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MAIN TEXT

End-ischemic hypothermic oxygenated machine perfusion does not improve renal outcome following liver transplantation from aged donors: A single-center retrospective report

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Abstract

Background: Organ transplantation using grafts from elderly donors entails a higher risk for severe ischemia-reperfusion injury (IRI). Advanced IRI after liver transplantation (LT) seems to be associated with the development of acute kidney injury (AKI). We studied if end-ischemic hypothermic oxygenated machine perfusion (HOPE) of liver grafts, aimed at mitigating liver IRI, impacts on the frequency and severity of AKI after LT.

Methods: LTs performed at our center between January 2017 and December 2022 using organs from deceased brain-dead donors aged 70 or older were reviewed. From November 2020 on, HOPE was performed routinely in this donor category. The frequency and severity of AKI (KDIGO criteria) within 48 hours of graft reperfusion and the model of early allograft function (MEAF) were compared between HOPE-LTs ($n = 30$) and control LTs ($n = 71$).

Results: AKI developed in 23/30 (77%) HOPE-LTs and in 40/71 (56%) control LTs ($p =$ n.s.), with no difference in severity and timing between groups. Renal replacement therapy was required in 3/30 (10%) HOPE-LTs and 6/71 (8%) control LTs. In addition, transaminase leak during the first week (marker of IRI) and MEAF were similar between groups. These findings persisted after propensity matching. Histology showed more hepatocyte vacuolization and higher Suzuki score in HOPE-LTs. Although this analysis could have been underpowered, no trends supporting the benefit of HOPE on liver and renal injury after LT were ever identified.

Conclusions: In conclusion, HOPE in this group of older donors does not seem to improve either graft IRI, or the incidence of early AKI after LT.

KEYWORDS

acute kidney injury, hypothermic oxygenated machine perfusion, liver transplantation, machine perfusion

1 | INTRODUCTION

The growing demand for organs for transplantation has gradually led to the increasing use of extended criteria donors (ECDs), previously deemed unsuitable for transplantation. Definition criteria for ECDs vary between studies and centers but always include elderly donors, donors with long intensive care unit stay prior to organ procurement, or donors after circulatory death (DCD), among others. Nevertheless, liver transplantation (LT) using organs from these donors in general and elderly donors in particular, entails a higher risk of developing a range of complications, including primary graft nonfunction or early allograft dysfunction (EAD), a more advanced ischemia-reperfusion injury (IRI), biliary complications and prolonged hospital stay[1,2]). Several approaches to reduce these risks have been explored, including changes in organ allocation as well as several types of interventions in the donors or on the organs aimed for transplantation. Among the latter, perfusion preservation strategies represent a key development, which may allow a safe increase in the number of transplantable organs by mitigating ischemia-reperfusion injury, reducing complications, and ultimately improving the clinical outcomes.

Renal impairment following LT is a well-recognized and frequent complication with multifactorial origin, including pre-, intra-, and postoperative factors.[3] Acute kidney injury (AKI) is an independent risk factor for death in the ICU and a major cause of morbidity and mortality after LT. In addition, it has been shown that early AKI has long-term repercussions for renal function. Previous reports have indicated a strong relationship between advanced hepatic IRI and AKI following liver transplantation.[4,5] We recently reported that early AKI in the recipient appears to be associated with complex molecular alterations found in the liver graft within minutes from reperfusion.[6] Hence, mitigating hepatic IRI may represent an important strategy in modifying the occurrence of renal dysfunction in this setting.

Hypothermic oxygenated machine perfusion (HOPE) of the liver has been repeatedly shown to significantly decrease the early allograft injury and improve post-transplant outcomes in liver transplantation from high-risk donors.[7–9] Expectedly, all available studies focused on liver IRI and liver-related outcomes, but data on renal outcomes are scarce. The limited information available reports anything from reduced incidence of AKI,[10] trends toward lower creatinine, and improved renal function following HOPE[9] to similar results compared to not-manipulated grafts.[8] Of note, almost all high-risk donors reported in these studies were aged less than 70 years and/or DCD. Herein, we examined the impact of HOPE performed on livers from donation after brain death (DBD) donors, aged 70 years or older on the early renal outcomes after liver transplantation at a large Scandinavian center.

2 | PATIENTS AND METHODS

2.1 | Patients

This was a single-center, retrospective study of all adults who underwent first, single-organ liver transplantation at Sahlgrenska University Hospital between January 1, 2017, and December 31, 2022, and received a liver from a DBD donor aged 70 years or older. Exclusion criteria were previous transplants, partial grafts, need for preoperative renal replacement therapy or combined liver-kidney transplant, and ABO-incompatible LTs.

Donor and recipient data were retrieved from the electronic medical records and a prospectively completed local database. Data review and collection were approved by the Regional Ethical Review Committee in Gothenburg (Dnr. 048-13). Given the anonymized, retrospective analysis patient consent was waived. Donor risk index (DRI) was calculated according to an acknowledged equation, using a calculator available online.[11] Concerning the "organ location" parameter in the formula, the organs procured in Gothenburg and in hospitals within 80 km were considered as "local", organs procured at other donor hospitals from our procurement area (Western, Southern, and Northern Sweden) were considered as "regional," whereas organs shared from other centers within the Scandiatransplant cooperation were inputted as "national." Recipient characteristics were documented at the time of admission for transplantation with the exception of glomerular filtration rate (GFR) which was measured using ^{51}chromium EDTA or iohexol at the time of listing for LT. The MELD (Model for end-stage liver disease) score was calculated using lab data at admission and calculated without exception points.

2.1.1 | Liver graft procurement and hypothermic oxygenated machine perfusion (HOPE)

Donor organs were perfused with either Custodiol® (Fresenius Kohler Chemie GmbH, Alsbach-Hähnlein, Germany), Viaspan-University of Wisconsin solution® (Bristol Myers Squibb, Solna, Sweden) or Institute Georges Lopez-1 (IGL-1) solution (Institute Georges Lopez, Lissieu, France) by aortic retrograde perfusion in situ, with additional portal perfusion of the liver graft on back table. Thereafter, the organs were put in isolation bags filled with the same type of solution used for perfusion, placed in an ice box, and transported to

our unit for transplantation. Cold ischemia time (CIT) was defined as duration from the start of cold perfusion in the donor to portal reperfusion of the graft in the recipient.

From November 2020, livers from donors aged 70 years or older, routinely underwent HOPE through the portal vein, using VitaSmart™ Machine Perfusion System (Bridge to life Europe Ltd, Wandsworth, London, UK) while recipient hepatectomy was performed. After standard back table graft preparation, the liver graft was perfused with one-liter Belzer MPS® solution (Bridge to Life Europe Ltd). The perfusion system was primed with 3 L of Belzer MPS® solution and perfusion was initiated through the cannulated portal vein. Both ends of the vena cava were left open to allow free outflow of the perfusate. The hepatic artery was prepared for implantation but was not cannulated. During HOPE, portal perfusion pressure (continuous) was set to maximal 3 mm Hg, the target oxygen concentration in the perfusate was 80–100 kPa and reservoir temperature was ~10°C. Gas analyses were obtained after 30, 60, and 120 min to check the oxygen pressure in the perfusate. At the end of HOPE, grafts were disconnected and immediately transplanted.

2.2 | Patient management

Anesthesia was induced by propofol and fentanyl or remifentanil and maintained with sevoflurane and either of the opiates used for induction. Apart from the autologous transfusion of blood collected intraoperatively, recipients received packed red blood cells, plasma platelets, and albumin according to the preference of the attending anesthesiologist. Norepinephrine was given intraoperatively to maintain a mean arterial pressure of >65 mmHg. Liver transplantation was performed with the preservation of the recipient retrohepatic vena cava and side-to-side cavo-caval anastomosis. Reperfusion of the liver graft was performed after completion of the portal vein anastomosis and before completion of arterial and biliary anastomoses.

Immunosuppression consisted of induction with steroids and intravenous basiliximab. Maintenance immunosuppression consisted of mycophenolate mofetil introduced on day 0 and tacrolimus introduced on postoperative day 3 (target levels 5–8 mg/mL) with additional oral corticosteroids for patients with primary sclerosing cholangitis or autoimmune hepatitis.

2.3 | Outcomes

The primary endpoint was defined as the occurrence of AKI in the first 2 days after LT according to the Kidney Disease: Improving Global Outcome (KDIGO) criteria and stages, excluding the incorporation of urine output in the creatinine-based formula.[12] The four stages are no AKI, AKI stage 1; rise in serum creatinine of 1.5–1.9 times baseline or an increase of ≥26.5 μmol/L within 48 h, stage 2; rise in serum creatinine of 2.0–2.9 times baseline and stage 3; 3 times baseline or an increase in serum creatinine to ≥353.6 μmol/L. or the initiation of renal replacement therapy (RRT).

Secondary outcomes were the difference in creatinine and transaminase serum levels during the first week between the two groups, peak transaminase levels, and the need for posttransplant RRT. The frequency of EAD defined as bilirubin ≥10 mg/dL on day 7, international normalized ratio ≥1.6 on day 7, and alanine or aspartate aminotransferases >2000 IU/L within the first 7 days was also analyzed.[13] Early graft dysfunction was also evaluated using the Model of Early Allograft Function (MEAF), a continuous score based on bilirubin, international normalized ratio, and alanine aminotransferase within the first 3 postoperative days.[14] MEAF was calculated as previously described: MEAF = ("score ALT" + "score INR" + "score bilirubin"), where "score ALT" = 3.29/(1 + e−1.9132(ln(ALTmax.3 days) − 6.1723), "score INR" = 3.29/(1 + e−6.8204(ln(INRmax.3 days) − 0.6658), "score bilirubin" = 3.4/(1 + e−1.8005(ln(bilirubinday3) − 1.0607). A score between 0 and 10 is obtained.

2.3.1 | Histological assessment

Ischemia-reperfusion injury and steatosis were assessed on formalin-fixed, paraffin-embedded liver biopsies obtained at the end of transplant. Sections (5 μm thick) were stained using hematoxylin and eosin and evaluated blindly by an experienced transplant pathologist. Macrosteatosis was graded on a scale from 0 to 3 (0 = 0%, 1 <10%, 2 = 10%–30%, 3 >30%) according to percentage of involved parenchyma. Ischemia-reperfusion injury was evaluated on a 0–4 scale for sinusoidal congestion, hepatocyte vacuolization, and parenchymal necrosis using the Suzuki score (Table 1).[15]

TABLE 1 The Suzuki histological criteria.

Score	Congestion	Cytoplasmic vacuolization	Parenchymal necrosis
0	No	No	No
1	Minimal	Minimal	Single-cell necrosis
2	Mild	Mild	<30%
3	Moderate	Moderate	<60%
4	Severe	Severe	>60%

2.4 | Statistical analyses

The number of test subjects (30) was limited in this study; therefore no power analysis was performed. Data are shown as median (interquartile range), mean (standard deviation), or as absolute and relative frequencies as appropriate. Continuous variables were compared using the Mann–Whitney U test. Categorical variables were compared using the Chi Square test or Fisher's exact test. Overall analyses were performed using GraphPad Prism v. 6 (GraphPad Software, San Diego, CA, USA), and a p-value <0.05 was considered significant.

To control for differing baseline characteristics, hence confounding, propensity score matching was performed on donor- and recipient variables that were considered to impact the outcome. Recipient age and MELD-score, pretransplant GFR, donor and recipient BMI, and DRI were taken into account. Group- or TT-Matching as well as a Caliper match were performed. An effect size of <0.2 was aimed for, whereas an effect size in the range 0.2–0.5 was considered to have a moderate biasing effect on the outcome analysis. The propensity score analysis is dependent on the uncertainty in the selection of variables in the propensity score equation. Moreover, residual imbalance between groups may still be present due to the small sample size of the matched cohort.

3 | RESULTS

From January 1, 2017, to December 31, 2022, 524 liver transplantations were performed at Sahlgrenska University Hospital. Of these, 124 (24%) were performed using livers from donors aged 70 or older. After applying the inclusion and exclusion criteria 101 patients were considered for this analysis: 71 recipients of livers undergoing static cold storage alone (SCS group) and 30 patients receiving additional end-ischemic HOPE (HOPE group).

The two study groups were largely similar in terms of demographics, diagnosis, and pretransplant status. However, there were significant differences in terms of recipient BMI, postviral cirrhosis as transplant indication, and donor age [73 (IQR 71, 76) in SCS group vs. 75 (IQR 73, 78) in HOPE group, $p<0.05$] and cold ischemia time [470 (IQR 379, 524) vs. 596 (IQR 503, 640) min; $p<0.001$]. Main donor and recipient characteristics are summarized in Table 2.

Group- or TT-Matching was performed to include as many controls as possible to the HOPE group while balancing the variables defined above. The best matching with low effect sizes resulted in 47 control patients matching the 30 patients of the HOPE group (Table 3A). Table 3B shows the Caliper-matching, performed to generate a control group of the same size as the HOPE group, matching on logit propensity score with an allowed variation of 0.2, rendering 27 patients in each arm.

3.1 | Renal outcomes

Creatinine increased significantly postoperatively in both groups compared to the baseline (preoperative) values and returned to baseline values after 4 days in HOPE graft recipients and 5 days in recipients of untreated, control grafts (Figure 1).

Overall, 62 (62%) recipients developed AKI within the first 2 days of LT: 40 (56%) recipients of control SCS grafts and 23 (77%) recipients of HOPE grafts. According to the severity of AKI, AKI1 occurred in 23 (58%) SCS recipients and 15 (68%) recipients of HOPE grafts, AKI2 was recorded in 9 (23%) SCS patients and 3 (14%) HOPE patients, whereas the threshold for AKI3 was reached in 8 (20%) control patients and 5 (17%) HOPE patients. Renal replacement therapy was required in 6 (8%) control patients and 3 (10%) HOPE patients. No patient required RRT beyond day 5.

Following TT- or Caliper-matching, we did not find any significant differences in severity or AKI stages between the two matched cohorts (Tables 4 and 5).

Relative risk (RR) for AKI in HOPE group was 1.36 [95% CI 1.02 to 1.80, $p=0.03$], whereas the RR for RRT was 1.18 [95% CI 0.32 to 4.42, $p=0.80$].

3.2 | Graft and patient outcomes

HOPE treatment did not result in any significant improvement regarding aminotransferase release during the first week after transplantation (Figure 2).

There were no cases of primary graft nonfunction, retransplantation, or postoperative deaths in the entire patient cohort. Moreover, the rate of EAD did not differ significantly between the patient groups receiving perfused and static stored allografts [incidence of EAD 4 (13%) HOPE vs. 10 (14%) SCS, p > 0.05].

MEAF score was similar between the SCS and HOPE group (6.547 ± 1.4 vs. 6.646 ± 1.195, $p=0.66$).

Similar findings and no significant difference between SCS and HOPE LT were observed when comparing graft-related outcomes in the two matched cohorts (Tables 4 and 5).

3.2.1 | Histology

Both the grafts undergoing SCS and the grafts receiving HOPE revealed postreperfusion liver damage

TABLE 2 Donor and recipient characteristics.

	SCS ($n=71$)	HOPE ($n=30$)	p-value
Donors			
Age (years)	73 (71, 76)	75 (73, 77.5)	**0.015**
BMI (kg/m^2)	26 (23, 29)	25 (22, 27)	0.253
BMI > 30, *n* (%)	13 (18)	3 (10)	0.328
Cause of death			
Trauma	5	1	0.493
ICB	55	25	0.557
Anoxia	11	4	0.829
Total CIT (min)	470 (379, 524)	596 (497, 641)	**<0.001**
Perfusion time (min)	–	125 (112–201)	
Macrosteatosis			
10%–30%	6/57	2/28	0.618
>30%	2/57	1/28	0.987
Donor risk index	2.1 (1,9, 2.1)	2.1 (2,2.1)	0.154
Recipients			
Age (years)	63 (56, 67)	63 (54, 66)	0.31
BMI (kg/m^2)	25 (22, 29)	27 (24, 33)	**0.02**
Diagnosis, *n* (%)			
Postviral cirrhosis	11 (15)	0	**0.02**
Cholestatic disease	14 (20)	3 (10)	0.236
Autoimmune	2 (3)	2 (7)	0.371
Alcoholic	15 (21)	7 (23)	0.807
HCC	15 (21)	10 (33)	0.196
NASH	4 (6	1 (3)	0.626
ALF	3 (4)	0	0.256
Other	7 (10)	7 (23)	0.073
Pretransplant creatinine (μmol/L)	85 (64, 106)	79 (67, 105)	0.469
Pretransplant mGFR	65 (51, 88)	70.5 (47, 85)	0.38
MELD	15 (9, 23)	17 (10, 25)	0.35
Bleeding (mL)	2800 (1790, 3500)	2000 (1000, 4000)	0.102

Note: Data are reported as numbers (percent), or median and interquartile range [IQR].

Abbreviations: ALF, acute liver failure; BMI, body mass index; HCC, hepatocellular carcinoma; ICB, intracerebral bleeding; MELD, model for end-stage liver disease; NASH, nonalcoholic steatohepatitis.

combining different degrees of sinusoidal congestion and vacuolization, and at least minimal, single-cell necrosis. (Figure 3). There was no statistical difference between groups in congestion (median 2, IQR 1, 2) or necrosis (median 0, IQR 0, 0) scores, but vacuolization was significantly more prominent in HOPE livers [median 3 (IQR 3, 4) vs. 2 (IQR 2, 4), $p<0.05$]. This resulted in an overall higher Suzuki score in HOPE-treated compared to SCS grafts [median 5 (IQR 5, 6) vs. 5 (IQR 3.5, 6), $p=0.038$ or in mean value 5.32 ± 1.69 vs. 4.54 ± 1.28, $p=0.035$].

4 | DISCUSSION

End-ischemic hypothermic oxygenated perfusion of liver grafts has been suggested as a relatively straightforward method to alleviate ischemia-reperfusion injury and reduce a range of early and late complications after LT, particularly in recipients of high-risk grafts. Remarkably, the current analysis did not find any significant differences in terms of early renal or liver outcomes after the transplantation of aged liver grafts undergoing HOPE compared to traditional SCS.

TABLE 3 (A) The main baseline donor and recipient characteristics in the two cohorts after group-matching (TT). (B) The main baseline donor and recipient characteristics in the two cohorts after Caliper matching.

(A)

Variable	HOPE ($n=30$)	SCS ($n=47$)	p-value	Difference between groups mean (95% CI)	Effect size
pre GFR	68.0 (25.4) 70.5 (47; 86)	69.2 (25.3) 62 (51; 90)	0.85	−1.19 (−12.94; 10.73)	0.047
MELD	17.6 (8.7) 17 (11; 24)	18.2 (9.2) 16 (10; 23)	0.80	−0.591 (−4.895; 3.647)	0.066
Recipient BMI	30.0 (7.6) 27.9 (24.4; 33.6)	28.9 (4.4) 28.4 (25.6; 31.4)	0.43	1.07 (−1.72; 3.80)	0.184
Donor BMI	25.9 (4.6) 26 (22.3; 28)	25.8 (4.1) 26.2 (22.4; 28.5)	0.98	0.024 (−2.024; 2.029)	0.005
DRI	2.09 (0.15) 2.12 (2.02; 2.18)	2.06 (0.26) 2.11 (1.9; 2.28)	0.59	0.028 (−0.078; 0.135)	0.123
Patient age (years)	60.4 (8.8) 62.5 (55; 67)	60.4 (8.9) 63 (54; 66)	1.00	0.008 (−4.050; 4.118)	0.001
Cold ischemia time	592 (96) 595 (521; 641)	483.5 (137.6) 485 (379; 527)	0.0006	108.3 (50.3; 164.9)	0.878

(B)

Variable	HOPE ($n=27$)	SCS ($n=27$)	p-value	Difference between groups mean (95% CI)	Effect size
pre GFR	68.9 (26.3) 77 (47; 87)	66.9 (27.0) 62 (50; 84)	0.79	1.93 (−12.64; 16.67)	0.072
MELD	18.0 (8.6) 17 (11; 24)	18.0 (9.1) 16 (11; 23)	0.99	0.074 (−4.700; 4.909)	0.008
Recipient BMI	28.4 (5.9) 27.2 (24.3; 30.9)	28.9 (5.5) 27.3 (24.9; 31.6)	0.75	−0.504 (−3.569; 2.598)	0.088
Donor BMI	25.8 (4.9) 25.1 (22.1; 28.1)	26.0 (4.4) 26.1 (23.1; 28.4)	0.83	−0.272 (−2.769; 2.219)	0.059
DRI	2.10 (0.15) 2.12 (2.02; 2.18)	2.04 (0.28) 2.08 (1.91; 2.23)	0.30	0.064 (−0.058; 0.183)	0.290
Patient age (years)	60.2 (9.2) 62 (55; 67)	59.5 (9.5) 63 (52; 66)	0.80	0.704 (−4.400; 5.818)	0.075
Cold ischemia time	585 (98) 592 (497; 641)	474.4 (135.6) 470 (379; 527)	0.0015	110.9 (45.4; 174.8)	0.937

Note: For continuous variables Mean (SD)/Median (IQR) is presented. For comparison between groups the Fisher's Non Parametric Permutation Test was used for continuous variables. The confidence interval for the mean difference between groups is based on Fishers nonparametric permutation test. Effect size is absolute difference in mean/pooled SD.

Abbreviations: BMI, body mass index; DRI, donor risk index score; HOPE, hypothermic oxygenated machine perfusion; IQR, interquartile range; MELD, model for end-stage liver disease; SCS, static cold storage.

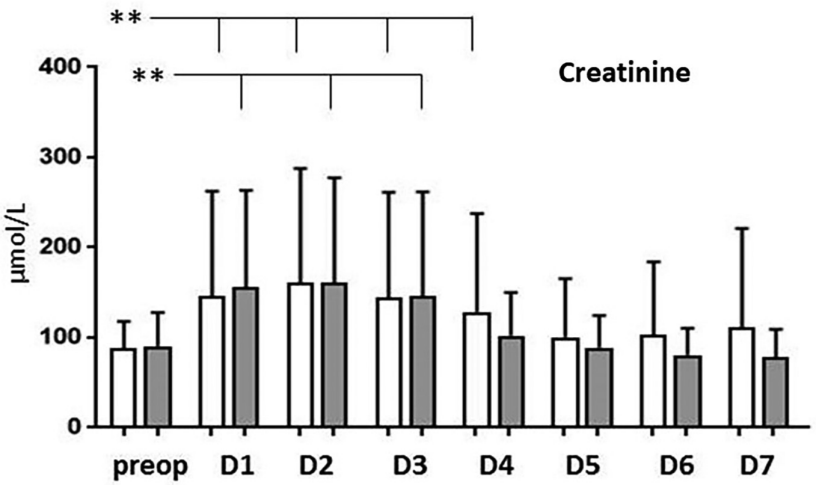

FIGURE 1 Daily serum creatinine in the recipients of livers undergoing SCS (white) and HOPE (gray). D: day; **$p<0.01$.

Remote organ injury may occur following sterile tissue injury, including IRI, when various cellular components (e.g., nucleic acids, histones, high-mobility group box 1 protein, and S100) or other pro-inflammatory mediators are released into the extracellular space or bloodstream and reach other organs.[16] These biomolecules may act as damage-associated molecular patterns (DAMPs) and stimulate multiple signaling pathways and cascades, ultimately leading to cell and organ dysfunction.[17] Therefore, we and others hypothesize that renal injury after liver transplantation may be partly driven by alterations occurring in the liver graft.[4–6] We have previously shown that grafts of LT recipients developing early AKI reveal distinct proteomic changes, mainly related to the innate immunity

TABLE 4 Postoperative outcomes after Group-Matching (TT) based on pretransplant GFR, model for end-stage liver disease (MELD), recipient body mass index (BMI), donor BMI and donor risk index.

Variable	HOPE ($n=30$)	SCS ($n=47$)	p-value	Difference between groups mean (95% CI)	Effect size
Peak AST 72 h (U/L)	931 (644) 810 (450; 1140)	1428 (1487) 1140 (534; 1860)	0.060	−496.2 (−1077.7; 12.4)	0.403
Peak AST 7 days (U/L)	933 (645) 810 (450; 1140)	1428 (1487) 1140 (534; 1860)	0.061	−494.2 (−1075.9; 14.2)	0.401
EAD	4 (13,3%)	9 (19.1%)	0.51		
MEAF	6.57 (5.8; 7.29)	6.8 (5.58; 7.94)	0.71	−0.114 (−0.713; 0.488)	0.089
AKI 0–3					
0	7 (23.3%)	16 (34.0%)			
1	16 (53.3%)	18 (38.3%)			
2	3 (10.0%)	7 (14.9%)			
3	4 (13.3%)	6 (12.8%)	0.81		

Note: For categorical variables numbers (percent) is presented. For continuous variables Mean (SD)/Median (IQR) is presented. For comparison between groups Fisher's Exact test was used for dichotomous variables and the Mantel–Haenszel Chi Square Exact test was used for ordered categorical variables and the Fisher's nonparametric permutation test was used for continuous variables. Effect size is absolute difference in mean/pooled SD.

Abbreviations: AKI, acute kidney injury; AST, aspartate aminotransferase; EAD, early allograft dysfunction; IQR, interquartile range; MEAF, model for allograft function; SCS, static cold storage.

response, inflammation, and ongoing neutrophil degranulation, compared to the liver grafts of patients retaining normal renal function after LT.[6] Given the lack of an obvious effect of HOPE on the development of AKI, we speculate that the altered proteome is not influenced by any of the putative protective mechanisms of HOPE,[17] which seem unable to override the multiple metabolic processes apparently steered from the graft.

Most studies using HOPE are performed on ECDs, and a majority report an improvement of the hepatocellular injury and milder transaminase leak in the recipients of HOPE-treated ECD grafts to levels corresponding to standard donors.[8] Although transaminase leak is a well-established marker of hepatocyte injury, it has been argued that in this particular setting, assessing transaminase levels as a quantitative indicator of graft injury may be misleading.[17] All transaminase leak that occurs during SCS will spill into the recipients of nonperfused grafts, whereas graft machine perfusion may wash out some of the leaked enzymes, thus decreasing the initial transaminase peak in the recipient of a perfused graft.[18]

The analysis of the whole cohort of patients could not identify any difference in terms of allograft (dys) function and postreperfusion transaminase leak between livers undergoing HOPE or not. Furthermore, no differences in liver biochemistry or occurrence of AKI were found after matching the patients according to several variables that may influence the development of early AKI or liver graft function. In addition, histology revealed a slightly more advanced tissue injury, primarily due to hepatocyte vacuolization in the grafts undergoing HOPE. These findings contrast with those of a recent murine study where necrosis was the dominant feature whereas vacuolization was absent in grafts undergoing SCS and HOPE.[19] Vacuolization is a common, potentially reversible finding after ischemia-reperfusion injury, echoing the energy (ATP) depletion, and is represented by dilated organelles, mainly endoplasmic reticulum.[20] It likely reflects a mild or early injury whereas necrosis represents severe energy loss and established, irreversible tissue injury due to advanced IRI. As the murine liver biopsies were sampled 24 hours after transplantation, those findings possibly reflect a different, later stage of IRI.

Hence, these findings add to the ongoing controversy concerning the ability of HOPE to modify the development of IRI or rate of several clinical outcomes such as EAD, intensive care unit, and hospital stay or biliary complications.[9,21] We believe part of these controversies stem from the heterogeneity of the ECD criteria used before, which combine widely different variables such as donor age, DCD, graft steatosis, or preprocurement donor biochemistry. We attempted to reduce this variability by grouping the transplants according to one single, major ECD criteria namely donor age over 70, whereas most other variables were similar between groups. This resulted in a median DRI over 2, which is numerically high, but which reflects "marginality" only from the perspective of age.

The overall incidence of AKI in this study was higher than in many other reports in unselected LT, where it hovers around 40%.[3] Yet, the proportion of patients requiring RRT was similar to the literature. This observation may

TABLE 5 Postoperative outcomes after Caliper-match logit Propensity Score 0.2 based on pretransplant GFR, model for end-stage liver disease (MELD), recipient body mass index (BMI), donor BMI and donor risk index.

Variable	HOPE ($n=27$)	SCS ($n=27$)	p-value	Difference between groups mean (95% CI)	Effect size
Peak AST 72 h (U/L)	870 (613) 720 (396; 1140)	1302 (1147) 1140 (486; 1860)	0.082	−431.1 (−922.0; 48.0)	0.469
Peak AST 7 days (U/L)	870 (613) 720 (396; 1140)	1302 (1147) 1140 (486; 1860)	0.082	−431.1 (−922.0; 48.0)	0.469
EAD	3 (11,1%)	3 (11,1%)	1		
MEAF	6.47 (5.71; 7.07)	7.03 (5.45; 7.94)	0.61	−0.166 (−0.823; 0.497)	0.138
AKI 0–3					
0	6 (22.2%)	8 (29.6%)			
1	14 (51.9%)	11 (40.7%)			
2	3 (11.1%)	3 (11.1%)			
3	4 (14.8%)	5 (18.5%)	1.00		

Note: For categorical variables numbers (percent) is presented. For continuous variables Mean (SD)/Median (IQR) is presented. For comparison between groups Fisher's Exact test was used for dichotomous variables and the Mantel–Haenszel Chi Square Exact test was used for ordered categorical variables and the Fisher's nonparametric permutation test was used for continuous variables. Effect size is absolute difference in mean/pooled SD.

Abbreviations: AKI, acute kidney injury; AST, aspartate aminotransferase; EAD, early allograft dysfunction; IQR, interquartile range; MEAF, model for allograft function; SCS, static cold storage.

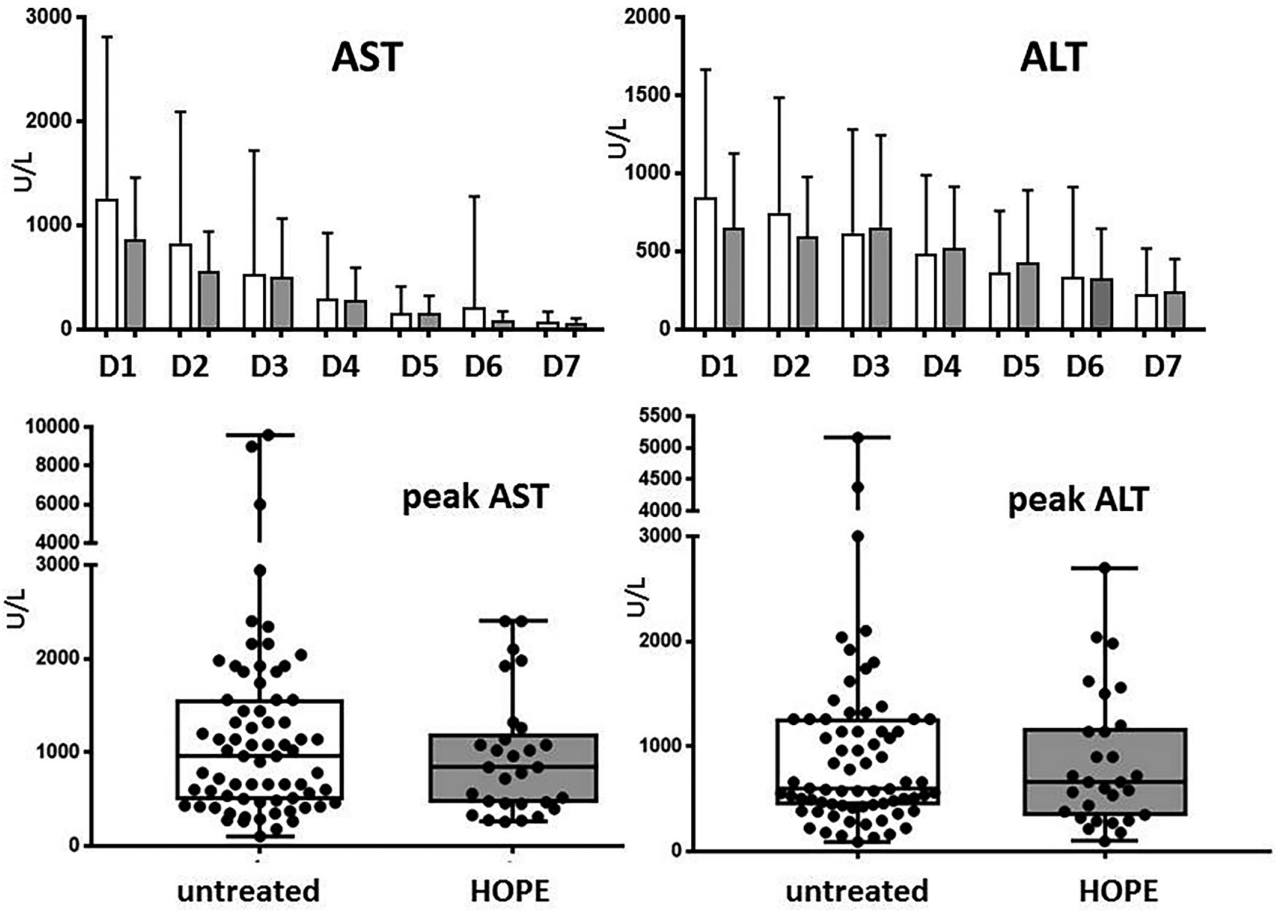

FIGURE 2 Daily and peak AST and ALT levels in the recipients of livers undergoing SCS (white) and HOPE (gray).

imply a higher predisposition for these older, higher-risk grafts to promote mild or moderate AKI. A previous study found that recipients of donor livers aged 60 years or over developed AKI in 25.2% of cases, while recipients of livers from donors with a BMI ≥30 kg/m2 or recipients of DCD grafts developed AKI in 24,3% and 14.7%, respectively.[4]

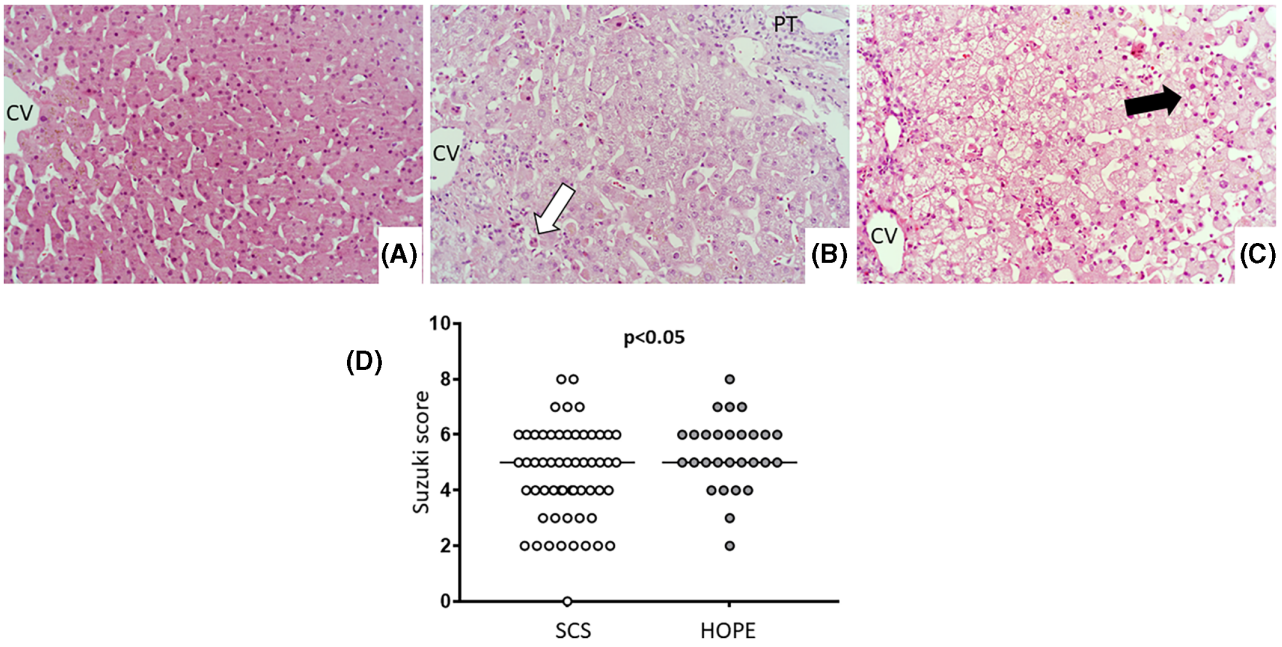

FIGURE 3 Representative light microphotographs (x200) demonstrating different levels of liver damage. Congestion (C), vacuolization (V) and necrosis (N) after ischemia-reperfusion injury were scored according to Suzuki. (A) Liver biopsy with grade 2 damage (2C + 0V + 0N); (B) grade 5 damage (2C + 2V + 1N); (C) grade 8 damage (2C + 4V + 2N). CV, centrilobular vein, PT, portal triad. White arrow: single-cell necrosis, black arrow: spotty area of necrosis. (D) Chart summarizing the Suzuki score in the exit biopsies of livers undergoing SCS (white) and HOPE (gray). [Color figure can be viewed at wileyonlinelibrary.com]

That analysis further confirmed in a multivariate analysis that recipients of older grafts (>60 years) had increased risk [OR 1.49 (1.07–2.09)] of developing AKI. Again, this may ultimately reflect the increased susceptibility of the aging liver to ischemia/reperfusion damage and the subsequent local and systemic response to it,[22] eventually suggesting that transplantation of older, high-risk liver grafts should be carefully weighed in general, and in particular when the recipient is frail or renal function is impaired.

Most studies reporting beneficial effects following HOPE are performed in DCD-LT, whereas the literature on HOPE and DBD-LT is more limited, and the results are inconsistent. An initial retrospective analysis of LTs using HOPE-treated livers from DBDs reported a lower frequency of AKI stage 2/3 and a tendency towards less reperfusion syndrome compared to matched LTs receiving livers undergoing SCS.[10] However, a subsequent larger analysis from the same group later showed somewhat different overall results, with no significant differences between the incidence of postoperative stage 2/3 AKI or need for RRT between HOPE-LTs and the control group (SCS).[21] However, the same study reported less need for early post-LT dialysis in the HOPE-LTs, compared to controls, when elderly (>75 years old) donors were considered. Besides these studies originating from the same large Italian center, two multicenter, randomized controlled trials on HOPE-treated DBD grafts have recently been published. The first trial reported a lesser peak ALT after LT in HOPE-LTs compared to control patients (interpreted as mitigated IRI) but no difference in the need for RRT.[9] The other study, although primarily focusing on the incidence of cumulative Clavien III complications, reported a similar need for post-LT dialysis among HOPE-LTs compared with control LTs.[23]

The clinical outcome in this patient cohort was very good, with no primary nonfunction and an incidence of EAD which was numerically below what is often reported in the literature.[13,24,25] The current results, which leave very little room for improvement, likely reflect the rigorous selection of all potential referrals before acceptance as liver donors and confirm that the use of elderly donors has become a safe and established practice.

This study has several limitations, stemming from the retrospective study design, whereas the single-center setting raises the issue of limited external validity. As liver and kidney injury were evaluated using routine laboratory tests, we lack a more detailed assessment of renal and graft dysfunction using molecular biomarkers in serum or urine.[5,26] Creatinine is an indirect marker of glomerular filtration but lacks specificity for damage to kidney tissue and it has a relatively late response. Another limitation is the rather low number of patients, particularly in the intervention group, which could have been too small to detect differences between groups.

Nonetheless, although the study may have been underpowered, no trends toward any difference were ever evident. Given the very well-defined inclusion criteria, this report still presents one of the largest cohorts to date studying LT from elderly donors and could be used as hypothesis-generating study. Among the advantages we count the same patient management and immunosuppressive protocol throughout the study, the histological evaluation of graft biopsies after reperfusion, and the completeness of overall data. Uniquely for this study, the delayed introduction of tacrolimus on post-transplant day 3 allows us to exclude calcineurin-inhibitor toxicity as a potential cause of AKI.

5 | CONCLUSIONS

Pretransplant HOPE of liver grafts from extended criteria, elderly donors did not provide any obvious short-term benefits in terms of frequency and severity of early AKI or alleviated reperfusion injury, as reflected by the transaminase leak or histology compared with similar historical controls and a matched cohort. These results partly contrast with the existing literature but have to be weighed against the very good results currently obtained using livers from well-selected very-aged donors.

AUTHOR CONTRIBUTIONS

Åsa Norén involved in concept or design, data collection, data analysis or interpretation, drafting article, critical revision of article, approval of article, and statistics. Johan Mölne involved in data collection, data analysis or interpretation, critical revision of article, and approval of article. William Bennet and Gustaf Herlenius involved in critical revision of article, approval of article, and other works. Gustaf Sörensen involved in data collection, data analysis, critical revision of article, and approval of article. Per Lindnér involved in concept or design, data interpretation, critical revision of article, approval of article, and statistics. Mihai Oltean involved in concept or design, data collection, data analysis or interpretation, drafting article, approval of article, statistics, and funding.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest with the contents of this article.

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REFERENCES

1. Thorsen T, Aandahl EM, Bennet W, Olausson M, Ericzon BG, Nowak G, et al. Transplantation with livers from deceased donors older than 75 years. Transplantation. 2015;99(12):2534–42.
2. Dickson KM, Martins PN. Implications of liver donor age on ischemia reperfusion injury and clinical outcomes. Transplant Rev (Orlando). 2020;34(3):100549.
3. Thongprayoon C, Kaewput W, Thamcharoen N, Bathini T, Watthanasuntorn K, Lertjitbanjong P, et al. Incidence and impact of acute kidney injury after liver transplantation: a meta-analysis. J Clin Med. 2019;8(3):372.
4. Leithead JA, Rajoriya N, Gunson BK, Muiesan P, Ferguson JW. The evolving use of higher risk grafts is associated with an increased incidence of acute kidney injury after liver transplantation. J Hepatol. 2014;60(6):1180–6.
5. Jochmans I, Meurisse N, Neyrinck A, Verhaegen M, Monbaliu D, Pirenne J. Hepatic ischemia/reperfusion injury associates with acute kidney injury in liver transplantation: prospective cohort study. Liver Transpl. 2017;23(5):634–44.
6. Norén Å, Oltean M, Friman S, Molinaro A, Mölne J, Sihlbom C, et al. Liver graft proteomics reveals potential incipient mechanisms behind early renal dysfunction after liver transplantation. Int J Mol Sci. 2022;23(19):11929.
7. Dutkowski P, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I, et al. First comparison of hypothermic oxygenated perfusion versus static cold storage of human donation after cardiac death liver transplants: an international-matched case analysis. Ann Surg. 2015;262:764–70.
8. Schlegel A, Muller X, Kalisvaart M, Muellhaupt B, Perera MTPR, Isaac JR, et al. Outcomes of DCD liver transplantation using organs treated by hypothermic oxygenated perfusion before implantation. J Hepatol. 2019;70(1):50–7.
9. Czigany Z, Pratschke J, Froněk J, Guba M, Schöning W, Raptis DA, et al. 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). Ann Surg. 2021;274(5):705–12.
10. Patrono D, Surra A, Catalano G, Rizza G, Berchialla P, Martini S, et al. Hypothermic oxygenated machine perfusion of liver grafts from brain-dead donors. Sci Rep. 2019;9(1):9337.
11. Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA, et al. Characteristics associated with liver graft failure: the concept of a donor risk index. Am J Transplant. 2006;6(4):783–90.
12. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin Pract. 2012;120(4):c179–84.
13. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010;16(8):943–9.
14. Pareja E, Cortes M, Hervás D, Mir J, Valdivieso A, Castell JV, et al. A score model for the continuous grading of early allograft dysfunction severity. Liver Transpl. 2015;21(1):38–46.
15. Suzuki S, Nakamura S, Koizumi T, Sakaguchi S, Baba S, Muro H, et al. The beneficial effect of a prostaglandin I2 analog on ischemic rat liver. Transplantation. 1991;52(6):979–83.

16. Zhai Y, Busuttil RW, Kupiec-Weglinski JW. Liver ischemia and reperfusion injury: new insights into mechanisms of innate-adaptive immune-mediated tissue inflammation. Am J Transplant. 2011;11(8):1563–9.
17. Bardallo RG, Da Silva RT, Carbonell T, Palmeira C, Folch-Puy E, Roselló-Catafau J, et al. Liver graft hypothermic static and oxygenated perfusion (HOPE) strategies: a mitochondrial crossroads. Int J Mol Sci. 2022;23(10):5742.
18. Martins PN, Rizzari MD, Ghinolfi D, Jochmans I, Attia M, Jalan R. Friend PJ; ILTS special interest group "DCD, preservation and machine perfusion". Design, analysis, and pitfalls of clinical trials using ex situ liver machine perfusion: the international liver transplantation society consensus guidelines. Transplantation. 2021;105(4):796–815.
19. Bonaccorsi-Riani E, Gillooly AR, Iesari S, Brüggenwirth IMA, Ferguson CM, Komuta M, et al. Delivering siRNA compounds during HOPE to modulate organ function: a proof-of-concept study in a rat liver transplant model. Transplantation. 2022;106(8):1565–76.
20. Gonzalez-Serratos H, Somlyo AV, McClellan G, Shuman H, Borrero LM, Somlyo AP. Composition of vacuoles and sarcoplasmic reticulum in fatigued muscle: electron probe analysis. Proc Natl Acad Sci U S A. 1978;75(3):1329–33.
21. Patrono D, Cussa D, Sciannameo V, Montanari E, Panconesi R, Berchialla P, et al. Outcome of liver transplantation with grafts from brain-dead donors treated with dual hypothermic oxygenated machine perfusion, with particular reference to elderly donors. Am J Transplant. 2022;22(5):1382–95.
22. Kireev RA, Cuesta S, Ibarrola C, Bela T, Moreno Gonzalez E, Vara E, et al. Age-related differences in hepatic ischemia/reperfusion: gene activation, liver injury, and protective effect of melatonin. J Surg Res. 2012;178(2):922–34.
23. Schlegel A, Mueller M, Muller X, Eden J, Panconesi R, von Felten S, et al. A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation. J Hepatol. 2023;78(4):783–93.
24. Jochmans I, Fieuws S, Monbaliu D, Pirenne J. "Model for early allograft function" outperforms "early allograft dysfunction" as a predictor of transplant survival. Transplantation. 2017;101(8):e258–64.
25. Lee DD, Croome KP, Shalev JA, Musto KR, Sharma M, Keaveny AP, et al. Early allograft dysfunction after liver transplantation: an intermediate outcome measure for targeted improvements. Ann Hepatol. 2016;15(1):53–60.
26. Nielsen MB, Krogstrup NV, Nieuwenhuijs-Moeke GJ, Oltean M, Dor FJMF, Jespersen B, et al. P-NGAL day 1 predicts early but not one year graft function following deceased donor kidney transplantation—the CONTEXT study. PloS One. 2019;14(2):e0212676.

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