 24 hours or cold preservation in University of Wisconsin solution for 4 hours followed by 20 hours normothermic preservation to achieve a total preservation time of 24 hours [6]. Livers that underwent normothermic preservation throughout had superior bile production, metabolic activity (base deficit and greater glucose use), and less hepatocellular damage (transaminase levels), and sinusoidal endothelial cell dysfunction (hyaluronic acid). The histology of livers that had been exposed to 4 hours of cold preservation before normothermia showed more necrosis and destruction of architecture. A similar study investigated 60 minutes of warm ischemia followed by 1 hour of cold preservation before 23 hours of normothermic perfusion [7]. This also showed evidence of increased hepatocellular injury, sinusoidal cell injury, but no detriment in terms of protein synthesis (factor V), bile production or histological features. These studies, therefore, demonstrated the need for the warm preservation device to be transportable so that normothermic preservation can be instituted with a minimal period of cooling at the time of organ retrieval.

In order to confirm these results in a preclinical model of organ transplantation, a series of liver transplants in a pig model was performed [8]. In these experiments pig livers were cold-preserved or warm-preserved (using the same machine perfusion methodology as before) for either 5 hours or 20 hours, followed by liver transplantation. As a model of DBD and DCD clinical scenarios, organs were cold-perfused *in situ* either at the time of cessation of circulation (as in a DBD organ donation) or after 40 and 60 minutes of warm ischaemia (simulating DCD organ donation). The two preservation

times were selected because 5 hours is comfortably within, and 20 hours substantially beyond, the limit of the conventional cold preservation technology in pigs (in which a generally accepted limit for survival is 12 hours). Similarly the 40 and 60 minute periods of warm ischaemia are considerably longer than would be acceptable in current clinical practice where warm ischaemia rarely exceeds 30 minutes. Indeed, success at 40 minutes would raise the realistic prospect of transplantation of donor livers from uncontrolled DCD donors.

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

Analysis of haemodynamic, synthetic and metabolic parameters showed that those groups of livers that subsequently went on to successful transplant were predictable before transplantation on the basis of portal flow/pressure, acid-base homeostasis and several other biochemical parameters [8]. It may be concluded that normothermic perfusion, in this context, is not only a more effective means of organ preservation than conventional cold storage, but also that this method can be configured to provide an effective means of viability assessment [9].

The prototype version of the automated clinical investigation device has been tested and demonstrated to be effective during pre-clinical studies in which human livers, discarded as unsuitable for transplantation, were perfused for 24 hours. 13 such livers were perfused with human blood and the perfusion characteristics and control algorithms have been shown to be equally applicable to human as to pig livers (manuscript in preparation). More recently, the clinical trials device has been tested, using livers declined for clinical transplantation, and all key functional aspects of the device shown to be operational, including particularly transport to the donor hospital, automation and 24 hour perfusion.

Phase 1 clinical trial data

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

## 3.1 Objectives

Hypothesis

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

Primary objective

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

Secondary objective

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

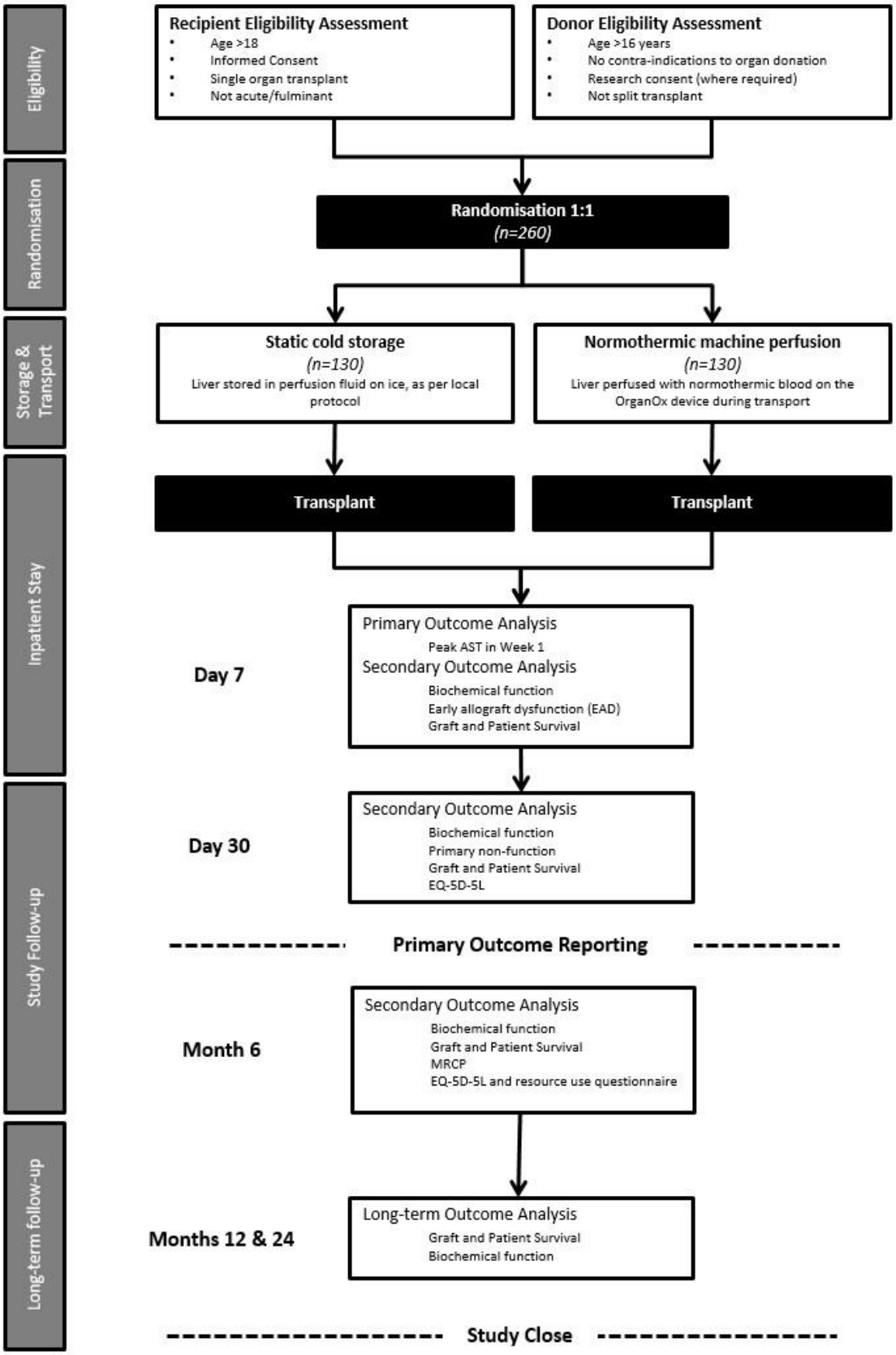
- To compare evidence of post reperfusion syndrome between NMP and SCS livers on implantation
- To compare evidence of ischaemia reperfusion injury between NMP and SCS livers
- To compare evidence of ischaemic cholangiopathy between NMP and SCS livers
- To assess the ability of perfusion parameters and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

## 3.2 Study Design

This is a randomised controlled, non-blinded, clinical trial comparing static cold storage (SCS) to normothermic machine perfusion (NMP) for organ preservation prior to liver transplantation. Following assessment of donor and recipient eligibility and confirmation of consent, the liver will be randomised to either NMP or SCS. At the end of preservation, the liver will be transplanted and the patient managed according to standard local practice and protocols.
Enrolled patients will participate in the study for 6 months, with outcomes assessed during the initial inpatient stay and at study visits at day 30 post-transplant and at month 6 post-transplant. Additional biochemical and survival data will be collected from routine clinical measurements taken in participating centres at 12 months and 24 months post-transplant.
Primary outcomes will be analysed and reported 30 days following enrolment of the last patient to the study. The study will close after the final patient has completed 24 months follow-up.
Anticipated flow of patients through the trial is depicted in Figure 1.

<u>Anticipated trial dates</u>

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




*Randomisation performed soon after organ retrieval by recipient centre

**Figure 1.** Flow of participants/livers through the trial

## 3.3 Eligibility

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

### 3.3.1 Donor criteria

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

### 3.3.2 Recipient criteria

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

### 3.3.3 Donor assessment

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

### 3.3.4 Recipient assessment

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

## 3.4 Treatment Interventions

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

### 3.4.1 NMP Group

If the liver is randomised to the NMP group, arrangements will be made to transport the OrganOx *metra* device to the donor hospital (see section 7.6). The recipient co-ordinator will also request that

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

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

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

#### 3.4.2 SCS group

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

### 3.5 Sample Size

Data from 416 liver transplant recipients from University Hospital Essen demonstrate the geometric mean of peak AST to be 608.59 IU/L (the geometric mean is used as peak AST is non-normally distributed). 220 transplants (110 per arm) would have 90% power at 5% significance level to detect a 33% reduction (to 401.67 IU/L) in the geometric mean of peak AST.
2011/12 NHSBT data suggest that 12% of livers retrieved are not transplanted. Assuming losses of 15%, randomisation of 260 livers into the trial will be required to achieve adequate power.
Data from a study of hypothermic machine perfusion of human livers demonstrate an approximate 35% reduction in the peak AST with machine preservation when compared to historical controls undergoing static cold storage [10]. It is expected that normothermic machine perfusion will be at least as effective as hypothermic machine preservation in preventing reperfusion injury. In studies of a porcine model of DBD liver transplantation, normothermic machine perfusion led to a 52% reduction in peak post-transplant AST [8]. In a DCD model, the reduction was 73%.
Given the relationship between peak AST and primary non-function, graft and patient survival described above [11, 12], a 33% reduction in peak AST is likely to represent a clinically significant difference in outcome between the study arms.
It is recognized that a proportion of DCD donor livers randomized in the study will not proceed to donation due to the donor not arresting within the time defined by local protocols. These livers will be replaced in the study so that the numbers above reflect the number of livers actually retrieved.

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

```
> exp(mean(log(peakast)))
```

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

```
[1] 608.5898
> exp(mean(log(peakast)))*0.66
[1] 401.6693
> control <- exp(mean(log(peakast)))
> study <- exp(mean(log(peakast)))*0.66
> log(control)-log(study)
[1] 0.4155154
> sd(log(peakast))
[1] 0.9456289
> (log(control)-log(study))/sd(log(peakast))
[1] 0.4394065
> pwr.t.test(d=0.4394, sig.level=0.05, power=0.90, type="two.sample", alternative="two.sided")
Two-sample t test power calculation
n = 109.8137
d = 0.4394
sig.level = 0.05
power = 0.9
alternative = two.sided
```

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

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

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

### 3.6 Strategies for achieving adequate recruitment

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

### 3.7 Randomisation

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

3.7.1 Sequence generation

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

3.7.2 Allocation concealment mechanism

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

3.7.3 Implementation

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

3.7.4 Blinding/Masking

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

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

## 3.8 Primary and Secondary Outcomes

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

A number of studies have demonstrated a relationship between peak AST in the early post-transplant period and patient survival, graft survival, early graft dysfunction and primary non-function following liver transplantation [11, 12]. Peak AST is also significantly elevated in liver allografts with histological evidence of moderate to severe perfusion injury [13, 14].

Secondary outcomes

| Objective | Outcome Measures |
|---|---|
| To compare graft and patient survival between NMP and SCS livers. | 1. Primary non-function: irreversible graft dysfunction requiring emergency liver replacement during the first 10 days after liver transplantation, in the absence of technical or immunological causes.<br>2. Graft survival at 30 days and 6, 12 and 24 months following transplantation.<br>3. Patient survival at 30 days and 6, 12 and 24 months following transplantation. |
| To compare biochemical liver function between NMP and SCS livers. | 1. Serum bilirubin, GGT, AST, INR and creatinine daily at days 1-7 following transplantation then at day 30 and months 6, 12 and 24 following transplantation.<br>2. Daily serum lactate at days 1-7 whilst in high level (ITU/HDU) care<br>3. Early allograft dysfunction (EAD) [15]; defined by any one of:<br>a. Bilirubin >170 µmol/l (10mg/dL) on day 7 post-transplant<br>b. INR >1.6 on day 7 post-transplant.<br>c. Peak aspartate transaminase (AST) >2000 IU/L within the first 7 days post-transplant |
| To compare the physiological response to reperfusion between NMP and SCS livers | 1. Post-reperfusion syndrome, defined as a decrease in mean arterial pressure (MAP) of more than 30% from the baseline value for more than one minute during the first five minutes after reperfusion. This will be assessed in the context of vasopressor use [16, 17]<br>2. Length of stay in high level (HDU/ITU) care<br>3. Length of hospital stay<br>4. Need for renal replacement therapy (haemodialysis, haemofiltration, haemodiafiltration) |
| To compare evidence of reperfusion injury between NMP and SCS livers. | Histological evidence of reperfusion injury in post-reperfusion biopsies (taken immediately prior to abdominal closure). These will be compared to baseline pre-reperfusion biopsies (on removal of the liver from SCS/NMP) and graded using standard histological criteria [13, 18] |
| To compare evidence of ischaemic cholangiopathy between NMP and SCS livers. | Evidence of biliary stricturing on magnetic resonance cholangiography (MRCP) at 6 months post-transplant. |
| To assess the ability of perfusion parameters and biomarkers in perfusion fluids to predict clinical outcomes following transplantation. | 1. Perfusion parameters (logged automatically by the device):<br>a. Arterial and caval pressures (in mmHg)<br>b. Arterial, portal and caval flow rates (in mmHg)<br>c. $pO_2$, $pCO_2$ and pH<br>d. Blood temperature (°C), Glucose (mmol/L) and bile production (ml/h)<br>2. Perfusate ALT and AST at 15 minutes, 1 hour and the end of NMP<br>3. Perfusate IL6, TNF, vWF at 15 minutes, 1 hour and the end of NMP<br>4. In addition to these pre-specified outcomes, additional biological samples will be taken for the COPE WP7 |

| | |
|---|---|
| | bioresource at specified timepoints as detailed in protocol appendix A1. |
| To assess the feasibility and safety of NMP as a method of organ storage and transportation. | 1. Organ discard rate<br>2. Perfusate culture. At the end of preservation a sample will be taken for microbiological culture (cold preservation or warm perfusate).<br>3. Adverse event rates and severity, graded according to the Clavien-Dindo classification [19] as described in Appendix 1: CLAVIEN DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS.<br>a. Recipient infection<br>b. Biopsy proven acute rejection<br>c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks)<br>d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis)<br>e. Reoperation rate<br>f. Technical complications/device failures |
| To assess the health economic implications of normothermic liver perfusion. | Full health economic analysis utilising:<br>1. Logistical costs, measured using national unit costs where available.<br>2. Healthcare resource use; measured by a combination of hospital episode records and a patient-completed resource use log.<br>3. Quality of life by delivery of the EQ-5D-5L questionnaire at baseline, day 30 and month 6 post-transplant. |

### 3.9 Outcomes Assessment Schedule

| **Activity** | **Pre-study Screening** | **Pre-study Baseline** | **Pre-storage** | **Pre-reperfusion** | **Post-reperfusion** | **Postoperative** | | | | | | | | | **Follow-up** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** | **D7** | **D10** | **D30** | **M6** | **M12** | **M24** |
| Informed consent | X | | | | | | | | | | | | | | | | |
| Meets inclusion/ exclusion criteria | X | | | | | | | | | | | | | | | | |
| Randomisation | | X | | | | | | | | | | | | | | | |
| Donor & recipient demographics | | X | | | | | | | | | | | | | | | |
| Perfusion parameters/samples | | | | X | | | | | | | | | | | | | |
| Surgical variables | | | | | X | | | | | | | | | | | | |
| Graft biopsy | | | X | X | X | | | | | | | | | | | | |
| CBD biopsy | | | | | X | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum AST | | | | | | X | X | X | X | X | X | X | | X | X | X | X |
| Serum Bilirubin | | | | | | X | X | X | X | X | X | X | | X | X | X | X |
| Serum GGT | | | | | | X | X | X | X | X | X | X | | X | X | X | X |
| Serum Creatinine | | | | | | X | X | X | X | X | X | X | | X | X | X | X |
| INR | | | | | | X | X | X | X | X | X | X | | X | X | X | X |
| Serum lactate* | | | | | | X | X | X | X | X | X | X | | | | | |
| Primary non-function | | | | | | | | | | | | | X | | | | |
| Graft survival | | | | | | X | X | X | X | X | X | X | X | X | X | X | X |
| Patient survival | | | | | | X | X | X | X | X | X | X | X | X | X | X | X |
| MRCP | | | | | | | | | | | | | | | X | | |
| Quality of life (EQ-5D-5L) | X | | | | | | | | | | | | | X | X | | |
| Resource use log | | | | | | | | | | | | | | X | X | | |
| Safety outcomes | | | | | X | X | X | X | X | X | X | X | X | X | X | X | |

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

### 3.10 Data Management Responsibility

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

## 4 QUALITY CONTROL AND DATA VALIDATION

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

## 5 DATA MONITORING COMMITTEE AND INTERIM ANALYSES

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

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

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

# 6 DESCRIPTIVE ANALYSES

## 6.1 Representativeness of Study Sample and Patient Throughput

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

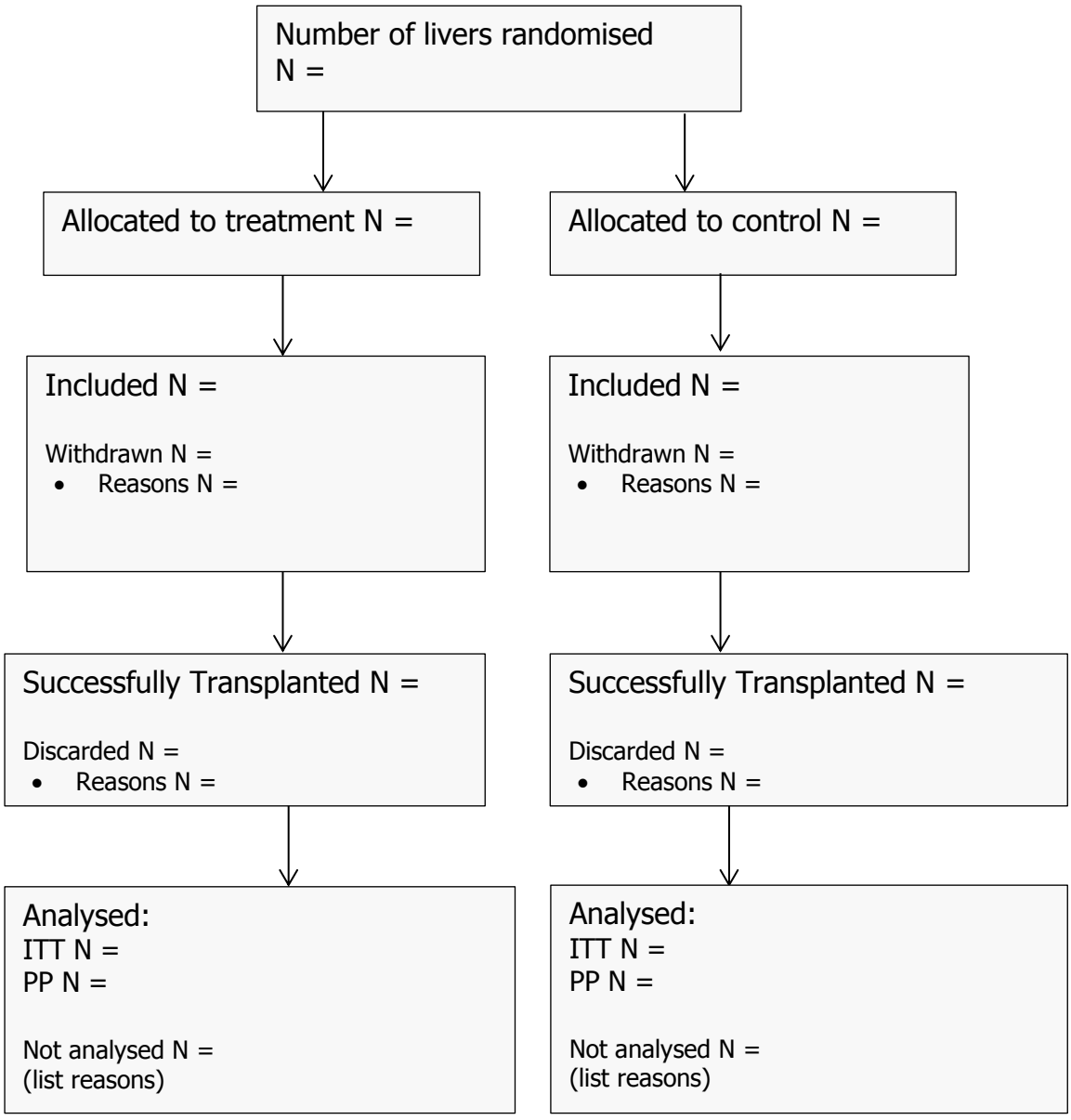



**Figure 2.** Adapted flowchart for WP2

## 6.2 Baseline Comparability of Randomised Groups

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

MELD Score will be described overall and separately by country due to variations in national donor rates causing likely differences in average MELD scores in different countries.
The formula to calculate MELD Score from serum creatinine, bilirubin and INR, developed and validated by Kamath and Kim [20] is the following:

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

The resulting score will be multiplied by 10 and rounded to the nearest whole number.
UNOS has made the following modifications to the score [21]:

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

DRI will be described overall and calculated using both the UK-DRI and the Eurotransplant DRI formulas. The UK-DRI formula has recently been developed and the relevant paper will soon be published in *Transplantation* (Collett D, Friend PJ and Watson CJE. *Factors associated with short and long term liver graft survival in the United Kingdom: development of a UK Donor Liver Index*.)

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

The ET-DRI formula has been adapted for the purposes of this study from the one developed by Braat et al [22].

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

The calculation of eGFR is based on the CKD-EPI Creatinine equation [23] as follows:

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

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

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

Numbers (with percentages) for binary and categorical variables and means (with standard deviations) or medians (with lower and upper quartiles) for continuous variables will be presented.
There will be no tests of statistical significance nor confidence intervals for differences between randomised groups for stratification factors and donor characteristics. However, due to the nature of the study (the randomisation unit is the donated liver and not the participant) and to a potential differential discard rate between the two groups, an appropriate statistical test will be applied for recipient characteristics and a p-value reported to demonstrate that the final recipients were well matched.

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

Royal Free Hospital, London, UK
University of Barcelona, Spain
University Hospital, Essen, Germany
University Hospitals, Leuven, Belgium

**Gender***

Male
Female

**Age^**

**Cause of Liver Failure***

Alcoholic
Auto-Immune Hepatitis
Drug Induced
Hepatitis B
Hepatitis C
Hepatocellular Carcinoma on background of Cirrhosis
Hepatocellular Carcinoma without Cirrhosis
Metabolic
Non-Alcoholic Fatty Liver Disease
Non-Alcoholic Steato-Hepatitis
Other Cancers
Primary Sclerosis Cholangitis
Primary Biliary Cirrhosis
Other

**BMI^**

**MELD score^**

UK
Essen, Germany
Leuven, Belgium
Barcelona, Spain

**eGFR^**

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

## 6.3 Comparison of Losses to Follow-up

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

## 6.4 Description of Available Data

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

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

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

## 6.5 Description of Intervention

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

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

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

## 6.6 Unblinding of Randomised Treatments

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

## 6.7 Reliability

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

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

## 7 PATIENT GROUPS FOR ANALYSIS

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

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

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

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

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

## 8 ANALYSES TO ADDRESS PRIMARY AIMS

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

### 8.1 Definition of Primary Outcome

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

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

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

### 8.2 Statistical Methods Used for Analysis of Primary Outcome

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

8.2.1 Secondary Analysis of Primary Outcome

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

## 8.3 Adjustment of P values for Multiple Testing

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

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

## 8.4 Missing Data

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

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

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

## 8.5 Pre-specified Subgroup Analysis

Subgroup analyses will be performed for donor type (DCD vs. DBD), donor risk index (ET-DRI) and MELD Score. Value intervals for ET-DRI will be 'low', 'medium' and 'high' based on the 33$^{rd}$ percentiles. Value intervals for MELD Score will be <9, 10-19, 20-29, 30-39, ≥40 based on the UNOS standard reference ranges[24].
The study is not powered to detect differences in the subgroups and should only be regarded as hypothesis-generating. Interaction methods will be used to look for consistency of treatment effect across the different subgroups and reported using forest plots.

### 8.6 Treatment by Centre Interaction

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

### 8.7 Sensitivity Analysis

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

## 9 ANALYSIS TO ADDRESS SECONDARY AIMS

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

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

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

### 9.1 Primary Non-Function

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

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

### 9.2 Graft and patient survival

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

graft at the end of the study will be censored at their last known date alive with functioning graft.

Patient overall survival is defined as time (in days) from transplant to patient death. Patients still alive will be censored at their last known alive date.[25]

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

## 9.3 Biochemical liver function

Liver function will be explored by assessing biochemical components of the blood serum. These are a number of serum biochemical tests: Biliburin, Gamma glutamyl transpeptidase (GGT), Aspartate transaminase (AST), International normalised ratio (INR), creatinine and lactate dehydrogenase.
All of these tests are interpreted using reference ranges.
The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the International Sensitivity Index (ISI) value for the analytical system used.[26]

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

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

### 9.3.1 Early Allograft Dysfunction

Early allograft dysfunction (EAD) has been defined as the presence of one or more of the following previously defined postoperative laboratory analyses reflective of liver injury and function [15]:

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

In case of missing data in any of the three variables required to define the EAD, this will be considered as present if any of the available values satisfies the specified criteria. In case of Bilirubin and/or INR missing due to the patient being discharged before Day 7 post-transplant, this will be considered as absence of EAD as the recipient would not be discharged earlier if the graft was not functioning. Subjects with missing data due to patient death or graft failure will be excluded from this analysis as expected to be few in numbers in the first 7 days post-transplant.
Presence of EAD will be compared across the two groups using the methods described above to adjust for potential confounders such as DRI and MELD Score.

## 9.4 Physiological response to reperfusion

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

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

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

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

## 9.5 Reperfusion injury

Graft biopsies will be taken immediately prior to abdominal closure and examined for evidence of reperfusion injury. These biopsies will be compared to the baseline biopsies prior to organ reperfusion (on removal of the liver from SCS/NMP) and graded according to standard histological criteria [13, 18]. The trial histopathologist, who will be blinded to the method of perfusion, will assess all biopsies.

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

## 9.6 Ischaemic cholangiopathy

All study participants will undergo magnetic resonance cholangiopancreatography (MRCP) with T2-weighted turbo-spin echo sequences at 6 months post-transplant unless contraindicated. The trial radiologist, who will be blinded to the preservation method, will assess all MRCPs. Evidence of ischaemic cholangiopathy will be taken as the presence of extra-anastomotic biliary structuring in the absence of hepatic artery thrombosis [27, 28].

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

## 9.7 Perfusion parameters and biomarkers

Perfusion parameters will be collected and used to give a summary of the intervention, as described in section 6.5.
Biomarkers will also be collected to be used with perfusion parameters as described in the Exploratory Analyses section (10.1).

### 9.8 Feasibility and safety of NMP

Organ discard rate and adverse events rates and severity will be assessed as described in the Safety Analysis section (11).

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

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

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

## 10 ADDITIONAL ANALYSES

### 10.1 Exploratory analyses

**Pre-specified exploratory analyses**

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

A model for the assessment of early allograft function was recently developed [29] and also showed to be associated with patient and graft survival. MEAF stands for Model of Early Allograft Function and is calculated by applying the formula below using the peak ALT and INR value from the first 3 post-operative days and the bilirubin value from the third postoperative day. The final MEAF score is the sum of the 3 values obtained rounded to the nearest integer.

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

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

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

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

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

Another recent publication reported that high AST levels on day 3 correlates with patient and graft survival [30]. The relationship of patient and graft survival with the AST on day 3 will be explored using survival analysis techniques as described in Section 9.

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

**Additional Exploratory Analysis Not Specified Prior to Receiving Data**

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

### 10.2 Blinded analysis

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

## 11 SAFETY ANALYSIS

Safety outcomes to be reported will be:

- Organ discard rate (number of livers not suitable for transplantation)
- Adverse event:
  a. Recipient infection (defined as a positive microbiological culture result)
  b. Biopsy-proven acute rejection episodes
  c. Biliary complications (biliary strictures - anastomotic and non-anastomotic, bile duct leaks)
  d. Vascular complications (bleeding, hepatic artery stenosis, hepatic artery thrombosis, portal vein thrombosis, portal vein stenosis)
  e. Reoperation rate
  f. Technical complications/device failures

Rates of adverse events and organ discards will be compared between NMP and SCS groups by examination of 95% confidence intervals for the difference in incidence. Their severity will be graded according to the Clavien-Dindo classification (Appendix 1: CLAVIEN DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS). Discarded organs will also be distinguished between declined (if accepted in a different hospital) and actually discarded.
The number of patients with any serious adverse events will be compared by assessment of the difference in incidence with 95% confidence interval. The number of serious adverse events per patient will be reported.

# 12 APPENDIX 1: CLAVIEN DINDO CLASSIFICATION OF SURGICAL COMPLICATIONS

| Grade | Definition |
|---|---|
| **I** | Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions. |
| **II** | Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included. |
| **III** | Requiring surgical, endoscopic or radiological intervention. |
| **IIIa** | Intervention not under general anaesthesia. |
| **IIIb** | Intervention under general anaesthesia. |
| **IV** | Life-threatening complications (including CNS complications) requiring HDU/ITU management. |
| **IVa** | Single organ dysfunction (including dialysis). |
| **IVb** | Multi-organ dysfunction. |
| **V** | Death of a patient. |
| **Suffix 'd'** | If the patient suffers from a complication at the time of discharge, the suffix 'd' (for disability) is added to the respective grade of complication. This label indicates the need for a follow-up to fully evaluate the complication. |

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