Routine End-ischemic Hypothermic Oxygenated Machine Perfusion in Liver Transplantation From Donors After Brain Death

A Randomized Controlled Trial

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Objective: To assess whether end-ischemic hypothermic oxygenated machine perfusion (HOPE) is superior to static cold storage (SCS) in preserving livers procured from donors after brain death (DBD).

Background: There is increasing evidence of the benefits of HOPE in liver transplantation, but predominantly in the setting of high-risk donors.

Methods: In this randomized clinical trial, livers procured from DBDs were randomly assigned to either end-ischemic dual HOPE for at least 2 hours or SCS (1:3 allocation ratio). The Model for Early Allograft Function (MEAF) was the primary outcome measure. The secondary outcome measure was 90-day morbidity (ClinicalTrials. gov, NCT04812054).

Results: Of the 104 liver transplantations included in the study, 26 were assigned to HOPE and 78 to SCS. Mean MEAF was 4.94 and 5.49 in the HOPE and SCS groups ($P = 0.24$), respectively, with the corresponding rates of MEAF > 8 of 3.8% (1/26) and 15.4% (12/78; $P = 0.18$). Median Comprehensive Complication Index was 20.9 after transplantations with HOPE and 21.8 after transplantations with SCS ($P = 0.19$). Transaminase activity, bilirubin concentration, and international normalized ratio were similar in both groups. In the case of donor risk index > 1.70, HOPE was associated with significantly lower mean MEAF (4.92 vs 6.31; $P = 0.037$) and lower median Comprehensive Complication Index (4.35 vs 22.6; $P = 0.050$). No significant differences between HOPE and SCS were observed for lower donor risk index values.

Conclusion: Routine use of HOPE in DBD liver transplantations does not seem justified as the clinical benefits are limited to high-risk donors.

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Ischemia-reperfusion injury (IRI) of the allograft is a direct cause of a series of adverse outcomes after liver transplantation (LT), ranging from transient early allograft dysfunction (EAD) to primary nonfunction and ischemic cholangiopathy.[1] This phenomenon involves a cascade of events occurring during ischemia and directly after restoration of blood flow, including ongoing ATP depletion, accumulation of metabolites, cellular necrosis before reperfusion, generation of reactive oxygen species, induction of proinflammatory response, and subsequent loss of viable cells.[1,2] To reduce IRI, static cold storage (SCS) of livers procured from deceased donors has been the gold standard for organ preservation for decades.[3]

The increasing gap between the demand for LT and the number of available donors has led to increased utilization of organs more prone to the deleterious effects of IRI and, thus, at a higher risk of negative outcomes. These include grafts with significant macrovesicular steatosis, procured from older donors or donors after cardiac death.[4] To improve post-LT outcomes with an increasing number of high-risk donors, numerous studies have focused on alleviating IRI or even eliminating ischemia.[5–8] Among the available methods, the introduction of machine perfusion has changed the landscape of organ preservation in deceased donor LT.[9] The spectrum of available protocols includes hypothermic (0–12°C) and normothermic (35–38°C) oxygenated machine perfusion after SCS (end-ischemic perfusion) or starting immediately after procurement, and normothermic regional perfusion, among others.[10–14] While end-ischemic normothermic perfusion is mainly aimed at viability assessment, the rationale for using hypothermic perfusion is based on reducing IRI.[15]

Hypothermic oxygenated machine perfusion (HOPE) may be performed either through the portal vein or through both the portal vein and hepatic artery (dual HOPE, dHOPE).[16,17] End-ischemic HOPE alleviates IRI through its protective effect on mitochondria by inducing a shift from high-flux to low-flux electron transfer, which prevents rapid postreperfusion

production of reactive oxygen species.[9] Downstream effects include increased hepatic adenosine triphosphate content, lower apoptosis, and necrosis rates, decreased oxidative damage, less endothelial injury, and reduced proinflammatory response.[18–24] The clinical benefits of HOPE, including decreased risk of non-anastomotic strictures, postreperfusion syndrome, and EAD, are predominantly reported for LTs from high-risk donors.[25,26] Therefore, the aim of this randomized trial was to evaluate the potential benefits of HOPE compared with SCS in LTs from donors after brain death (DBD) in general.

METHODS

This randomized parallel controlled trial compared dHOPE and SCS in organ preservation for DBD LT (clinicaltrials.gov: NCT04812054). All patients undergoing LT in the department were screened for inclusion. Recruitment took place between April 2021 and May 2022, and follow-up for short-term outcomes was completed on August 18, 2022. The inclusion criteria were age above 18 years, deceased donor LT, and provision of informed consent. LT from donors after cardiac death or utilization of either reduced or split grafts was set as the exclusion criteria. The study was conducted in accordance with the ethical standards of the Helsinki Declaration of 1975, and the institutional review board approved the protocol (KB/6/2020). All patients provided informed consent before their inclusion.

Immediately after acceptance, the organs were randomly assigned to either dHOPE or SCS by one of the investigators in the department. Randomization was performed by drawing a sealed envelope with a computer-generated allocation code with a 1:3 allocation ratio in blocks of 8. Procured livers were flushed with 1 L of cold perfusate during back-table preparation. Organs allocated to dHOPE underwent at least 2 hours of HOPE through both the portal vein (continuous flow: pressure 3–5 mm Hg) and hepatic artery (pulsatile flow: systolic pressure 30 mm Hg; diastolic pressure 20 mm Hg) with University of Wisconsin machine perfusion solution (12°C) using a Liver Assist device (Organ Assist, now XVIVO), which was performed by an additional team of 2 physicians. A flow of at least 500 mL/min of 100% oxygen was supplied to the oxygenators to maintain a perfusate partial oxygen pressure of $\geq$450 mm Hg. In the case of ongoing hepatectomy after 2 hours of dHOPE, it was continued until the beginning of implantation. Organs allocated to SCS were stored in Institut Georges Lopez-1 solution at 4°C until implantation. While the patients were unaware of the group assignment, the surgical team was not blinded due to the nature of the intervention.

The Model for Early Allograft Function (MEAF), based on serum bilirubin concentration and alanine transaminase activity, and international normalized ratio during the first 3 posttransplant days was the primary outcome measure.[27] The primary hypothesis was that dHOPE improves early allograft function in comparison with SCS, as reflected by a decrease in mean MEAF from 5.0 to 3.5. With an SD of 1.99 and thresholds for type I error of 5%, and power of 90%, the numbers of enrolled patients required to detect such difference were 26 in the dHOPE group and 78 in the SCS (total of 104 patients). The mean MEAF of 5 was based on the original study and validated in our retrospective cohort of 144 transplantations (similar value of 5.2). Decrease to 3.5 was chosen arbitrarily, in search for a clinically meaningful difference. Complications occurring over the 90-day postoperative period graded according to the Clavien-Dindo classification were the secondary outcome measure.[28] Cumulative morbidity was expressed as the Comprehensive Complication Index (CCI®).[29] The occurrence of liver allograft-related complications, as previously classified, was analyzed separately.[30] Data on the remaining secondary outcome measures, namely 2-year patient and graft survival, as well as the occurrence of biliary complications, are not available yet. In addition, a 7-day liver graft assessment following transplantation (L-GrAFT$_7$) risk score was calculated.[31] Postreperfusion syndrome was defined as a decrease in mean arterial pressure by at least 30% within 5 minutes after reperfusion, lasting for at least 1 minute.[32] Extended-criteria donors were identified according to a previously published definition.[25]

Baseline recipient-related, donor-related, and transplant procedure-related factors were compared between the groups. The donor risk index (DRI) was calculated for graft quality assessment.[33] Graft steatosis was assessed in routine wedge biopsies by an experienced pathologist.

Quantitative and qualitative variables are presented as medians with interquartile ranges (IQRs) or means with SDs or SEs and numbers with percentages, respectively. Student t test, Mann-Whitney U test, and Fisher exact test were used for comparisons. Mixed models were applied for the longitudinal analyses of laboratory tests. Associations between MEAF and the occurrence of severe complications and mortality were analyzed using logistic regression. Odds ratios (ORs) were calculated with 95% CIs. Statistical significance was set at P value <0.05. SAS/STAT, version 15.2 (2020; SAS Institute Inc.) was used to perform statistical analyses.

RESULTS

A total of 104 patients were included, with 26 assigned to the dHOPE group and 78 to the SCS group (Supplemental Digital Content, Figure 1, http://links.lww.com/SLA/E821). The baseline characteristics of the patients are presented in Supplemental Digital Content, Table S1 (http://links.lww.com/SLA/E820). DRI was higher in the dHOPE group, with a significantly higher donor body mass index and similar other donor characteristics. Patients in the SCS group were more frequently in Child-Turcotte-Pugh classes B and C but had a similar model for end-stage liver disease. The total duration of hypothermic organ preservation was longer and the duration of static cold ischemia was shorter in the dHOPE group.

In patients assigned to the dHOPE group, the median duration of the procedure was 120 minutes. Perfusion was prolonged due to ongoing hepatectomy in 12 patients (46.2%) up to 135 to 220 minutes. The median arterial and portal flow rates were 95 mL/min (IQR: 78–124 mL/min) and 284 mL/min (IQR: 168–394 mL/min), respectively. The median flows throughout the first 120 minutes of dHOPE are presented in Supplemental Digital Content 1 (http://links.lww.com/SLA/E794).

There was no difference in the mean MEAF score between the groups (P = 0.24), which was 4.94 (SD: 1.72) in the dHOPE group and 5.49 (SD: 2.14) in the SCS group (Table 1). The proportions of patients with MEAF $\geq$7 and $\geq$8 were 15.4% (4/26) and 3.8% (1/26), respectively, in the dHOPE group, and 24.4% (19/78; P = 0.42) and 15.4% (12/78; P = 0.18), respectively, in the SCS group. The mean L-GrAFT$_7$ score was −1.76 (SD: 1.40) in the dHOPE group and −1.75 (SD: 1.58) in the SCS group (P = 0.99). Postreperfusion syndrome occurred in 23.1% (6/26) of recipients of transplantations with dHOPE and in 19.2% (15/78) of recipients of transplantations with SCS (P = 0.78). No significant differences were found between the groups with respect to the 7-day posttransplant serum bilirubin concentration (P = 0.89), aspartate transaminase activity (P = 0.50), alanine transaminase activity (P = 0.81), and international normalized ratio (P = 0.13; Fig. 1). There were no cases

TABLE 1. Comparisons of LT Recipients Assigned to Organ Preservation With dHOPE and SCS With Respect to Primary and Secondary Prespecified and Not Prespecified Outcome Measures

Outcome measures	HOPE (N = 26)	SCS (N = 78)	*P*
Primary outcome measure			
Model for early allograft function	4.94 (1.72)	5.49 (2.14)	0.24
Secondary outcome measures			
Any complication	17 (65.4)	59 (75.6)	0.32
Grade ≥2 complications	15 (57.7)	53 (67.9)	0.35
Grade ≥3 complications	8 (30.8)	36 (46.2)	0.25
Grade ≥4 complications	4 (15.4)	18 (23.1)	0.58
Mortality	1 (3.8)	6 (7.7)	0.68
CCI®	20.9 (0–39.7)	21.8 (8.7–47.6)	0.19
Other (not prespecified) outcome measures			
Postreperfusion syndrome	6 (23.1)	15 (19.2)	0.78
Model for early allograft function ≥8	1 (3.8)	12 (15.4)	0.18
L-GrAFT$_7$	−1.76 (1.40)	−1.75 (1.58)	0.99
Intensive care unit stay	5.0 (4.0–6.0)	4.5 (4.0–6.0)	0.25
Primary nonfunction	0 (0.0)	3 (3.8)	0.57
EAD*	7 (26.9)	26 (33.3)	0.63
EASE score†	0.72 (0.82)	0.72 (0.95)	>0.99

Data are presented and n (%), mean (SD), or median (IQR).
*Defined according to criteria by Olthoff et al.[34]
†Calculated according to Avolio et al.[35]

of primary nonfunction with dHOPE and 3 (3.8%) with SCS ($P = 0.57$).

A total of 76 patients developed at least 1 complication: 17 (65.4%) in the dHOPE group and 59 (75.6%) in the SCS group ($P = 0.32$; Supplemental Digital Content 2, http://links.lww.com/SLA/E794). There were no differences in the frequencies of Clavien-Dindo grade ≥2 ($P = 0.35$), grade ≥3 ($P = 0.25$), grade ≥4 ($P = 0.58$), or mortality between the groups ($P = 0.68$; Table 1). Furthermore, no significant differences were found in the median CCI® ($P = 0.19$). Grade ≥3 graft-related complications occurred in 3 (11.5%) and 15 (19.2%) patients in the dHOPE and SCS groups, respectively ($P = 0.37$).

In the entire cohort, increasing MEAF was a significant risk factor for both the development of grade ≥3 complications (OR: 1.38 per 1-point increase; 95% CI: 1.11–1.70; $P = 0.003$) and mortality (OR: 2.61 per 1-point increase; 95% CI: 1.40–4.88; $P = 0.003$). Significant associations between MEAF and both severe morbidity (OR: 1.32 per 1-point increase; 95% CI: 1.05–1.66; $P = 0.017$) and mortality (OR: 3.92 per 1-point increase; 95% CI: 1.43–10.80; $P = 0.008$) were confirmed in patients after transplantation with SCS. In the dHOPE group, the association between MEAF and severe morbidity was above the level of significance (OR: 1.61 per 1-point increase; 95% CI: 0.92–2.82; $P = 0.096$). The effect of MEAF on mortality in the dHOPE group was not analyzed because there was only 1 death.

In post hoc analysis of transplantations with high DRI (>1.70), dHOPE was associated with significantly lower MEAF ($P = 0.037$) and lower CCI® ($P = 0.050$; Table 2). No significant effects of dHOPE were found in patients who underwent LT with DRI ≤1.70.

DISCUSSION

The results of this randomized study indicate that the routine use of HOPE does not improve early graft function after DBD LT. Patients undergoing LT preceded by dHOPE and SCS had similar composite scores, indicating early allograft function, namely, MEAF, L-GrAFT$_7$, and Early Allograft Failure Simplified Estimation (EASE). Furthermore, dHOPE did not improve the laboratory results.

This is the fourth randomized trial to compare HOPE with SCS in DBD LT. Contrary to the results of the present study, Czigany et al[25] found significantly lower transaminase activity after DBD LT preceded by HOPE. A comparison of patient outcomes after 23 procedures preceded by HOPE and 23 preceded by SCS also revealed that the former is associated with a lower risk of severe morbidity, decreased CCI®, and shorter intensive care unit stay. However, that study was restricted to LTs from extended-criteria donors. In the present study, including both standard-criteria and extended-criteria donors, no significant benefits of dHOPE with respect to 90-day morbidity were found. This is in line with the recently published results of a multicenter randomized trial with 85 HOPEs and 85 controls, showing a similar 1-year rate of severe morbidity, CCI®, and laboratory results.[30] Nevertheless, the authors of that study performed post hoc analysis, which revealed that the number of grade ≥3b graft-related complications and graft-related CCI® were lower after HOPE. The third published randomized study on HOPE in DBD LTs, including 110 recipients predominantly with hepatocellular carcinoma, was also limited to extended-criteria donors.[36] The results pointed towards fewer EADs, fewer intermediate or high EASE score values, fewer complications, and improved short-term graft survival. Several retrospective studies have also pointed towards a reduced risk of morbidity, postreperfusion syndrome, EAD, and lower activity of transaminases after transplantations with HOPE.[37–39] Although the present study failed to provide evidence for a significant reduction in general and graft-related severe morbidity, the rates were remarkably yet nonsignificantly lower after dHOPE.

Considering the results of the present study and previous randomized trials, we hypothesized that HOPE improves LT outcomes, yet only in cases of selected high-risk grafts. This was confirmed in a post hoc analysis, which showed significant improvement in early allograft function and lower cumulative morbidity after HOPE in transplantations with high DRI. Regardless of the statistical significance, early allograft function and severe complication rates were almost identical after LT with HOPE and SCS in the case of low DRI. Therefore, this analysis confirms the benefits of HOPE for livers procured from high-risk donors and explains the lack of a significant effect on predefined outcome measures for this and a single previous all-in DBD trial.[30]

The choice of primary outcome measure based on laboratory findings in machine perfusion trials is being widely criticized for unknown clinical relevance.[30,40] However, MEAF was chosen as a primary outcome measure for this study as it is a previously well-validated surrogate of early allograft function independently associated with the strongest clinical end-point – transplant survival.[41–43] Importantly, MEAF was a significant predictor of both severe morbidity and mortality in all the patients included in the present study. Although the level of significance was not reached in the dHOPE group, the ORs for the association between MEAF and the development of severe complications were consistent in both arms. Furthermore, the potential effect of machine perfusion on laboratory values with no clinical relevance, such as washout of transaminases, seems contradictory to the similar laboratory results obtained after dHOPE and SCS in the present study. The 2 other quantitative indicators of early allograft function, namely EASE score, and L-GrAFT$_7$, were previously reported to outperform MEAF.[31,35] Nevertheless, these were also similar after dHOPE and SCS.

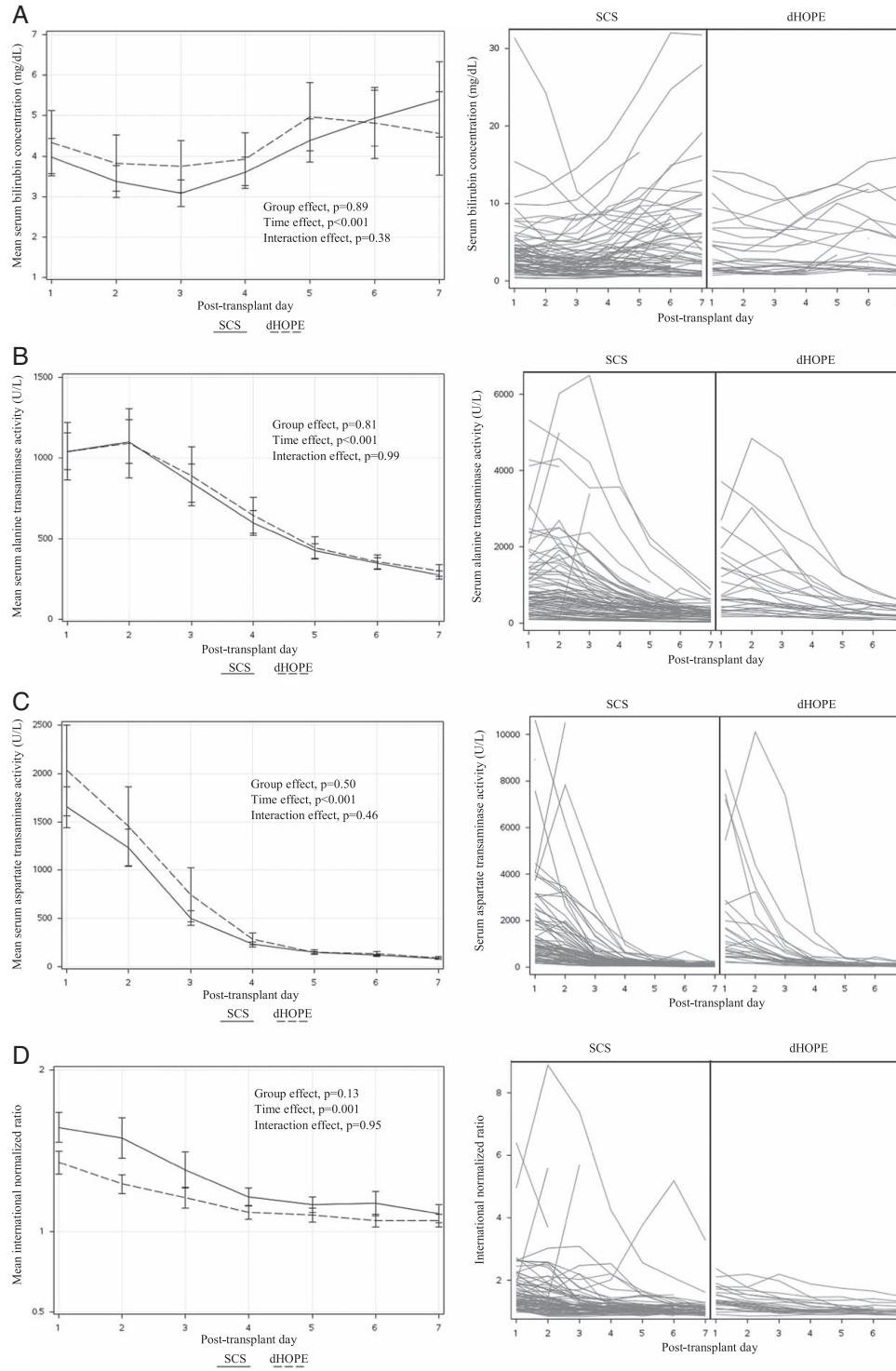

FIGURE 1. Mean values with SEs (left panel) and spaghetti plots (right panel) of serum bilirubin concentration (A), activity of alanine transaminase (B), aspartate transaminase (C), and international normalized ratio (D) in recipients of LT after dHOPE.

While the present study points towards the selective use of HOPE in LT for high-risk donors, the results focus on the early clinical perspective. Further studies are needed to determine the impact of HOPE on long-term outcomes in this setting, such as the occurrence of biliary complications, the incidence of cancer recurrence, and survival rates.[26,39,44–46] Nevertheless, considering that the potential protective mechanism is the reduction of IRI for all of these clinical endpoints, there is currently no evidence for the routine application of HOPE in DBD LT.

Another important issue associated with HOPE is logistics, depending on the local policy regarding the timing of donor and recipient procedures. Its use may increase costs, requires more human resources, and may prolong the duration of the transplantations that start before the return of the procurement

TABLE 2. Comparisons of LT Recipients Assigned to Organ Preservation With dHOPE and SCS With Respect to Primary and Secondary Outcome Measures According to DRI

	DRI ≤1.70			DRI >1.70		
Outcome measures	**HOPE (N = 12)**	**SCS (N = 52)**	***P***	**HOPE (N = 14)**	**SCS (N = 26)**	***P***
Primary outcome measure [mean (SD)]						
Model for early allograft function	4.98 (1.86)	5.08 (2.07)	0.88	4.92 (1.67)	6.31 (2.07)	0.037
Secondary outcome measures						
Any complication	10 (83.3)	38 (73.1)	0.71	7 (50.0)	21 (80.8)	0.071
Grade ≥2 complications	9 (75.0)	34 (65.4)	0.74	6 (42.9)	19 (73.1)	0.089
Grade ≥3 complications	5 (41.7)	24 (46.2)	>0.99	3 (21.4)	12 (46.2)	0.18
Grade ≥4 complications	2 (16.7)	10 (19.2)	>0.99	2 (14.3)	8 (30.8)	0.45
Mortality	1 (8.3)	2 (3.8)	0.47	0 (0.0)	4 (15.4)	0.28
CCI®	21.8 (14.8–39.7)	20.9 (0.0–43.5)	0.91	4.35 (0.0–22.60)	22.60 (8.70–58.10)	0.050

Data are presented as n (%) or median (IQR).

team. In contrast, prolonged use of HOPE has been shown to safely facilitate logistics in case of unexpected delays.[47]

The present study has several limitations. Despite randomization, patients in the dHOPE group had a lower Child-Turcotte-Pugh class. However, this would enhance, rather than prevent, the beneficial effect of the procedure. There were minor differences in the donor data between the groups, yet were addressed by a post hoc analysis with stratification by DRI. Furthermore, the study was adequately powered for primary and not for secondary outcome measures. Nevertheless, the findings of the post hoc analysis with almost identical morbidity after transplantations with low DRI and significantly lower morbidity associated with HOPE when DRI was high, indicate that potential type II error does not seem to be an important bias. Higher patient numbers would probably increase the chance of reaching significance, yet this would be driven by the high-risk donor subpopulation. Finally, the study results should be interpreted in the context of a limited number of dHOPE procedures.

In conclusion, our results do not support the routine use of HOPE in all DBD LTs from the early outcomes perspective. However, the results of the exploratory analysis suggest, in line with previously published studies, that HOPE exerts beneficial effects on early LT outcomes from high-risk DBDs.

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DISCUSSANT

Hugo Pinto Marques (Lisbon, Portugal)

Routine end-ischemic hypothermic oxygenated perfusion has proven beneficial in selected donors after cardiac death. The placement of hypothermic oxygenated perfusion in brain dead donors is still a topic of debate. The paper is well-written, and the study is well-designed. It focuses on a relevant, and contemporary subject. The paper generally concludes that the systematic application of HOPE in donors after brain death has no benefit, although a subgroup analysis concludes that for it may be beneficial DRI >1.7.

I have several remarks and questions: First, the study population comprises of a significant proportion of CHILD A patients with a mean BMI of 24 to 26. I am not sure this reflects the reality in most transplant centers. Please comment.

Second, hypothermic perfusion under these conditions demands more human resources. Were the teams modified for the procedure using HOPE?

Third, although short-term complications were addressed, long-term complications, such as biliary complications, were not studied. Considering the follow-up time, this should be assessed.

Fourth, in a sub-analysis, the authors found HOPE to be beneficial in high-risk donors. Since this was not the primary endpoint, could the authors please comment on the robustness of the result? The authors acknowledge this limitation in the discussion.

Fifth, I also note that there is an important lack of homogeneity between the two groups (35% of DRI >1.7 patients had HOPE, compared to only 18% of patients in the DRI <1.7 group).

Finally, the authors describe a 10-15% reduction of all 90-day complications with HOPE, and a reduced mortality rate of 3.8% from 7.7%. Although such findings did not achieve significance, they are relevant and should not automatically lead to the conclusion that the perfusion of donor livers with a DRI of <1.7 is not needed.

The conclusion that HOPE is not necessary for liver transplantation (LT) using donors with a low DRI is very important, although some benefits cannot be completely excluded. The use of HOPE in LT, using donors with a high DRI, can be beneficial, though we still have to consider the limitations of the study.

Response From Michał Grąt (Warsaw, Poland)

Thank you for your important remarks. First, regarding the study population, in our opinion, it reflects the real-world scenario. In the most recent randomized trials published in the *Journal of Hepatology*, the overall proportion of CHILD A patients was 29%, very similar to the 31% rate in the cold storage group. We do, however, acknowledge that the proportion of CHILD A patients was higher in the HOPE group. Yet, as we stated in our discussion, this could favor the HOPE group, instead of increasing the chances of having a false negative study. Considering the recipient BMI, the median value in the recent ELTR-based study, which included over 46,000 liver transplant patients, was 26. This is almost identical to what we saw in our study.

Regarding your second remark on human resources, we completely agree. A separate team of at least two people, usually residents, was involved in the HOPE procedure over the course of the project.

Third, regarding long-term outcomes, we do not feel comfortable illustrating the long-term data in the manuscript, as the follow-up is still ongoing. In our viewpoint, we will need to await the complete follow-up.

In terms of the sub-analysis, we completely agree that randomization failed to provide fully homogenous groups in our study. On the other hand, we have demonstrated an important benefit of HOPE in the high DRI group, despite low numbers. The clinical and biochemical outcomes were not only statistically not significant, but they were also nearly identical in the subgroup of patients receiving livers from donors when the DRI was under 1.7.

Finally, regarding the insignificance of the 10-15% reduction of all 90-day complications and reduced mortality with HOPE, when we noticed this, we tried to determine what was driving this difference; however, again, in the low-risk donor group, the values were almost identical.

Pierre-Alain Clavien (Zurich, Switzerland)

Thank you for this important presentation. I have two concerns that I need to express. First, this study is largely underpowered, and therefore, yields a high risk of ending up negative. Enrolling only 26 patients, in the absence of a proper sample calculation (i.e. arbitrarily chosen delta of the primary endpoint) may lead to grossly incorrect results, particularly when dealing with so many confounding factors inherent to the procedure of liver transplantation. In a recently published study on HOPE by our group, which included more than 80 patients per group, covering a 1-year follow-up, we failed to reach significance with a highly clinically relevant endpoint: the comprehensive complication (CCI®). This was due to the high recipient morbidity in this population. We only found a benefit for HOPE in a subgroup analysis for higher risk liver grafts. RCT offers only one primary endpoint. Consequently, a sample size that is too small may fail to capture differences and lead to a misleading, negative study, called a Type II error. Based on this, I must disagree with your interpretation. You did not reach the main endpoint, but you found that HOPE is beneficial in higher-risk patients, defined arbitrarily by DRI >1.7. Likewise, a higher caseload would lead to different DRI cut-offs.

Second, I must call for caution when it comes to your conclusion that HOPE should not be routinely used. We all have experience with assumed "near-perfect grafts" which unexpectedly fail after implantation. What we need during HOPE is an easily available marker that reliably predicts the outcome after reperfusion. The recent advent of mitochondrial biomarker measurements, such as flavin mononucleotide (FMN), easily assessed by a real time photometric assay, may provide information on the quality of the graft. Personally, at this stage, I would lobby for the routine use of HOPE for graft assessment besides treatment until a conclusive RCT is made available, including quality assessment, such as FMN measurements, because preventing the transplantation of a "failed liver" is important.

Response From Michał Grąt (Warsaw, Poland)

I have great respect for you, Prof. Clavien, but I fully disagree with what you said. First, when we have an endpoint based on laboratory results in perfusion trials, the main criticism is a high-risk of acquiring false positive results. In this study, we showed that the MEAF was nearly identical, regardless of whether machine perfusion was used. Regarding the power calculations, I agree that this study was severely underpowered for the secondary outcome measures. However, for the primary outcomes, it was accurately calculated. We have 90% power, with 5% of Type I error for detecting a difference in only 1.5 in mean MEAF. Therefore, the risk of type II error in detecting clinically relevant difference in post-transplant graft function seems negligible.

Second, concerning the RCT that you mentioned, it was also a negative study. None of the primary and secondary endpoints were reached, and therefore, both studies seem consistent. Please note that in the low DRI group, we did not fail to detect non-significant differences; rather, we found absolutely no differences when looking at the numbers. Accordingly, I agree that increasing the sample size would probably lead to a significant difference that would, however, be driven only by patients receiving high DRI transplants.

Mickaël Lesurtel (Clichy, France)

This topic is becoming increasingly interesting. In France, we've just completed a study on HOPE versus no HOPE, including 133 patients in each group. We should have the results ready by the end of summer. Since you have interesting results with a DRI of more than 1.7, would you be able to look at only the extended criteria donor to see whether there is something of interest to be drawn from this subgroup analysis?

Response From Michał Grąt (Warsaw, Poland)

We haven't published this yet, but we've already done it. We have chosen the same definition of extended criteria donors that was utilized in the 2021/2022 *Annals of Surgery* study, in order to keep the data homogeneous. I don't recall the actual number, but it was about 60% of extended criteria donors in one group and 50% in the other group. Using DRI for subgroup analyses worked well for finding significant benefits of HOPE in higher risk transplants. Using the definition of extended criteria donors for subgroup division failed to reveal significant benefits of HOPE. This was probably driven my multiple aspects. This definition was based on several factors, e.g. hospital distance. So, in our population, the DRI seemed to work better when identifying high-risk donors.

Antonio D. Pinna (Weston, United States)

Let's talk about the good grafts. Good grafts had a total *ex vivo* time, which was higher than cold ischemia. So, most likely, the next step could be extended HOPE for good grafts, i.e. pumping for 10 hours on HOPE. Do you agree? Also, why did you opt for DHOPE over the simple HOPE?

Response From Michał Grąt (Warsaw, Poland)

It's a tricky question. In all honesty, when we bought the perfusion machine, since we had two cannulas, we decided to use them both for the hepatic artery and portal vein, especially since there is currently no robust data on the superiority of either perfusion technique.

Regarding the prolonged use of HOPE, this is certainly one way to avoid nighttime operations. We can use it for logistical purposes. However, we need to know what our aim is, and this study aimed to reveal the clinical benefits of HOPE in DBD transplantations.