Hypothermic Oxygenated Machine Perfusion Reduces Early Allograft Injury and Improves Post-transplant Outcomes in Extended Criteria Donation Liver Transplantation From Donation After Brain Death

Results From a Multicenter Randomized Controlled Trial (HOPE ECD-DBD)

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Objective: The aim of this study was to evaluate peak serum alanine aminotransferase (ALT) and postoperative clinical outcomes after hypothermic oxygenated machine perfusion (HOPE) versus static cold storage (SCS) in extended criteria donation (ECD) liver transplantation (LT) from donation after brain death (DBD).

Background: HOPE might improve outcomes in LT, particularly in high-risk settings such as ECD organs after DBD, but this hypothesis has not yet been tested in a randomized controlled clinical trial (RCT).

Methods: Between September 2017 and September 2020, 46 patients undergoing ECD-DBD LT from four centers were randomly assigned to HOPE (n = 23) or SCS (n = 23). Peak-ALT levels within 7 days following LT constituted the primary endpoint. Secondary endpoints included incidence of postoperative complications [Clavien-Dindo classification (CD), Comprehensive Complication Index (CCI)], length of intensive care- (ICU) and hospital-stay, and incidence of early allograft dysfunction (EAD).

Results: Demographics were equally distributed between both groups [donor age: 72 (IQR: 59–78) years, recipient age: 62 (IQR: 55–65) years, labMELD: 15 (IQR: 9–25), 38 male and 8 female recipients]. HOPE resulted in a 47% decrease in serum peak ALT [418 (IQR: 221–828) vs 796 (IQR: 477–1195) IU/L, $P = 0.030$], a significant reduction in 90-day complications [44% vs 74% CD grade ≥ 3, $P = 0.036$; 32 (IQR: 12–56) vs 52 (IQR: 35–98) CCI, $P = 0.021$], and shorter ICU- and hospital-stays [5 (IQR: 4–8) vs 8 (IQR: 5–18) days, $P = 0.045$; 20 (IQR: 16–27) vs 36 (IQR: 23–62) days, $P = 0.002$] compared to SCS. A trend toward reduced EAD was observed for HOPE (17% vs 35%; $P = 0.314$).

Conclusion: This multicenter RCT demonstrates that HOPE, in comparison to SCS, significantly reduces early allograft injury and improves post-transplant outcomes in ECD-DBD liver transplantation.

Keywords: extended criteria donation, HOPE, hypothermic oxygenated machine perfusion, liver transplantation, machine perfusion

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Liver transplantation (LT) is the only curative treatment for end-stage liver disease. Despite the success of this life saving procedure, receding organ donation rates have led to a severe shortage of donor organs, prompting clinicians to accept extended criteria donation (ECD)-allografts that were previously deemed unsuitable for transplantation.[1] Although the transplantation of ECD-organs increases the donor pool, these pre-damaged organs are susceptible to injury during organ storage and transplantation. Static cold storage

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(SCS) comprises the transport of procured donor livers on packed ice and has remained the gold standard for organ preservation for decades. As SCS is unable to meet the challenges of ECD LT, machine perfusion (MP) is increasingly recognized as an important strategy to protect donor livers from preservation and ischemia-reperfusion injury (IRI).[1,2] Two main dynamic preservation techniques have shown promising clinical results. Firstly, normothermic machine perfusion (NMP) supports the cellular metabolism by preserving the organ at physiological temperatures with oxygen and nutrient supply.[1,3] Secondly, hypothermic (oxygenated) machine perfusion (HMP/HOPE) with cooled, oxygenized perfusate restores tissue energy reserves before *in-situ* reperfusion, thus mitigating the effects of IRI.[2,4–9] Although HOPE holds promise to improve outcomes in human LT, no data from a randomized controlled trial (RCT) are currently available on HOPE in ECD donation after brain death (DBD) liver transplantation. We therefore conducted an RCT comparing early allograft injury and clinical outcomes after HOPE versus SCS in ECD-DBD liver transplantation assessing the peak level of serum alanine aminotransferase (ALT) within 1 week after LT as the primary and surgical complications, length of intensive care unit (ICU)- and hospital stay as well as early allograft dysfunction (EAD) as secondary endpoints.

METHODS

Trial Design and Ethics

This investigator initiated, two-arm, parallel, open-label, multicenter RCT was conducted to evaluate the effects of HOPE versus SCS in patients receiving ECD-allografts from DBD between September 2017 and September 2020. We hypothesized that HOPE is superior to SCS with regards to early allograft injury. The study was conducted according to the principles of the Declaration of Helsinki, the Declaration of Istanbul, and the good clinical practice guidelines (International Conference on Harmonization-Good Clinical Practice) and was reported on the basis of the Consolidated Standards of Reporting Trials (CONSORT) statement.[10] The protocol of the trial was approved by the local ethics committee (EK 049/17), registered at clinicaltrials.gov (NCT03124641) and published before enrollment of the first participant.[11]

Participants

Patients >18 years of age, suffering from end stage-liver disease and/or malignant liver tumors, listed for liver transplantation and receiving ECD organs were eligible for the study (Supplemental Digital Content Table 1; http://links.lww.com/SLA/D355). Allocation of the allografts followed national (Deutsche Stiftung Organ Transplantation; DSO – Germany/Koordinacni Stredisko Transplantaci; KST – Czech Republic) and European (Eurotransplant; ET) policies. Specific details of the trial design were defined in our *ex-ante* published study protocol.[11] The trial recruited patients at four European liver transplant centers (University Hospital RWTH Aachen, Aachen; Charité-Universitätsmedizin Berlin, Campus Charité Mitte I Campus Virchow Klinikum, Berlin; University Hospital Ludwig-Maximilians-University Munich, Munich; Institute for Clinical and Experimental Medicine, Prague, Czech Republic). No amendments to the trial design were made after the inclusion of the first patient.

Control and Intervention Groups (SCS vs HOPE)

Group SCS ''control'': patients randomized to the control SCS group received ECD livers that were preserved using SCS according to current local standard practice.

Group HOPE ''intervention'': HOPE was conducted after regular organ procurement, transport and back table preparation based on a protocol previously described by Dutkowski et al.[12] End-ischemic HOPE (Liver Assist; Organ Assist b.v., Groningen, The Netherlands) was applied through the portal vein for a minimum of 1 hour before implantation in a pressure controlled system (2–3 mm Hg) with 3 to 4 L of oxygenized (target pO_2 of 60–80 kPa) and cooled (10°C) re-circulating UW-MPS (Belzer MPS, Bridge to Life, London, United Kingdom) solution. In cases wherein recipient hepatectomy was prolonged, HOPE was continued to avoid repeated SCS. Donor allografts were pre-flushed with UW-MPS perfusion solution before HOPE-treatment to wash out the residual cold storage solution (HTK or UW). The allografts were again flushed after HOPE and shortly before implantation using 1 L of HTK (Custodiol, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) to wash out residual UW-MPS solution.

Outcome Measures

The primary endpoint was defined as the difference in early allograft injury assessed by the difference between the two treatment arms in the peak level of serum ALT within the first 7 days after LT. To correct for an assumed washout effect of machine perfusion, the relative changes of serum peak ALT (delta peak ALT) were assessed.[13] Peak ALT levels were corrected per protocol to the values measured in the routine blood analysis after reperfusion at the time point of admission to the ICU (within 3 hours after graft reperfusion, followed by the next (Day 1) measurement 24 hours after reperfusion).[11] Delta peak ALT = peak ALT Days 1 to 7/1st post-LT ALT <3 hours. Perfusate transaminase levels were measured serially during HOPE as well as at the termination of the perfusion.

Secondary endpoints included: incidence of post-operative complications as assessed by the Clavien-Dindo (CD) classification and the Comprehensive Complication Index (CCI)[14,15]; further serum laboratory parameters[11,16]; EAD as defined by the Olthoff criteria (bilirubin ≥10 mg/dL on day 7, international normalized ratio (INR) ≥1.6 on day 7, and alanine or aspartate aminotransferases (AST) >2000 IU/L within the first 7 days)[17]; length of intensive care unit (ICU) stay; length of hospital stay; 1-year recipient and graft survival; analysis of serum, tissue, bile, and perfusate biomarkers of IRI (to be analyzed and reported separately as translational aspects of the trial).

Sample Size Calculation

The trial was designed to detect a significant difference in early allograft injury assessing the peak level of serum ALT within 1 week after LT as the primary endpoint as described previously in the HOPE ECD-DBD study protocol.[11] Sample size estimation was performed using the G*Power software (Version 3.1.9.6, Heinrich-Heine-University, Dusseldorf, Germany) for the primary endpoint. The nonrandomized DBD trial of Guarrera et al demonstrated the mean peak ALT following SCS to be 1358 IU/mL.[18] Presuming a comparable 59% (ALT) to 65% (AST) reduction in mean peak transaminase levels following machine perfusion treatment and on the basis of a power of 80%, a 5% significance level for two-sided testing in two independent groups as well as a 15% drop-out rate or invalid data, we arrived at a sample size of 23 patients per group ($\Sigma n = 46$).[11,18]

Randomization

Randomization was performed upon arrival of the allograft in the transplant center and after visual acceptance by an experienced member of the study and transplant team, using a web-based randomization service for clinical trials (www.randomizer.at).[11,19] A stratified randomization model (ratio 1: 1) was used to ensure a balance of prognostic variables between treatment groups [stratification criteria were defined as cold ischemia time (CIT): <8 or ≥8 and graft macrovesicular steatosis <30% or ≥30%].

Blinding

Due to the nature of the intervention using a large and complex perfusion device, blinding of the investigators and surgical team was not feasible. This is in accordance with the design of other open-label machine perfusion RCTs in kidney and liver transplantation.[2,20]

Organ Procurement and ECD-Criteria

All randomized allografts were retrieved by ET or national procurement teams according to local standards. Liver allografts were considered ECD based on the recommendations of the German Medical Chamber (Bundesärztekammer)[21,22] (Supplemental Digital Content Table 1; http://links.lww.com/SLA/D355).

Surgical- and Perioperative Approach

All study centers used their local standard surgical techniques of deceased donor LT. Apart from the study interventions, patients were treated according to the institutional protocols, including the use of a standardized immunosuppressive regimen based on induction therapy with intravenous basiliximab and methylprednisolone followed by corresponding oral doses of prednisolone, tacrolimus, and mycophenolate mofetil.

Data Collection and Statistical Methods

Informed consent and patient screening were carried out in the local outpatient clinics. All collected data were documented by trained members of the study team on paper case report forms and considered as source data. A study database was created based on the CRFs and data correction, record keeping, and archiving were performed according to the ICH-GCP guidelines. Recipient data was pseudonymized and analyzed in the group to which they were originally randomly assigned according to an intention-to-treat concept. All patients received their allocated intervention following randomization. Continuous variables were compared with the Mann-Whitney *U* test. For analysis of categorical data, the *chi-square* and the Fisher exact tests were used. The Pearson correlation coefficient was used to express the associations between serum and perfusate transaminase levels. Log-rank test was used for comparisons between Kaplan-Meier curves. Cost estimation was performed according to Staigner et al, using a validated online cost-assessment tool for LT (www.assessurgery.com/cost-prediction).[23] The alpha-niveau for the primary endpoint was set to 0.05, all secondary analyses are explorative, and *P* values will be interpreted descriptively. Values are displayed as median (interquartile range), mean (standard deviation-SD) for metric parameters or absolute and relative frequencies for nominal data. Statistical analysis was performed using SPSS Statistics v24 (IBM Corp., Armonk, NY).

RESULTS

Patient Flow

Supplemental Digital Content Figure 1; http://links.lww.com/SLA/D357 illustrates patient recruitment according to CONSORT guidelines. Of 59 patients screened for eligibility, 46 were randomized. Ten otherwise eligible patients did not receive allografts fulfilling the predefined ECD-criteria and further three consented patients were not randomized and included due to logistical reasons (no perfusionist available or maintenance requirements of the perfusion device). Twenty-three patients were randomized either to the HOPE or SCS arms. All 46 patients were included in the final analysis. In the present trial, no drop-outs, reallocations, or allograft discards were registered following randomization.

Donor, Recipient, Intraoperative, and Perfusion Characteristics

Forty-six recipients [8 (17%) women, median age 62 (55–65) years] were randomly assigned to HOPE (n = 23) and SCS (n = 23). The median calculated Eurotransplant Donor Risk Index (ET-DRI[24]) score indicated an elevated allograft-related risk in our ECD-DBD donor population [ET-DRI 2.050 (1.878–2.218)]. Donor, recipient, intraoperative, and perfusion characteristics were equally distributed between the trial arms.[25] There were no relevant differences among the baseline parameters (Supplemental Digital Content Table 2; http://links.lww.com/SLA/D356).

Peak ALT Within the First 7 Days Postoperatively

Peak ALT during the first 7 days following LT, the primary endpoint, was reduced by 47% in the HOPE compared to the SCS-group [peak ALT days 1–7 418 [221–828] HOPE vs 796 (477–1195) SCS, IU/L, $P = 0.030$, Table 1 and Fig. 1]. An analysis of delta peak ALT was performed; however, in 52% of the allografts the peak ALT represented also the first post-reperfusion ALT-value resulting in a delta value of 1.00 [median delta peak ALT 1.00 (1.00–1.53) HOPE vs 1.08 (1.00–1.89) SCS, $P = 0.262$].

Further Biochemical Findings

Serum ALT remained lower in HOPE patients compared to SCS over the course of the first week (Fig. 1D). Serum AST showed similar characteristics with a 50% decrease of the peak AST levels following HOPE, however, with a marginal nonsignificant difference between the trial arms [peak AST days 1–7: 652 (415–1322) HOPE vs 1312 (576–2514) SCS, IU/L, $P = 0.091$, Table 1 and Fig. 1). Perfusate transaminase levels showed a relatively mild elevation at the end of perfusion in most cases and a good correlation with the serum peak transaminase levels (ALT: $r = 0.687$, $P = 0.001$; AST: $r = 0.897$, $P < 0.001$, Supplementary Figure 1, http://links.lww.com/SLA/D299).

Despite favorable trends in the serum markers of renal function in the HOPE group compared to SCS, no significant difference was found in the early estimated glomerular filtrations rate (eGFR) or peak creatinine values [eGFR days1–7 nadir: 50 (28–75) HOPE vs 28 (18–56) SCS, mL/min/1.73m^2, $P = 0.072$; peak creatinine days 1–7: 1.45 (1.07–2.20) HOPE vs 2.28 (1.33–3.47) SCS, mg/dL, $P = 0.071$, Table 1]. Six months after transplantation, HOPE patients showed slightly but significantly lower total bilirubin levels when compared to SCS, even though the median values remained within normal ranges in both groups [bilirubin 6 months 0.49 (0.41–0.73) HOPE vs 0.87 (0.60–1.18) SCS, mg/dL, $P = 0.018$, Table 1].

Graft- and Patient Outcomes

The rate of major postoperative complications (CD grade ≥ 3) after LT was 44% in HOPE treated allografts versus 74% in the SCS arm, postoperative major complication rates were significantly different between the groups ($P = 0.036$, Table 1). The median 90-day CCI was reduced by 38% in the HOPE group compared to SCS (32 HOPE vs 52 SCS, 90-day CCI points, $P = 0.021$, Table 1 and Fig. 1). This marked difference in cumulative morbidity remained significant at 6 months after the exclusion of patients deceased within the first 90 days (35 HOPE vs 56 SCS, 6 months CCI points, $P = 0.025$, Table 1). The length of the median ICU-stay (5 HOPE vs 8 SCS days; $P = 0.045$) and hospital-stay (20 HOPE vs 36 SCS days; $P = 0.002$) were reduced in the HOPE- compared to the SCS-group (Table 1 and Fig. 1). The rate of early allograft dysfunction did not differ significantly between the patient groups receiving perfused and static stored allografts [incidence of EAD 4 [17%] HOPE vs 8 [35%] SCS, absolute and relative frequency, $P = 0.314$, Table 1).

TABLE 1. Trial Outcomes

Outcome	All Patients (n = 46)	SCS (n = 23)	HOPE (n = 23)	*P*
Biochemical findings and surrogate parameters				
Peak ALT primary trial outcome days 1–7, IU/L, median (IQR)	668 (350–1018)	796 (477–1195)	418 (221–828)	*0.030*
Delta peak ALT days 1–7, IU/L, median (IQR)*	1.00 (1.00–1.60)	1.08 (1.00–1.89)	1.00 (1.00–1.53)	0.262
Peak AST days 1–7, IU/L, median (IQR)	927 (456–1928)	1312 (576–2514)	652 (415–1322)	0.091
Bilirubin day 7, mg/dL, median (IQR)†	1.84 (1.02–3.88]	1.75 (0.83–3.85)	2.39 (1.10–3.96)	0.597
Bilirubin 6 mo, mg/dL, median (IQR)	0.68 (0.43–0.90)	0.87 (0.60–1.18)	0.49 (0.41–0.73)	*0.018*
INR day 7, median (IQR)	1.12 (1.06–1.18)	1.10 (1.06–1.15)	1.12 (1.07–1.19)	0.488
Peak lactate days 1–7, mmol/L, median (IQR)	2.25 (1.60–3.20)	2.20 (1.50–2.85)	2.40 (1.60–3.70)	0.672
Platelet counts day 7, nL, median (IQR)	95 (56–131)	83 (54–121)	96 (79–141)	0.223
Albumin days 1–7 nadir, g/dL, median (IQR)	2.30 (2.16–2.70)	2.39 (2.20–2.60)	2.30 (2.00–2.70)	0.355
eGFR days 1–7 nadir, mL/min/1.73 m^2, median (IQR)‡	36 (21–69)	28 (18–56)	50 (28–75)	0.072
Peak creatinine days 1–7, mg/dL, median (IQR)	1.79 (1.12–2.98)	2.28 (1.33–3.47)	1.45 (1.07–2.20)	0.071
Peak urea days 1–7, mg/dL, median (IQR)	96 (65–151)	118 (75–174)	75 (48–129)	0.053
Other outcomes				
Need for RRT within 90 days, n (%)	14 (30%)	9 (39%)	5 (22%)	0.337
Early allograft dysfunction, n (%)§	12 (26%)	8 (35%)	4 (17%)	0.314
90 Day complications, n (%)‖				
No complications	2 (4%)	0 (%)	2 (8.5%)	0.489
CD 1–2	17 (37%)	6 (26%)	11 (48%)	0.221
CD 3–4	22 (48%)	14 (61%)	8 (35%)	0.077
CD 5	5 (11%)	3 (13%)	2 (8.5%)	>0.999
Major complications (CD ≥3)	27 (59%)	17 (74%)	10 (44%)	*0.036*
Complication type, n (%)				
Primary non-function	2 (4%)	1 (4%)	1 (4%)	>0.999
Hepatic artery thrombosis	2 (4%)	2 (9%)	0 (0%)	0.489
Retransplantation	3 (7%)	2 (9%)	1 (4%)	>0.999
Acute rejection	10 22%)	6 (26%)	4 (17%)	0.722
Biliary (clinical, radiological)	10 (22%)	6 (26%)	4 (17%)	0.722
Pulmonary	7 (15%)	4 (17%)	3 (13%)	>0.999
90-day cumulative CCI, median (IQR)¶	43 (23–90)	52 (35–98)	32 (12–56)	*0.021*
6 mo cumulative CCI, median (IQR)¶	46 (25–82)	56 (36–97)	35 (17–61)	*0.025*
Cost estimation, TEuro, median (IQR)#	45 (35–86)	52 (41–99)	39 (30–55)	*0.016*
Length of ICU stay, days, median (IQR)**	6 (4–15)	8 (5–18)	5 (4–8)	*0.045*
Length of hospital stay, days, median (IQR)/	26 (18–41)	36 (23–62)	20 (16–27)	*0.002*
1-year graft survival	85%	78%	91%	0.253††
1-year patient survival	87%	83%	91%	0.442††
Deaths with/due to the consequence of graft failure‡‡	2	1	1	NA
Deaths with functional grafts§§	4	3	1	NA
Graft loss without death‖‖	1	1	0	NA

*To correct for an assumed washout effect of machine perfusion, besides the absolute values, relative changes of serum peak ALT were assessed. Peak ALT was corrected to the values measured in the routine blood analysis after reperfusion at the time point of admission to the ICU[13].

†Based on total serum bilirubin levels.

‡Based on CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation[16].

§Based on Olthoff et al.[17]

‖Based on Clavien et al.[14]

¶Based on Slankamenac et al, patients with Clavien-Dindo 5 complication (death) within 90 days (n = 5) were excluded from the analysis of 6 months cumulative CCI. [15].

#Based on Staiger et al.[23]

**Length of ICU stay represents the initial stay after the LT procedure until the transfer of the patient to the standard care transplantation unit.

††Kaplan-Meier estimates of survival and log-rank p value. Patients who are yet to complete 1-year follow up were censored at the last study visit at 6 months (n = 4).

‡‡Group SCS: n = 1 early HAT with unsuccessful recanalization -> Candida sepsis -> Not eligible for retransplantation -> MOF (2 months post-LT); Group HOPE: n=1 PNF -> retransplantation -> septic complications (17 days post-LT).

§§Group SCS: n = 1 early recurrent HCC -> rapid progress with metastases (10 months post-LT), n = 1 unclear domestic death after discharge from hospital (2 months post-LT), n = 1 septic complications; Group HOPE: n = 1 cardiac complications.

‖‖Group SCS: n = 1 PNF; Group HOPE: n = 0.

CKD-EPI indicates Chronic Kidney Disease Epidemiology Collaboration; INR, international normalized ratio; NA, not applicable; RRT, renal replacement therapy; TEuro, thousand Euros.

In line with these findings, cost estimation analysis yielded a 25% decrease in the treatment costs over the first 3 months in the HOPE treated patients compared to SCS, not including the approximately 5000 Euro running costs for machine perfusion disposables and perfusion solution [cost estimation 39 (30–55) HOPE vs 52 (41–99) SCS, thousand Euro, $P = 0.016$, Table 1]. The Kaplan-Meier estimates of 1-year graft and 1-year overall survival did not differ between both groups. Patients who did not complete 1-year follow up were censored at the time point of the last study visit at 6 months (n = 4). Additional secondary trial outcomes are summarized in Table 1.

DISCUSSION

This is one of the first RCTs comparing the clinical effects of HOPE with SCS in human liver transplantation, and the first randomized trial focusing on the use of ECD-allografts after DBD. The present trial demonstrates that the beneficial effects of HOPE are not

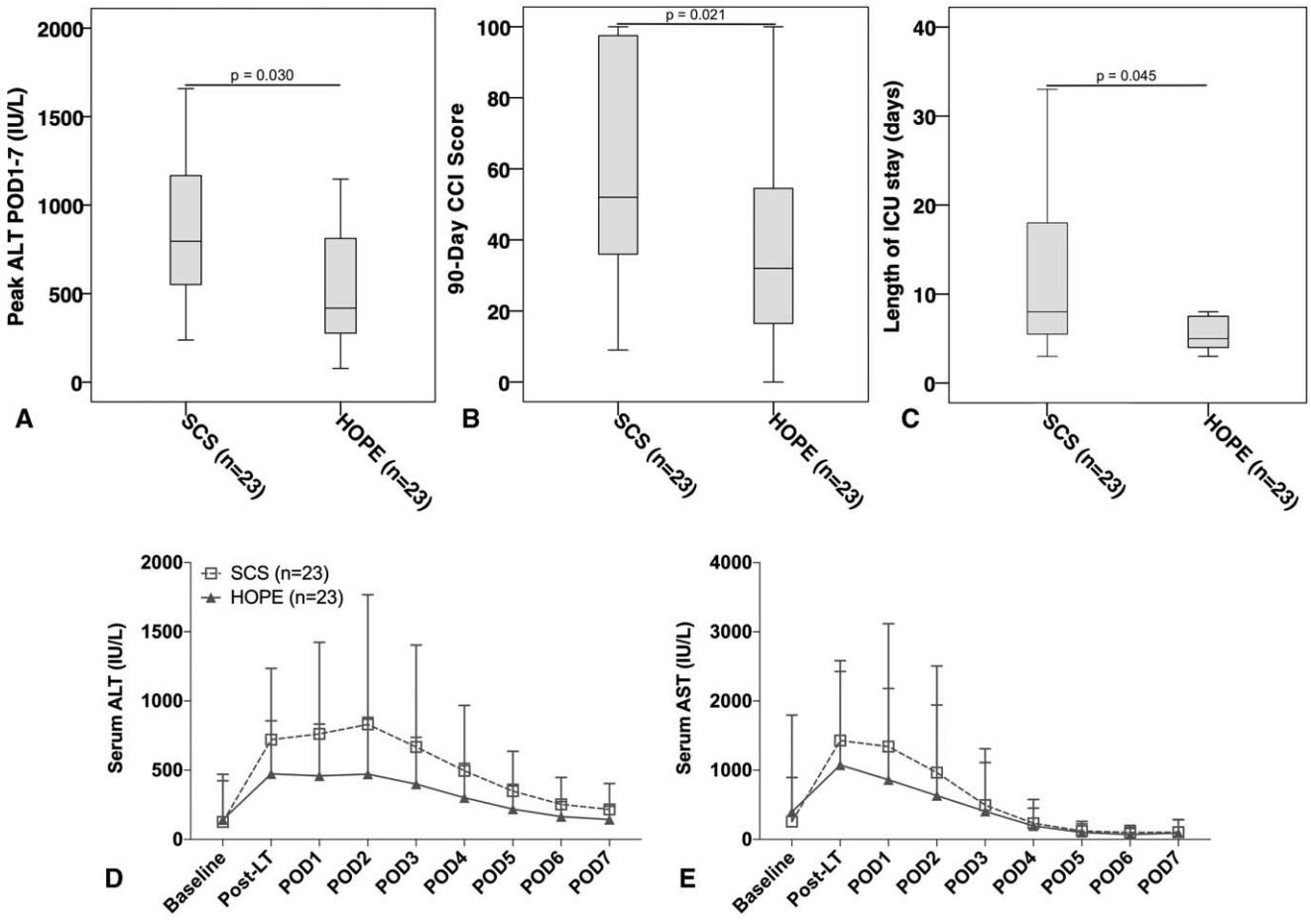

FIGURE 1. Serum peak ALT values over the first week after liver transplantation (A), Cumulative Comprehensive Complication Index 3 months after liver transplantation (B), Length of intensive care unit stay (C), Cumulative ALT and AST levels over the first week following LT (D, E). Data presented as median and interquartile range (A, B, C) or mean and standard deviation (D, E). ICU indicates intensive care unit; IU, international unit; POD, postoperative day.

limited to a reduction of serum ALT and its mitigating effects on early allograft injury but are also associated with reduced perioperative morbidity and shorter ICU- and hospital stays.

In an attempt to further extend the donor pool and to improve clinical outcomes after LT, dynamic organ preservation techniques were introduced.[2,18] The first RCT on *ex-vivo* machine perfusion by Nasralla et al compared upfront NMP to SCS, demonstrating an improved organ utilization rate and lower graft injury after NMP.[2] The first nonrandomized clinical series for end-ischemic HMP by Guarrera et al[18] and HOPE by Dutkowski et al[26] advanced the clinical application of machine perfusion and provided evidence that NMP and HOPE do not only mitigate the effects of IRI, but also improve the overall safety of organ transplantation.[3,7,27–30] Although the beneficial effects of MP were demonstrated in preclinical animal series,[4,31–33] as well as in case-matched human studies[5,12,18] and retrospective single-center analyses,[34,35] data from RCTs comparing HOPE with SCS are not available, yet. Three of six active RCTs on HOPE in human liver transplantation have recently been completed. Although the Groningen and Zurich groups investigated the effects of HOPE in donation after circulatory death (DCD) and DBD, the present trial focuses on HOPE in DBD liver transplantation using allografts with an increased donor risk.[11]

This RCT reveals several important findings. First, end-ischemic treatment with HOPE led to a significant decrease in the primary endpoint, serum peak ALT, indicating a reduced allograft injury after reperfusion. Interestingly, serum ALT levels remained tendentially lower in the HOPE-group throughout the entire observation period of the first postoperative week. The characteristics of transaminase release and the lack of a secondary increase after reperfusion, as it was the case in most of our HOPE recipients, are in contrast to reports by other groups,[13,18] suggesting the absence of a clinically relevant effect of the MP-related *ex-vivo* washout phenomenon on the serum transaminases after reperfusion. This observation may be explained at least in part by relatively short HOPE-perfusion periods, whereas previous studies used significantly longer MP times of 2 to 4 hours. Although choosing ideal endpoints in clinical MP-trials is subject of ongoing debate,[36,37] circulating levels of hepatocellular enzymes are well-recognized as a quantitative markers of hepatocellular injury and were used as surrogates in the past.[2,17,36,38] As such, the peak transaminase release was recently used as the primary endpoint in the first RCT of NMP by the Oxford group.[2]

Another significant finding of our trial is the observation that lower peak levels of ALT after HOPE also correlate with superior post-transplant outcomes. This is particularly important taking into

account that MP is associated with a considerable logistical effort and causes higher operational costs for public health care providers and as such is currently not reimbursed by medical insurances in most countries.[2] In our study, patients receiving HOPE-treated allografts showed markedly reduced overall complications as assessed by the CCI and presented with fewer major complications (CD grade $\geq$3) within the first 3 months following LT. Likewise, patients receiving HOPE-treated ECD-allografts also showed significantly shorter post-transplant ICU- and hospital-stays. Although the costs of a MP-program are substantial (in average five-thousand Euros running-costs per case), our data suggest that the overall procedural costs after transplanting patients with HOPE was 13,000 Euros lower compared to the SCS group (Table 1). Even though no statistical difference was found between the HOPE and SCS groups with regards to biliary complications, our 3 months' biliary complication rate of 22% was slightly higher than the 18% benchmark cut-off defined by Muller et al and as such may also reflect our high-risk allograft population.[39] Furthermore, the vast majority of our recipients had a T-drain cholangiogram performed 6 weeks after LT, which facilitates the detection of clinically or biochemically less apparent biliary abnormalities and may therefore explain the higher rate of diagnosed biliary complications. In this context, the Groningen group recently reported the results of the DHOPE (dual-HOPE) study, the first RCT of hypothermic machine perfusion in human liver transplantation.[27,40] In this large RCT of 160 patients undergoing OLT using DCD allografts, the authors showed a significant reduction of non-anastomotic biliary structures within 6 months after transplantation (6% after dual-HOPE vs 18% following SCS, $P = 0.03$).[27,40] Based on these findings, future studies are warranted to explore whether DHOPE has a significant effect on the incidence of biliary complications in high-risk ECD-DBD livers.

In contrast to previous reports using upfront machine preservation protocols such as the COPE-NMP study by Nasralla et al and the COMPARE trial by Jochmans et al, with 48 and 182 discarded organs after randomization respectively, no allografts discards or drop-out were observed in our trial.[2,41] This is also in line with the recent DHOPE trial,[40] reporting only 4 discarded organs in 160 randomized patients. This may be attributed to a ''back-to-base'' end-ischemic MP-approach that significantly simplifies trial logistics allowing a randomization at the latest possible timepoint (back-table organ preparation) in such trials.

Certain limitations to this trial need to be declared. First, during the recruitment phase of the study, a number of patients in all participating hospitals were not assessed for eligibility. This was mostly attributed to a limited availability of a trained perfusionist and perfusion logistics, and eventually resulted in a prolonged interval for recruitment for the trial. This effect is well known in surgical RCTs and has been described elsewhere in detail.[19,42] Second, standard national and international allocation policies had an indirect selection effect on our patient recruitment. Although ECD-allografts were less frequently allocated to severely ill high- model for end-stage liver disease (MELD) recipients, resulting in a relatively low-MELD study population, the overall donor risk in this cohort was markedly higher compared to previous HMP and NMP trials (donor age 72 vs 39 years in the HMP cohort study by Guarrera et al;[18] and ET-DRI of 2.05 vs 1.71 in the NMP RCT by Nasralla et al, respectively).[2] Third, although HOPE ECD-DBD was designed 5 years ago with no RCT data available at that time, our surrogate primary endpoint does not necessarily reflect recommendations for future MP-trials, as highlighted by the recent consensus guidelines of the International Liver Transplantation Society (ILTS) on machine perfusion.[43] In this regard, our newly designed ''cold versus warm'' end-ischemic HOPE-NMP trial (NCT04644744) uses the Comprehensive Complication Index (CCI), a cumulative scoring system of all major and minor postoperative complications weighted by severity, as the primary endpoint.

Global organ shortage limits the expansion of organ transplantation. Although DCD liver transplantation programs are becoming more popular, the majority of transplants in Europe (91%) are still being performed using allografts derived from DBD,[44] highlighting the global importance and applicability of our findings. Although HOPE is a simple, practical, and cost-efficient pre-implantation perfusion technology, this trial provides for the first-time level-Ib evidence that HOPE, in comparison to SCS, significantly reduces early allograft injury and improves post-transplant outcomes in ECD-DBD liver transplantation.

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The authors dedicate this manuscript to Professor Xavier Rogiers. The authors mourn his passing both as a scientist and a role model.

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DISCUSSANTS

Paolo Muiesan (Birmingham, United Kingdom)

I must congratulate you for this timely and very well-written manuscript that supports the use of HOPE for all ECD-DBD grafts, both in terms of clinical outcomes and from a financial perspective. In addition, I have a quick comment, which regards early allograft dysfunction (EAD). EAD is one of the secondary outcomes and it is only mentioned in the abstract as it trends towards reduction in the HOPE group. My personal opinion is that we should modify this EAD definition because it does not truly reflect EAD for ECD grafts. This is because most ECD grafts meet EAD criteria on transaminases levels only and almost never (never in this manuscript) on day 7 INR or Bilirubin. I agree that transaminases levels have been used by previous machine trials as an expression of graft injury, allowing for a comparison using the same factor. However, I would rather see graft survival as primary outcome, even though it needs a greater number of cases.

I have the following questions: First, the median donor age was 72 years, which represents 1 criterion for an ECD. What were the other criteria to allocate such grafts as ECD (DBD)? Second, this is the first large, clinical, multicenter study on machine perfusion in Germany and your group has also done experimental research in Aachen, and now, in Berlin. Which specific challenges did you see during the implementation of machine perfusion in your country and what would you do differently? Third, the total cold preservation time was comparable between the groups (ca 500 minutes). Did the cold preservation time include the HOPE treatment in the perfusion group, and could the subsequently shorter cold storage duration have impacted on the better results in this group? Finally, acute kidney injury is frequently seen after liver transplantation with ECD livers with high peak transaminases levels and it increases the cost of liver transplantation. Did you observe any differences in postoperative kidney function, the grading of AKI or just the need for hemofiltration after surgery between the study groups?

Response From Georg Lurje (Berlin, Germany)

Dr. Muiesan, thank you very much for your valuable comments. We certainly agree with you on the pitfalls of current binary EAD definitions and on the fact that graft- and patient survival are clinically relevant endpoints in MP-trials, even though they require a very large sample size to reach adequate power. Endpoint selection for MP trials has been a subject of an intense scientific debate in the last years, in which our group has actively participated. This is highlighted by the fact that all four major RCTs on clinical liver MP used a different primary endpoint. When our HOPE ECD-DBD trial was designed 5 years ago, there were no data from RCTs available and circulating levels of transaminases as surrogate parameter for allograft injury have not only been used in the Guarrera HMP series, but also in the Oxford NMP trial, published in Nature in 2018. Even though previous studies have often failed to show a direct link between peak transaminases and clinical outcomes, one strength of our trial is that the beneficial effects of HOPE are not limited to a

reduction of serum ALT but are also associated with better clinical outcomes (reduced perioperative morbidity and shorter ICU- and hospital-stays). This might be attributed to the relatively high-risk allograft population used in our trial (median ET-DRI of 2.050).

With regards to your first question, ECD criteria were adopted in our trial according to the recommendations of the German Medical Chamber, and as also mentioned in our manuscript, they were not only based on the age of 65+ but also included a prolonged ICU stay of the donor, donor obesity, and so on (see Supplemental Digital Content Table 1; http://links.lww.com/SLA/D355).

Regarding your second question, after I was fortunate enough to implement ex-vivo machine perfusion technology in two major German University Hospitals, I see some specific challenges and pitfalls which are predominantly associated with the organization of organ donation and liver transplantation in Germany. First, as in most other countries funding of machine perfusion programs is still largely based on research funding and/or internal funds of the university or the hospital. Second, due to the decentralized nature of retrieval activity with >800 retrieval surgeons in Germany, it is very difficult to implement national standards and logistics for upfront machine preservation techniques as it was done in the UK for the Oxford trial.

Concerning your third question, in our paper we described cold preservation time first as ''total cold preservation time'' including static and dynamic cold preservation with HOPE and second as cold preservation time before HOPE which does not include HOPE treatment in the intervention group. I think a combination of the reduction of static cold storage time plus dynamic reconditioning of the allograft have an additive effect on the outcome showing that the application of even short periods, 2 to 3 hours of end-ischemic HOPE performed during recipient preparation and hepatectomy is still superior compared to conventional static cold storage.

With regards to your last question, I certainly agree with you that acute kidney injury has a very high clinical significance. Although there was a clear – although insignificant – trend toward improved renal function after HOPE, our study was neither powered nor designed to investigate this specific question. In addition, our study participants also presented with significant preoperative renal impairment, complicating the interpretation of these findings.

Antonio Pinna (Weston, FL, USA)

How long was the static cold storage time in the HOPE group?

Response From Georg Lurje (Berlin, Germany)

Dr. Pinna, thank you very much for your question. Median total cold preservation time including dynamic preservation was 495 minutes (8 hours, 15 minutes) and the median time of static preservation before HOPE was 375 minutes (6 hours, 15 minutes).

Pierre-Alain Clavien (Zurich, Switzerland)

Dr. Lurje, congratulations for being able to complete this demanding RCT on the potential benefits of HOPE in ECD liver transplantation. However, you chose ALT peak levels as the primary endpoint, which does not inform us on liver injury or function. In addition, a loss of ALT may occur during machine perfusion, which may distort the results. Can you comment on the clinical relevance of such an endpoint?

Response From Georg Lurje (Berlin, Germany)

Dr. Clavien, thank you very much for your important comment. As I have already mentioned above in my reply to Dr. Muiesan, endpoints of MP trials have been the subject of an ongoing scientific debate over the past years. In 2016, when we designed our trial, most, if not all, active trials used exactly this or very similar surrogate endpoints. Even though such endpoints have important limitations, transaminases are probably still relevant in marginal livers, especially since we could see that clinical outcome in terms of morbidity correlated well with the primary surrogate endpoint in our RCT. As recommended in the recent ILTS consensus paper on endpoints in MP-trials, our new HOPE-NMP (NCT04644744, hopeliver.com) machine perfusion trial uses the broadly recognized Comprehensive Complication Index as the primary end-point.

Mickaël Lesurtel (Lyon, France)

How long did you perfuse the livers? Was it single or dual perfusion?

Response From Georg Lurje (Berlin, Germany)

Perfusion was performed as single HOPE via the portal vein for at least 1 hour as per protocol (median 145 minutes).

Gabriel Oniscu (Edinburgh, United Kingdom)

Very nice work. Did you see a difference in the incidence of acute rejection (as seen in the COPE kidney study)?

Response From Georg Lurje (Berlin, Germany)

Dr. Oniscu thank you for your interesting comment. Over the first 3 months, we could not find any significant difference in acute rejection episodes (Table 1). We will investigate this further in our subsequent article during the whole follow-up period as well as in the ongoing HOPE-NMP trial (hopeliver.com).

Diethard Monbaliu (Leuven, Belgium)

First, I would like to have more information on cost reduction. Was the cost of HOPE included? Second, what was the incidence of nonanastomic biliary strictures?

Response From Georg Lurje (Berlin, Germany)

Dr. Monbaliu, thank you for your important questions. Cost-reduction was reported as cost estimation based on the 90-day CCI values of a previous work completed by the Zurich group (Staiger RD et al, *Ann Surg*. 2018, PMID: 30272585) and did not include costs for HOPE itself (approx. 5000 Euro per case in our center). If we include this, we still yield in a relevant cost reduction. A proper cost analysis of the ''real'' costs, which also includes the expenses of the perfusion consumables and logistics of this RCT, is on its way. Even though our study protocol did not include an MRCP after 6 months, the total incidence of all biliary complications was 22% in our cohort of marginal DBD allografts, without a significant difference between the intervention and control groups.