Normothermic Machine Perfusion of Donor Livers for Transplantation in the United States

A Randomized Controlled Trial

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Objective: To compare conventional low-temperature storage of transplant donor livers [static cold storage (SCS)] with storage of the organs at physiological body temperature [normothermic machine perfusion (NMP)].

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Background: The high success rate of liver transplantation is constrained by the shortage of transplantable organs (eg, waiting list mortality >20% in many centers). NMP maintains the liver in a functioning state to improve preservation quality and enable testing of the organ before transplantation. This is of greatest potential value with organs from brain-dead donor organs (DBD) with risk factors (age and comorbidities), and those from donors declared dead by cardiovascular criteria (donation after circulatory death).

Methods: Three hundred eighty-three donor organs were randomized by 15 US liver transplant centers to undergo NMP (n = 192) or SCS (n = 191). Two hundred sixty-six donor livers proceeded to transplantation (NMP: n = 136; SCS: n = 130). The primary endpoint of the study was "early allograft dysfunction" (EAD), a marker of early posttransplant liver injury and function.

Results: The difference in the incidence of EAD did not achieve significance, with 20.6% (NMP) versus 23.7% (SCS). Using exploratory, "as-treated" rather than "intent-to-treat," subgroup analyses, there was a greater effect size in donation after circulatory death donor livers (22.8% NMP vs 44.6% SCS) and in organs in the highest risk quartile by donor risk (19.2% NMP vs 33.3% SCS). The incidence of acute cardiovascular decompensation at organ reperfusion, "postreperfusion syndrome," as a secondary outcome was reduced in the NMP arm (5.9% vs 14.6%).

Conclusions: NMP did not lower EAD, perhaps related to the inclusion of lower-risk liver donors, as higher-risk donor livers seemed to benefit more. The technology is safe in standard organ recovery and seems to have the greatest benefit for marginal donors.

Key Words: liver, normothermic, perfusion, preservation, transplantation

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The success of liver transplantation in recent decades has created an increasing supply problem: the number of patients referred for transplantation has increased more rapidly than the number of suitable grafts from deceased organ donors. The donor organ shortage constitutes a serious risk for patients and leads to the death of patients on the waiting list worldwide. In

the United States, 12,409 patients were added to the national waiting list in 2020, compared with 11,514 in 2017 and 10,636 in 2015.[1] Adult liver transplantation continues to be challenged by declining organ quality and increased mortality rates on the waiting list. Based upon the OPTN/SRTR 2022 Annual Data Report for liver transplants,[1] 3-year outcomes for adults listed for liver transplantation in 2017: 58.0% underwent liver transplantation (including 2.3% with living donor livers), 10.1% died, and 23.4% were removed from the list without undergoing transplantation, leaving 8.6% still waiting.

In an effort to decrease waitlist mortality, surgeons have expanded the acceptance criteria for liver grafts. These include liver grafts from donors declared dead by cardiovascular criteria ["donation after circulatory death" (DCD)] and other "extended criteria" organ donors—those with known risk factors for poorer outcome, including older age, steatosis, higher body mass index, cardiovascular disease, and diabetes.

The safety and utilization rates of higher-risk donor organs would be greatly improved by a reliable test to predict outcomes after transplantation. For example, in the United States in 2020, livers retrieved from DCD donors were four times more likely to be discarded than donor brain death (DBD) donors (26.6% vs 7.1%, respectively, SRTR data). An effective means of pretransplant viability assessment would not only allow greater use of higher-risk donors but also minimize the risk of primary nonfunction by identifying and excluding nonviable organs before subjecting a patient to the risk of surgery.

Organs retrieved for transplantation undergo injury at several consecutive stages: (1) warm ischemia before preservation, (2) cold preservation injury, (3) ischemic rewarming during surgical implantation, and (4) reperfusion injury. These consecutive events lead to a cumulative cellular injury that may not be compatible with life-sustaining function after transplantation.

The standard clinical practice involves flushing and cooling the liver in situ with a preservation solution; the University of Wisconsin solution is used most commonly, although Histidine-Tryptophane-Ketoglutarate solution is also widely used.

As soon as the flow of oxygenated blood ceases, the supply of oxygen, cofactors, and nutrients stops along with the means of disposal of metabolic waste products. Anaerobic metabolism continues (at a temperature-dependent rate), leading to the depletion of energy stores, mainly adenosine triphosphate (ATP), with a concomitant build-up of an acidotic milieu. Prevention of ATP depletion is, therefore, an important target of innovative preservation methods. It has been shown that providing an oxygen supply to the organ can prevent ATP depletion and preserve viability after cardiac arrest in a porcine liver transplant model.[2,3]

There is accumulating evidence of the benefits of an approach using perfused oxygenated blood at normal body temperature [normothermic machine perfusion (NMP)]. The quality of preservation can be improved substantially by warm perfusion, by combining the avoidance of prolonged cold ischemia with the maintenance of a supply of oxygen and nutrition.[4] Preclinical liver transplant experiments in the pig model from a number of centers have shown that the normothermically preserved liver can be transplanted reliably and successfully after warm ischemic injuries that do not allow survival using cold preservation.[2,3] Several clinical studies have since demonstrated evidence that NMP not only improves subsequent graft performance but also allows for graft viability assessment, thus potentially optimizing allograft selection and allocation.[5]

NMP of the liver has been developed by a number of groups worldwide.[6] After a feasibility study carried out in the UK,[7] the first multicenter randomized controlled trial (RCT) was conducted in 7 European liver transplant centers to compare NMP with a standard of care [static cold storage (SCS)] of adult deceased donor liver transplants.[8] The study confirmed that NMP is associated with a significant reduction in graft injury. The primary endpoint was defined as the difference between the two treatment arms in the peak level of serum aspartate transaminase (AST) within 7 days posttransplantation: this is a validated surrogate marker of graft survival.[9] Peak AST during the first 7 days posttransplantation was reduced by 49.4% in the NMP group compared with SCS. Similarly, the incidence of "early allograft dysfunction" (EAD), a further acute injury marker and validated surrogate for graft survival,[10] was reduced by 74%. The rate of organ discard was lower in the NMP arm (12% vs 24%), and the mean preservation time was 54% longer. At 1 year, there was no significant difference in patient and graft survivals.

The potential for organ viability assessment during NMP to increase organ utilization was shown in a UK study, which recruited livers discarded by all UK centres that also met one or more defined high-risk criteria.[11] Twenty-two of 31 enrolled livers were transplanted, all with primary function, although with substantial rates of posttransplant complications, including EAD (32%), acute kidney injury (18%), and nonanastomotic biliary strictures (3 out of 10 cases of DCD transplantation).

The study reported here represents the first use in the United States of the OrganOx *metra* NMP device, an updated version of the system that was used in the European studies referred to above. As with the European RCT, the study was designed to test the effect of delivering NMP continuously from the donor to the recipient operating room, thereby limiting the cold exposure of the donor organ to a short time during retrieval and preparation for perfusion (at the donor hospital) and a short time at the transplant hospital (during implantation).

METHODS

The OrganOx *metra* is a device for sustaining donor livers outside the body using blood at normal body temperatures. It is transportable by road but not yet certified for transport by air, although designed with this specification. It is intended to be used to transport and preserve donor livers for transplantation, with a maximum perfusion duration of 24 hours. The system incorporates a centrifugal pump, an oxygenator, an oxygen concentrator, a heat exchanger, a reservoir, flow probes, pressure sensors, infusions, and a blood gas analyzer, together with tubing and connectors. It is a fully-cannulated system (hepatic artery, portal vein, inferior vena cava, and bile duct) with perfusion parameters designed to replicate the physiological environment with respect to pressure and flow.

The perfusate was a combination of packed red blood cells (3 units) and 5% human albumin (total of 1.5 liters). At the time of priming the disposable set before perfusion, the additives given as bolus injections included: cefuroxime, heparin, sodium bicarbonate, and calcium gluconate. Throughout the perfusion, the following were automatically infused at a constant rate through onboard syringe drivers: insulin, heparin, epoprostenol, and sodium taurocholate. Parenteral nutrition (Clinimix) was infused through an onboard infusion pump once the perfusate glucose concentration had fallen below 180 mg/dL.

Real-time data provided to the user include the following: arterial and inferior vena cava pressures, portal, arterial, and inferior vena cava flow rates, pO_2, pCO_2, and pH, perfusate temperature, bile production, and elapsed perfusion time. Perfusate samples tested during perfusion included: lactate, glucose,

transaminase, hemoglobin, and hematocrit. The decision to transplant any given liver was made by the transplanting surgeon based on a combination of clinical and laboratory parameters. The perfusion duration allowed within the protocol was between 4 hours (minimum) and 24 hours (maximum).

The study was conducted at a total of 15 US liver transplant centres (of which 14 enrolled patients during the trial). An independent Data Safety Monitoring Board, Clinical Events Committee (CEC), and Medical Monitor were appointed to oversee the conduct of the study and ensure patient safety. The study protocol was approved by the FDA to support a class III medical device PMA review. The trial was registered with ClinicalTrials.gov Identifier: NCT02478151.

Trial Eligibility Criteria

Donor Inclusion:

- Liver allograft from DBD or DCD donors
  DBD donors aged ≥40 years.
  DCD donors aged ≥16 years.

Donor Exclusion:

- Living donor liver.
- Liver intended for split transplantation.
- Liver, which the investigator is unwilling to randomize to either arm.

Recipient Inclusion:

- Aged ≥18 years.
- Registered as an active recipient on the UNOS liver transplant waiting list.
- Able and willing to give informed consent.
- Able and willing to comply with all study requirements (in investigator's opinion).

Recipient Exclusion:

- Patient requiring all of the following at the time of transplantation:
  Oxygen therapy through a ventilator/respirator.
  Inotropic support.
  Renal replacement therapy.

- Diagnosis of acute/fulminant liver failure (UNOS status 1A).
- Simultaneous transplantation of more than one organ (eg, liver and kidney).
- Pregnancy (confirmed by urine or serum test) or breastfeeding.
- Concurrent enrollment in another clinical trial, with the exception of enrollment in observational trials where no test device or drug is used.

Trial Design

The primary objective of the trial was to compare the effectiveness of NMP to that of SCS in preventing preservation-related graft injury. The primary outcome measure was the incidence of EAD as defined by Oltoff et al.[10]

Secondary objectives included the following: graft and patient survival, the incidence of postreperfusion syndrome (as defined by Aggarwal et al 1993),[12] biochemical liver function,

histologic evidence of ischemia-reperfusion injury, biliary complications, feasibility and safety, organ utilization, and health economics.

This was a randomized, controlled, nonblinded clinical trial comparing SCS with NMP. After the assessment of donor and recipient eligibility and recipient informed consent, the donor liver was randomized to either NMP or SCS (Fig. 1). If the liver was randomized to NMP, the perfusion device was transferred to the donor hospital, and the perfusion established immediately after (conventional) retrieval, back-table preparation, and cannulation. The organ was transported to the transplant hospital while undergoing perfusion. At the end of preservation, the liver was transplanted, and the enrolled recipient was managed according to standard local practice and protocols. Control group livers were managed according to the local standard of care (SCS).

The point of enrollment to the study was defined as that of knife-to-skin contact in the operating room during the recipient transplant procedure. Any patient who was transplanted with a randomized liver remained in the study for 12 months. If a liver was randomized but not transplanted, it was not considered enrolled. If an intended original recipient did not receive the randomized liver, the liver was offered to the next recipient in accordance with the UNOS matching sequence.

Surgeons at the trial sites were supported by clinical specialists from OrganOx, who provided training in the use of the system at the start of the trial, hands-on assistance for early cases, and online/telephone support as needed for subsequent cases.

Randomization

Sequence Generation

Donor livers were randomly assigned to NMP or SCS with 1:1 allocation as per a computer-generated randomization schedule using variable block randomization using the following stratification factors: participating (recipient) center and donor type (DBD or DCD). The randomization schedule was created by the study statistician.

Allocation Concealment Mechanism

This was achieved by the use of central computerized randomization. Allocation was not revealed until the patient had been recruited and donor and recipient eligibility confirmed and recorded. Random permuted block length was used; block sizes were not disclosed.

Implementation

Before randomization, the local investigator confirmed the availability of the perfusion device. Once the informed consent and donor/recipient eligibility had been confirmed, the local recipient investigative site staff proceeded with randomization. The perfusion system was only transported to the donor site if the liver had been randomized to NMP.

Randomization Blocks

The size of the randomization blocks was known only to the study statistician and the Data Safety Monitoring Board statistician unless documented otherwise with justification.

Data Collection

The following data were collected at the following time points: days 1 to 7, day 30, month 3, and month 6:
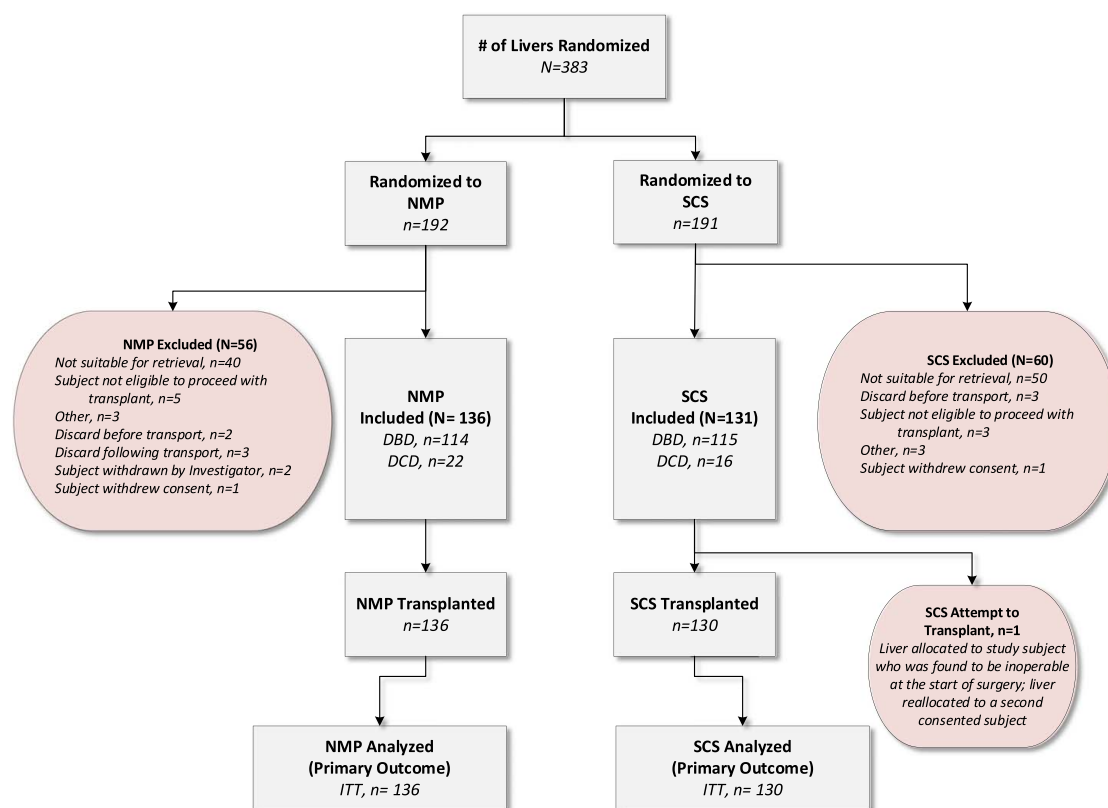
FIGURE 1. Randomized liver flowchart.

- Biochemistry: total bilirubin, alkaline phosphatase, gamma-glutamyl transferase, AST, alanine aminotransferase (ALT), International normalized ratio, albumin, and creatinine.
- Additional assessments: graft and patient survival, a requirement for renal replacement therapy, readmission, health care resource use (hospital admissions data and medical records), and quality of life (EQ-5D-5L questionnaire).
- Safety data: infections, rejection episodes, biliary investigations (magnetic resonance cholangiopancreatography, endoscopic retrograde choloangiopancreatography, and percutaneous transhepatic cholangiogram), biliary interventions (surgical, radiologic, and endoscopic), vascular complications, reoperation for graft-related complications, and adverse events.

Data collection at 12 months was limited to the following: graft and subject survival, a requirement for renal replacement therapy for more than a total of 5 days, readmission (s), and health care resource use.

Of the total, 90% of NMP recipients and 92% of SCS recipients completed 12-month follow-up; the reasons for withdrawal before this timepoint included: recipient mortality (n = 14), retransplantation (n = 7), and withdrawal of consent (n = 1).

Reporting of Outcomes

The definitive analysis of the study was based on "intention-to-treat" (ITT). The primary outcome (incidence of EAD) was also compared using "per-protocol" and "as-treated" analyses:

- ITT analysis: this included all transplanted patients (ie, those with reperfusion of a donor liver) analyzed in the groups to which the liver was randomly assigned, irrespective of whether the assigned method of preservation was actually used.
- Per-protocol analysis: this included all transplanted patients who were managed according to the protocol procedures with no major deviations.
- As-treated analysis: this included all transplanted patients analyzed according to the method of preservation that was actually used.

P values were calculated only for the primary endpoint.

RESULTS

Fifteen investigator sites were initiated, and a total of 383 livers were randomized into the NMP group (n = 192) and SCS group (n = 191) between October 9, 2016 and February 3, 2020. One hundred thirty-six livers (NMP) and 130 (SCS) livers were transplanted, of which 114 (NMP) and 114 (SCS) were DBD organs.

One hundred sixteen randomized livers were excluded from the study (56 NMP and 60 SCS) (Fig. 1). Ninety donor livers were declined before retrieval: 46 DCD donors did not proceed or did so outside local acceptance criteria, 40 organs were discarded for other donor-related reasons, 2 livers were retrieved for research, and 2 livers were declined but then accepted by other centers and transplanted into nontrial patients.

Of the remaining 26 livers that were excluded from the study, 8 were excluded because the patients were not medically suitable to proceed on the day, 5 were discarded after retrieval before transport (for liver quality reasons), 3 were discarded after transport, due to steatosis (1) and suboptimal perfusion

parameters (2). Two livers were excluded because the patients were withdrawn by the investigator (both for cardiac reasons), 2 because the patients withdrew consent, and 6 were excluded for other reasons.

Ten livers (within the 116 described) were randomized and, having been deemed suitable for transplantation, were subsequently transplanted into patients outside the study. In 4 cases, the original recipients were judged on the day to be medically unfit for transplantation; in 2 cases, the liver was judged too large for the recipient; 2 recipients withdrew consent; and 2 livers were deemed unsuitable for transplantation but then accepted and transplanted by another center. None of these livers underwent perfusion (ie, the decision to transplant a different recipient was made before placing the liver on the perfusion device).

A total of 14 device malfunctions were reported: 9 of these were related to issues with the disposable set (eg, leakage from luer-lock connector) and 5 related to the perfusion device itself (eg, function/calibration of blood gas analyzer). In 4 of the 14 cases, the mode of preservation was switched to SCS: none of these required emergency transfer of an organ to SCS, and none was associated with graft loss. Twelve of these livers were transplanted, and 2 occurred in livers that were subsequently not transplanted, one because the recipient was unfit for surgery and the other because the DCD donor did not proceed to donation. The 2 deaths in this group were due to recurrent malignancy (on day 263 posttransplant) and hepatic artery hemorrhage (on day 9 posttransplant), both adjudicated as unrelated to the perfusion device. More details of the device malfunctions are given in Supplemental Table (Supplemental Digital Content Table 5, http://links.lww.com/SLA/E698).

In 266 cases (136 NMP and 130 SCS), liver transplantation proceeded. There were no notable demographic differences between the two groups with respect to donor characteristics (Supplemental Table (Supplemental Digital Content Table 1, http://links.lww.com/SLA/E698) or recipient characteristics (Supplemental Table (Supplemental Digital Content Table 2, http://links.lww.com/SLA/E698).

Preservation time: the trial protocol stipulated that NMP-preserved livers should be perfused for a minimum of 4 hours and a maximum of 24 hours and that SCS livers should be preserved according to local unit practice. The mean total preservation time using the "as-treated" population was 75% longer in the NMP arm of the study compared with the SCS arm (NMP: 553.8 ± 115.9 minutes and SCS: 316.9 ± 94.1 minutes). The NMP total preservation time included a mean of 135 ± 35.7 minutes cold and 356 ± 106 minutes normothermic preservation.

Primary Endpoint

- The primary endpoint of the study was the incidence of EAD. The comparison between the arms was conducted using "ITT," "per-protocol," and "As Treated" analyses (Table 1). Missing data points were handled by imputation.
- In the "ITT" analysis, the NMP arm had a numerically lower imputed EAD rate of 20.6% (NMP) versus 23.7% (SCS); this difference did not achieve statistical significance ($P = 0.275$).

Additional Exploratory Analyses of Primary Endpoint Data

- The most pronounced difference between the two arms was seen in the "as-treated" analysis, with imputed EAD rates of 18.7% (NMP) and 24.9% (SCS) ($P = 0.115$).

- There was a marked effect of donor type (DBD vs DCD), with a greater effect size in DCD donor livers (22.8% NMP vs 44.6% SCS) than in DBD donor livers (17.9% NMP vs 22.0% SCS). These results are based on "as-treated" analysis after imputation. (Table 2).
- There was evidence for a "learning curve.". EAD rates in the NMP arm decreased after enhanced on-site training (23.9% vs 15.4%), whereas no such effect was seen in the SCS arm (23.4% vs 24.4%).
- EAD events were compared at different tiers of the Donor Risk Index. In the lower quartiles of donor risk index (DRI), EAD rates were similar between the randomization arms, whereas a large difference (19.2% NMP vs 33.3% SCS) was observed in the highest quartile of DRI. (Fig. 2, Supplemental Digital Content Table 3, http://links.lww.com/SLA/E698)
- There was no correlation between the duration of NMP and observed EAD rates. EAD rates in 3 tertiles of perfusion time (< 228 minutes, 228–381 minutes, and >381 minutes) were 14.3%, 26.2%, and 17.5%, respectively.

Secondary Endpoints

Graft Survival

Four graft failures were reported in the NMP arm and 3 in the SCS arm (Supplemental Digital Content Fig. 1, http://links.lww.com/

TABLE 1. EAD—ITT, Per-protocol, and As-treated Analysis Populations

	NMP*	SCS	Superiority *P*
ITT analysis†			
Analysis population (N)	136	130	—
Incomplete EAD information (N)	9	3	—
EAD before imputation (%)	20.5 (26/127)	22.8 (29/127)	—
EAD using imputation‡(%)	20.6 (14.5, 28.5)	23.7 (17.1, 31.9)	0.275
Per-protocol analysis§‖			
Analysis population (N)	133	130	—
Incomplete EAD information (N)	9	3	—
EAD before imputation (%)	18.5 (23/124)	22.8 (29/127)	—
EAD using imputation‡ (%)	18.6 (12.7, 26.4)	23.8 (17.2, 31.9)	0.158
As-treated analysis§‖			
Analysis population (N)	133	132	—
Incomplete EAD information (N)	9	3	—
EAD before imputation (%)	18.5 (23/124)	24.0 (31/129)	—
EAD using imputation‡ (%)	18.7 (12.8, 26.5)	24.9 (18.2, 33.1)	0.115

*Three patients had elevated day 7 International normalized ratio values due to anticoagulation: these values were imputed

†ITT population.

‡Multiple imputation was used for patients with missing laboratory values that were required to determine EAD status. Imputation was not used to determine EAD status when: (1) the patient already had one or more laboratory values meeting EAD criteria, (2) the patient had been discharged before day 7 with laboratory values below the EAD threshold, or (3) the patient's last available International normalized ratio value was below EAD threshold, and there was no hospital readmission.

§One patient met exclusion criteria and was excluded from this analysis.

‖Two patients who received livers that were randomized to NMP but were transported using SCS were excluded from the per-protocol analysis and included as SCS in the as-treated analysis.

TABLE 2. EAD Analyzed by Donor Type

	DBD		DCD	
	NMP*	**SCS**	**NMP**	**SCS**
Primary endpoint†				
Analysis population (N)	114	114	22	16
Incomplete EAD information (N)	8	2	1	1
EAD before imputation (%)	18.9 (20/106)	20.5 (23/112)	28.6 (6/21)	40.0 (6/15)
EAD using imputation‡ (%)	18.7 (12.5, 27.2)	21.3 (14.7, 29.8)	30.1 (14.5, 52.4)	41.0 (19.8, 66.0)
Per-protocol analysis§‖				
Analysis population (N)	113	114	20	16
Incomplete EAD information (N)	8	2	1	1
EAD before Imputation (%)	18.1 (19/105)	20.5 (23/112)	21.1 (4/19)	40.0 (6/15)
EAD using imputation‡ (%)	17.9 (11.8, 26.4)	21.3 (14.7, 29.9)	22.3 (8.8, 46.2)	41.1 (19.9, 66.1)
As-treated analysis§‖				
Analysis population (N)	113	115	20	17
incomplete EAD information (N)	8	2	1	1
EAD before imputation (%)	18.1 (19/105)	21.2 (24/113)	21.1 (4/19)	43.8 (7/16)
EAD using imputation‡ (%)	17.9 (11.8, 26.4)	22.0 (15.3, 30.5)	22.8 (9.1, 46.6)	44.6 (23.1, 68.3)

*Three patients had elevated day 7 International normalized ratio values due to anticoagulation: these values were imputed.

†ITT population.

‡Multiple imputation was used for patients with missing laboratory values that were required to determine EAD status. Imputation was not used to determine EAD status when: (1) the patient already had one or more laboratory values meeting EAD criteria, (2) the patient had been discharged before day 7 with laboratory values below the EAD threshold, or (3) the patient's last available International normalized ratio value was below EAD threshold, and there was no hospital readmission.

§One patient met exclusion criteria and was excluded from this analysis.

‖Two patients who received livers that were randomized to NMP but were transported using SCS were excluded from the per-protocol analysis and included as SCS in the as-treated analysis.

SLA/E699), with 12-month graft survival rates of 97.0% and 97.7%, respectively. These graft losses included 3 cases of primary graft nonfunction (1 in the NMP arm and 2 in the SCS arm). Further details are given in Supplemental Table (Supplemental Digital Content Table 6, http://links.lww.com/SLA/E698).

Patient Survival

Ten patient deaths were reported in the NMP arm and 4 in the SCS arm, and 12-month patient survival rates were 92.5% and 96.6%, respectively (Supplemental Digital Content Fig. 2, http://links.lww.com/SLA/E699). Although there was a numerical difference between the two arms, there was no consistent pattern with respect to timing or cause of death, and no deaths were adjudicated (by the CEC) to be related to the perfusion device. Further details are given in Supplemental Tables (Supplemental Digital Content Tables 7 and 8, http://links.lww.com/SLA/E698).
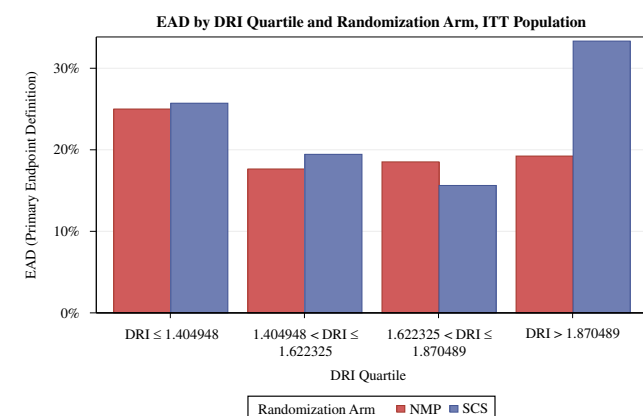
Postreperfusion Syndrome

Nineteen patients experienced this phenomenon in the SCS arm (14.6%) compared with 8 patients in the NMP arm (5.9%). The difference was greatest in DBD livers (14.0% SCS vs 4.4% NMP).

Biochemical Liver Function

Additional analyses of biochemical data, which were not prespecified in the protocol, were performed as a point of reference to other published data. Comparison of peak AST levels (Fig. 3) in the first 7 days postoperatively showed a lower level in the NMP group overall (722.4 IU/L SCS vs 540.3 IU/L NMP—25.2% reduction) with the largest effect seen in the DCD cohort (1543.8 IU/L SCS vs 604.2 IU/L NMP—60.9% reduction).

The same pattern is seen with ALT (Table 3). There is a greater difference in ALT levels between NMP and SCS in DCD (887.3 IU/L SCS vs 420.6 IU/L NMP—52.6% reduction)
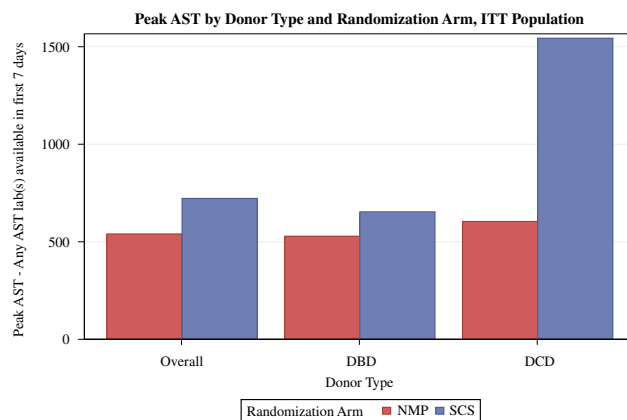

FIGURE 2. EAD analyzed by donor risk.

FIGURE 3. Peak transaminase levels during first 7 days postoperatively.

TABLE 3. Peak ALT During Days 1–7

	Overall				DBD				DCD			
	NMP		SCS		NMP		SCS		NMP		SCS	
	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)
Peak ALT—any ALT laboratory(s) available in first 7 d	136	381.8 (326.4, 446.5)	129	500.9 (432.7, 579.8)	114	374.7 (316.4, 443.7)	114	464.6 (397.4, 543.1)	22	420.6 (270.4, 654.4)	15	887.3 (642.9, 1224.8)
Peak ALT—day 1 ALT available	133	378.0 (322.9, 442.6)	129	500.9 (432.7, 579.8)	112	368.1 (310.7, 436.0)	114	464.6 (397.4, 543.1)	21	435.8 (275.5, 689.3)	15	887.3 (642.9, 1224.8)
Peak ALT—at least 2 ALT laboratories available	135	382.1 (326.3, 447.4)	129	500.9 (432.7, 579.8)	113	375.0 (316.2, 444.8)	114	464.6 (397.4, 543.1)	22	420.6 (270.4, 654.4)	15	887.3 (642.9, 1224.8)

compared with DBD livers, although a clear effect is shown in both cohorts (500.9 IU/L SCS vs 381.8 IU/L NMP—23.8% reduction overall).

Other postoperative laboratory assessments (bilirubin, International normalized ratio, alkaline phosphatase, gamma-glutamyl transferase, and albumin, lactate) showed no statistically significant differences.

Renal Function

The median level of creatinine (days 1–7) was reduced in the NMP arm (1.2 vs 1.4 mg/dL). The incidence of renal replacement therapy required during the first 30 days posttransplantation was 4.5% (6/132) in the NMP arm compared with 8.6% (11/128) in the SCS arm (Supplemental Digital Content Table 4, http://links.lww.com/SLA/E698).

Histologic Evidence of Ischemia-reperfusion Injury

Postreperfusion biopsies were compared with baseline prereperfusion biopsies and graded for ischemia-reperfusion injury according to standard histologic criteria. 255/266 (95.9%) of livers were assessed. There were no notable differences in the degree of ischemia-reperfusion injury (Supplemental Digital Content Fig. 3, http://links.lww.com/SLA/E699). However, there was a small but notable difference in the proportion of livers with mild/moderate/severe lobular inflammation when comparing the postreperfusion to prestorage biopsies between the arms. In the NMP arm, there was a 26.5% increase between prestorage (52.4%) and postreperfusion (78.9%) biopsies, whereas, in the SCS arm, there was a 44.7% increase between prestorage (43.1%) and postreperfusion (87.8%) biopsies.

Biliary Complications

The cumulative incidence of biliary investigations and biliary interventions (Table 4) was used as a surrogate measure for biliary complications of all types. The number of biliary interventions was effectively a subset of the biliary investigations (because all patients undergoing an intervention had prior investigation). Data have been expressed as the number of patients experiencing the events rather than the total number of events (noting that some patients may have had more than one investigation or intervention).

Biliary investigations between day 7 and month 6 occurred in 12.6% (16/127) of NMP patients and 13.4% (17/127) of SCS patients. Biliary interventions were reported for 9.4% (12/127) and 8.7% (11/126) of NMP and SCS patients, respectively. Although there are no notable differences between the groups overall, there is a marked numerical reduction in interventions in the NMP arm in the DCD cohort, acknowledging that the sample size for this group is small (biliary interventions: 19.0% NMP; 28.6% SCS). A diagnosis of ischemic cholangiopathy was reported within 12 months in 2 patients in the NMP arm and 1 patient in the SCS arm.

Organ Utilization

The number of livers randomized but not transplanted did not differ notably between NMP (56/192, 29.2%) and SCS (61/191, 31.9%). The majority of nontransplanted livers were not retrieved: this was for a number of reasons (Fig. 1), of which the commonest were a failure of a DCD donor to proceed to donation (n = 47) and donor liver appearance upon visualization by the retrieval surgeon (n = 21). No direct advice was given to the clinicians regarding the decision to accept or to proceed with any individual liver.

TABLE 4. Biliary Investigations and Interventions From 7 Days to 6 Months

	Overall (%)		DBD (%)		DCD (%)	
	NMP	SCS	NMP	SCS	NMP	SCS
Biliary investigations	11.0 (14/127)	12.7 (16/126)	8.5 (9/106)	10.7 (12/112)	23.8 (5/21)	28.6 (4/14)
Biliary interventions	9.4 (12/127)	8.7 (11/126)	7.5 (8/106)	6.3 (7/112)	19.0 (4/21)	28.6 (4/14)

Denominators are patients with completed 6-month visits or at least one event occurring between 7 days and 6 months posttransplantation.

Health Economic Analysis

The median length of intensive care unit stays after transplantation was lower in the NMP (2 days) compared with the SCS arm (3 days) (Supplemental Digital Content Table 4, http://links.lww.com/SLA/E698). The median total length of hospital stay was the same in both treatment arms (9 days). There were no substantial differences with respect to the measured quality of life (EQ-5D) between the two groups.

Safety

All adverse events collected through follow-up were reviewed by an independent Medical Monitor. A CEC adjudicated the most critical adverse events, and a Data Safety Monitoring Committee reviewed aggregate safety data. The rates of serious adverse events were similar in the 2 arms throughout the study: 74 NMP versus 71 SCS patients experienced at least 1 serious adverse event within 30 days, and 95 NMP versus 93 SCS patients did so within 12 months.

DISCUSSION

Machine perfusion of the liver has moved from the status of an experimental method, the focus of a small number of enthusiastic pioneers, to that of a rapidly emerging technology that stands to change the scale and scope of liver transplantation. In particular, the claimed advantages of NMP include: (1) longer safe preservation, leading to improved transplant logistics, (2) organ repair—enabling the organ to recover from reversible injury, particularly hypoxia at the time of retrieval, (3) viability assessment, enabling better utilization of high-risk organs, and (4) therapeutic interventions—the ability to treat a donor organ. There is increasing evidence to support the first 3 of these: trials of novel organ therapy protocols are underway, although at an early stage.

The study described here was designed to demonstrate that NMP reduces the amount of acute liver injury that is associated with the process of transplantation and that the procedure is feasible and safe. The study did not achieve its primary outcome target: with an overall reduction in EAD from 23.7% (SCS) to 20.6% (NMP): it did not, therefore, reproduce the results of the two previous RCTs, which have provided evidence that NMP reduces acute liver injury across a broad range of donor livers. The European multicentre RCT[8] showed a statistically significant reduction in liver injury, measured by the peak transaminase level during the first 7 days (primary endpoint) and EAD (secondary endpoint). A more recently published multicentre US trial using EAD as a primary endpoint also showed a statistically significant effect.[13]

There is a clear trend in the current study in the direction of a similar effect, albeit not achieving statistical significance—that is, evidence of reduced effect size. Compared with the European RCT, the DRI and the recipients were of higher risk modified end-state liver disease The overall preservation times were shorter in the US study, which is likely to have further reduced any effect of NMP. It is likely, therefore, that the current US study, the design and power of which were largely based on the European experience, was underpowered to show what was a smaller difference between the two arms. This is supported by two subgroup analyses (carried out for exploratory purposes), which suggest that there was a larger effect in two higher-risk cohorts: (1) the donor organs in the highest quartile of DRI and (2) DCD donors.

Much has been written about the appropriateness of the primary endpoint in this and other NMP studies.[14] Although EAD has been shown to be prognostic of subsequent graft outcome in livers preserved by SCS,[10] it is the case that its use in perfused (as opposed to SCS) livers has never been validated (and the same is true of peak transaminase used in other studies). What all investigators agree is that graft or patient survival is no longer a feasible endpoint for a trial of organ preservation: this is because the key event (death/graft failure) is both uncommon and of multifactorial etiology. A recent publication calculated that the necessary size of a trial with such an endpoint in liver transplantation would be of the order of 4000 patients.[15] Some investigators have reasonably argued that postoperative complications may be a better marker of any benefit that accrues to perfused organs: the "Comprehensive Complications Index" has been shown to be the best predictor of outcome after liver transplantation in a European multicenter analysis[16] and is the primary outcome measure of a currently-recruiting RCT in Germany comparing NMP with oxygenated hypothermic machine perfusion and SCS.[17]

The results of the secondary endpoint analyses are of interest. In line with the two published studies, there was a marked and clinically relevant reduction in the incidence of postreperfusion syndrome. As defined,[12] this is a life-threatening cardiovascular collapse triggered by reperfusion of the graft, which is known to be related to the quality of the donor organ. For this reason, organs, which are at high risk of generating postreperfusion syndrome, are not used in very sick patients. A recent study carried out at the University of Birmingham has explored the use of NMP to enable high-risk organs to be transplanted into complex, high-risk recipients.[18]

The issue of biliary complications is of key importance in liver transplantation as this is both a frequent and often serious issue, particularly in DCD liver transplantation. There has been much debate about the possible benefits of organ perfusion on the occurrence of intrahepatic (nonanastomotic) biliary stricture formation (ischemic cholangiopathy): data from the European randomized trial (to date the only randomized trial to mandate postoperative magnetic resonance cholangiopancreatography imaging) showed a reduction in the radiologic evidence of this complication (from 26% to 12%) in DCD livers, but no difference in DBD organs.[8] The current study suggests a possible benefit in the DCD cohort (biliary interventions reduced from 29% SCS to 19% NMP) but shows no difference in the overall incidence across the study. The previous US trial[13] showed a reduction in overall "ischemic" biliary complications from 8.5% (SCS) to 1.3% (NMP) at the 6-month timepoint, based on clinical/biochemical diagnosis. Current thinking is that the incidence of nonanastomotic biliary strictures is related to

micro-thrombi in the peribiliary vascular plexus and that normothermic perfusion might provide a suitable platform to deliver thrombolytic therapy before transplanting the organ: this strategy has been explored in discarded human livers,[19] but remains untested in a clinical trial.

The current trial followed the same model of "continuous NMP" as the previously published studies—that is, transporting the perfusion device to the donor hospital and reducing the cold exposure of the organ to a period at the time of retrieval (in situ flushing with cold preservation solution and back-table preparation for perfusion) and at the end of perfusion (cooling the organ with cold preservation solution to cover the period of implantation). This model ("device-to-donor") creates logistic challenges: it requires not only the device to be transported (in the current trial, this was always by road, but in the future, potentially also by air) but also the retrieval team to include the necessary skills (surgical and technical) to initiate perfusion. Although this is the logically superior way to use the technology because it minimizes exposure to cooling and prevents the organ from ever achieving ice temperature, there is increasing experience of the simpler method of transporting the organ in an ice box and initiating NMP immediately on arrival at the transplant hospital or organ procurement center (known as "back-to-base"). Although not subjected to an RCT, published evidence suggests that, at least for the majority of organs, there is little difference between the two methods in terms of efficacy (as measured by acute liver injury markers). A single-arm study recruited 30 patients in the UK, using the same protocol as the recently reported European RCT except for the back-to-base method of use compared with device-to-donor. There was no difference in peak AST, the rate of EAD, or graft outcome.[20] It is, however, possible that a clinically important difference would be experienced in very high-risk donors (eg, marginal DCD or severely steatotic organs)—this hypothesis is supported by the relatively high complication rate reported in the Birmingham "VITTAL" study in which organs declined by every liver transplant centre in the UK were perfused and assessed by NMP after substantial periods of SCS.[11] At this point, therefore, there is still a relative lack of evidence regarding the use of NMP in back-to-base as opposed to device-to-donor mode.

The current study suggests that NMP may be beneficial in high-risk organs, with a much larger effect on EAD in donor organs in the highest quartile of DRI and also a much larger effect in DCD, compared with DBD organs (in both cases compared with the overall trial outcomes). Any advantage of NMP in lower-risk organs is, therefore, mainly within the domain of logistic benefits—the ability to preserve organs for longer before transplantation. Prolonging preservation has substantial value in its own right: liver transplantation is a long and technically challenging operation and one that is often conducted during the night because of the timing of donor organ retrieval: the process of transplantation of SCS-preserved livers is dominated by time constraints. Although the advent of modern SCS solutions in the 1990s enabled longer preservation, the correlation between preservation time and postoperative performance remains, and higher-risk organs are particularly vulnerable. Indeed, with the use of DCD and other marginal organs, the timing of liver transplantation increasingly resembles the pre-University of Wisconsin era: a high proportion of liver transplants take place overnight.

As well as the greater staffing costs of overnight operating, many transplant units suffer from the effects of the "burn-out" of the surgical team: this is increasingly recognized as an important issue of sustainability. The potential to extend the preservation duration and the effect of doing so have not been evaluated in this study, in which clinical sites were requested to treat the 2 arms in the same way as far as possible. The longer preservation time reported in the NMP arm was a function of the need to perfuse livers in the NMP arm for no <4 hours: a greater prolongation of preservation time would be possible within the current label of the perfusion device.

There is increasing evidence not only that NMP allows a clinically significant increase in overall preservation time but also that this advantage does not come at the price of inferior outcomes. Enabling preservation times of up to 24 hours (as practiced in a number of European centers) can transform the logistics of transplantation, not only allowing organs and recipients to travel greater distances but also allowing transplant teams to operate at times of their choosing. Experience in Europe already shows the size of the opportunity: NMP can enable liver transplantation routinely to become a daytime procedure, and with no apparent detriment to the outcomes.[21]

The European COPE RCT reported a 20% improvement in organ utilization in the NMP arm of the study:[8] the same effect was not seen in the current study. The most likely explanation for this difference is that the decline rate among US liver transplant programs of organs once accepted is already very low compared with Europe. This may reflect a more selective approach to organ offers in the United States, where distances are greater and retrieval costs are higher: the decision to decline a donor is more likely to be made at the time of offering in the United States, whereas in Europe the decision may be more commonly deferred until the organ has been visualized at retrieval.

Although head-to-head randomization of NMP against SCS can provide valuable evidence regarding organ utilization, it is nonetheless not an appropriate method to test the size of any such effect because the effect of randomization excludes some donor organs that might benefit. The enrollment criteria for this and the two previous RCTs require that all donor organs recruited must be within acceptable criteria for either method of preservation. If NMP is effective in limiting the injury sustained during retrieval, preservation, and reperfusion or if it enables a more reliable selection of viable organs, then it is logical that the acceptability limits for NMP-preserved organs will be broader than those of SCS organs. The current study was neither designed nor expected to show a difference in organ utilization.

A number of published series now illustrate the importance of functional assessment. The ability to assess the viability of a donor organ before committing a patient to the risks of a transplant gives the clinician greater information on which to base the decision to proceed. Currently used parameters include markers of hepatocellular injury (transaminase levels), hepatocellular function (lactate clearance, acid-base stability), and cholangiocyte function (biliary pH, glucose). Increasing evidence supports the claim that organ utilization is enhanced by the application of NMP in this way in donor organs of marginal quality.[22] This is the strategy being utilized in the recently reported interim analysis of a single-center US trial assessing declined liver grafts for transplantation as part of the RESTORE trial.[23]

What is the next technological development in this field? Organ assessment is a likely area for rapid evolution: the use of NMP to assess the viability of marginal donor organs is currently based on simple markers of function and injury (as discussed). Much current research is addressing a wide range of functional, genetic, transcriptomic, and metabolomic markers that might prove more predictive of subsequent graft outcomes. Similarly, biological interventions might modify the graft to abrogate ischemia-reperfusion or later rejection: experimental studies have

shown the potential for treatment with drugs,[24] cells,[25] and genes.[26] The development of specialized organ perfusion laboratories to deliver complex interventions may not be far off.

NMP is not the only emerging organ perfusion technology that is making an impact in liver transplantation. A recently published RCT studied the effect of oxygenated hypothermic machine perfusion (HMPO$_2$) on DCD livers.[27] This is a technology, which is intrinsically less complex and expensive than NMP, and it showed a clinically important reduction in the incidence of nonanastomotic biliary strictures (6% vs 18%). However, HMPO$_2$ provides limited viability assessment: flavin mononucleotide[28] gives a measure of mitochondrial injury, but its value as a predictor of medium-term graft outcome remains to be proven. Although HMPO$_2$ is increasingly widely used in Europe and the subject of an ongoing clinical study in the United States, there is as yet no evidence that it increases the use of organs that are currently discarded (ie, organ utilization).

There is good evidence of the benefit of normothermic regional perfusion—the technique of in situ reperfusion of donor organs following the declaration of death in DCD donors—with lower levels of postreperfusion organ injury, nonanastomotic biliary strictures, and improved utilization.[29] Although seen as an alternative technology, there is increasing consensus that normothermic regional perfusion and NMP are complementary technologies: there are situations where one or the other is indicated, and others (eg, the highest risk DCD donors) when the combination is needed to optimize the use and outcome of the precious resource of donated organs.

Finally, the issue of cost-effectiveness is important. NMP is inherently more expensive than the SCS method that it replaces and comes with high development and regulatory costs. There is clear value in transplanting more organs safely, not only on humanitarian grounds but also by reducing the high cost of caring for patients on liver transplant waiting lists.[30] Although the studies conducted to date have (rightly) focused on demonstrating that the technology is safe and that it delivers a clinically worthwhile benefit, it is now important to demonstrate that the cost of that benefit is acceptable. Health care payers worldwide assess cost-benefit in different ways, but there is consensus that future studies should collect data that enable this important evaluation.

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