Supplementary material

Long-Term Outcomes of Hypothermic Oxygenated Machine Perfusion in Extended Criteria Donor Liver Transplantation

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Study population and design

This single-center, observational cohort study analyzed data from adult recipients (age >18 years) of livers preserved with HOPE at the Rennes Transplant Center performed between January, 2018 and November 2021. All consecutive cases of HOPE were carried out within the framework of the PERPHO study,[1] a prospective single-arm pilot study designed to assess the short-term benefits of HOPE in ECD LT. After the conclusion of the PERPHO study, HOPE was prospectively implemented into clinical practice at our center, following the same protocol with continued data collection and follow-up. To compare outcomes with SCS, a control group was selected after propensity score matching among patients transplanted with ECD grafts in the same institution between 2010 and 2021 and data were collected up until November 30, 2024 to ensure a maximum follow-up period for all included patients.

The Medical Research Ethics Committee of the University Medical Center Rennes reviewed the study and waived the need for informed consent since the study used de-identified data. The study was conducted according to the declaration of Helsinki, was approved by the center's ethics committee (No. 25.41) and was reported according to STROBE guidelines.[2]

Inclusion criteria

HOPE group

All HOPE-perfused liver grafts included in this study met identical inclusion criteria to those defined in the PERPHO study, i.e. adult patients eligible for a first orthotopic liver

transplantation (LT) receiving ECD grafts from DBD donors. A graft was defined as ECD when meeting at least one of the following criteria, as previously reported[1]: (i) donor age >65 years, (ii) body mass index (BMI) >30, (iii) intensive care unit (ICU) stay >7 days prior to liver procurement, (iv) serum sodium (natremia) >155 mmol/L, (v) liver enzymes more than three times the normal value (aspartate aminotransferase [AST] >150 IU/mL, alanine aminotransferase [ALT] >170 IU/mL), (vi) cardiac arrest occurring before liver procurement and (vii) biopsy-proven macro vesicular steatosis >30%. The PERPHO study protocol is summarized in the Supplementary material.

Control group

To ensure a robust and balanced comparison with the experimental group the control group was selected based on a propensity score matching (PSM) (1:2 ratio) among LT with ECD grafts preserved using static cold storage (SCS). To minimize confounding, propensity score included all forementioned ECD-definition characteristics and other recipient and donor patient characteristics including age, gender, BMI, MELD and Child-Pugh score, indication for LT, cirrhosis aetiology, bridge treatment to LT, portal hypertension severity[3] presence of ascites or use of preoperative transjugular intrahepatic portosystemic shunt (TIPS) and the duration of cold ischemia which was included as a critical variable.

Exclusion Criteria

Patients requiring emergency transplantation for acute liver failure, requiring combined organ transplantation, receiving split grafts or grafts from donors after circulatory death (DCD) and those undergoing retransplantation were excluded from the PERPHO study and thus from the current analysis.

PERPHO study protocol and patient management

Procurement and Machine Perfusion Settings

After graft acceptance and standard procurement, the liver grafts were immediately prepared on the back table upon arrival at the transplantation center. The grafts were then connected to the Liver Assist® perfusion machine (CE-certified, XVivo®, Sweden) for hypothermic oxygenated perfusion (HOPE) via the portal vein only. This perfusion process occurred in parallel with the recipient's hepatectomy and lasted between 1 and 4 hours. Machine perfusion was performed using Machine Perfusion Solution (Belzer-MPS, CE-certified) and initiated immediately following the back-table preparation of the graft. To ensure adequate machine perfusion time, the graft was placed on the perfusion device either before or at the time of the recipient's incision. Perfusion continued during the recipient's native liver hepatectomy and was stopped just before the graft was implanted.

Liver Transplantation and Postoperative Care

All patients underwent orthotopic liver transplantation with preservation of the inferior vena cava. Following a standard abdominal incision and liver pedicle dissection, the native liver was removed with meticulous hemostasis. The perfused graft was then removed from the machine, flushed with 500 mL of 5% albumin, and implantation commenced. The procedure included a side-to-side caval anastomosis followed by an end-to-end portal vein anastomosis. Vascularization of the graft was achieved prior to completing the arterial and biliary anastomoses. Postoperatively, all patients received a standardized

immunosuppression regimen, including a calcineurin inhibitor (typically tacrolimus), mycophenolate mofetil and a short course of corticosteroids. Routine Doppler ultrasonography was performed on postoperative days (POD) 1 and 7, or earlier if hepatic dysfunction or vascular complications were suspected. In cases of suspicion, a CT scan was systematically conducted to confirm vascular complications, and treatment strategies (medical, radiological, or surgical) were determined through multidisciplinary discussions. Patients were discharged only when they demonstrated stable liver graft function, therapeutic immunosuppressive drug levels, and sufficient autonomy for safe discharge.

Definitions and study endpoints

The primary endpoint of this study was death-censored graft survival, defined as the survival of the liver graft with and without considering death as a competing risk. Secondary endpoints included incidence of early allograft dysfunction (EAD) according to the Olthoff criteria,[4] primary graft nonfunction (PNF) defined as liver failure requiring retransplantation or leading to death within 7 days after transplantation,[5] incidence of biliary complications, including nonanastomotic and anastomotic strictures as previously defined[1] and incidence of vascular complications. Moreover, short-term postoperative outcomes, the duration of stay in the intensive care unit as well as the total length of hospital stay were compared. Postoperative morbidity was graded according to the Clavien-Dindo (CD) classification within 90 days and severe morbidity was defined as CD ≥ grade 3.[6] Liver transplantation technique and postoperative patient care are detailed in the Supplementary material.

Statistical analysis

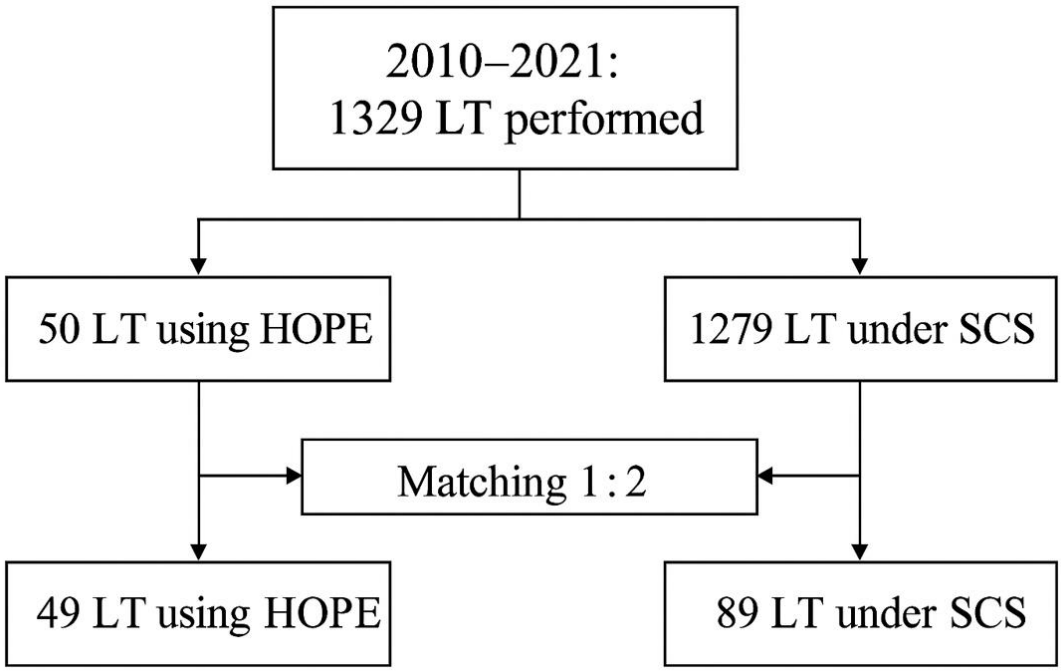
Continuous variables are expressed as medians with 25-75 interquartile range (IQR) and were compared using the Wilcoxon Signed-Rank test for paired data. Categorical variables are summarized in frequency/ percentage and were compared using the McNemar's test for paired data. Patient matching was based on a 1:2 nearest neighbour matching (caliper restriction adjusted at 0.2) without replacement according to the propensity score and balance of matching variables was assessed using standardised mean differences (SMD). SMD values ≤ 0.1 indicated very small differences; values between 0.101 and 0.300 indicated small differences; values between 0.301 and 0.500 indicated moderate differences and values above 0.500 indicated considerable differences.

Death-censored graft survival was calculated from the time of LT to the time of graft loss or last follow-up and compared using Kaplan-Meier curves and log-rank Mantel-Cox tests. Competing risk analysis was also used to estimate the probability of graft loss in the presence of the competing risks of patient death to avoid overestimating graft survival. The association of variables with death-censored graft loss was calculated using multivariable Cox-proportional hazards models, Fine and Gray models and associations were expressed using adjusted Hazard ratios (aHR) and subhazard ratios with their 95% confidence intervals (CI), respectively. All models were adjusted for age, sex and ECD-, patient- and LT-related variables included in the univariable Cox or Fine and Gray analyses and presenting a p-value < 0.2. Covariate multi-collinearity was assessed with a variance inflation factor and proportional hazards assumptions were tested using Schoenfeld residuals. No missing data were reported, except for liver macro vesicular steatosis (20 per cent of missing values) since liver biopsy was not performed systematically during LT.

The latter missing data were managed using multiple imputation chained methodology, all models were performed in each imputed data set (n=20), and the estimates and standard errors were pooled into a final point estimate with robust standard errors according to Rubin's rule.[7] Predictors for the imputation of liver macro vesicular steatosis data included donor patient characteristics (sex, age, BMI, liver blood test results) as well as liver and spleen unenhanced CT scan density, already reported to be a robust predictor of pathologic liver fat content.[8] All statistical tests were based on two-tailed P values, with $P<0.05$ considered to indicate statistical significance. All analyses were performed using RStudio statistical software (Version 2024.04.2+764 © 2024 RStudio, Inc).

Supplementary Figures and Tables

Figure S1: Patient flowchart



Abbreviations: LT, liver transplantation, HOPE, Hypothermic Oxygenated Machine Perfusion, SCS, Static Cold Storage

Table S1. Propensity score matched cohort covariate balancing between hypothermic oxygenated machine perfusion and static cold storage for patients with extended criteria donor undergoing orthotopic liver transplantation.

Characteristics	Overall, N = 138[1]	Static Cold Storage n = 89[1]	Hypothermic oxygenated machine perfusion, n = 49[1]	Difference (95% CI)[2,3]	Missing values[4]	p-value[1]
Recipient patient characteristics						
Age †*	62 (56.0, 64)	61 (56.0, 64)	62 (56.0, 64)	0.13 (-0.22, 0.48)	0 (0)	0.957 ‡
Sex, male *	115 (83.3)	73 (82.0)	42 (85.7)	0.10 (-0.25, 0.45)	0 (0)	0.640
Smoking	66 (47.8)	33 (37.1)	33 (67.3)	-0.64 (-0.99, -0.28)	0 (0)	**<0.001**
Diabetes type 2	47 (34.1)	25 (28.1)	22 (44.9)	-0.35(-0.71, 0.00)	0 (0)	0.060
Arterial hypertension	62 (44.9)	42 (47.2)	20 (40.8)	0.13 (-0.22, 0.48)	0 (0)	0.482
Indication for LT *				0.40 (0.13, 0.70)	0 (0)	**0.012**
HCC	60 (43.5)	46 (51.7)	14 (28.6)			
Cirrhosis	78 (56.5)	43 (48.3)	35 (71.4)			
Cirrhosis, aetiology *				0.24 (-0.11, 0.59)	0 (0)	0.528
Alcohol	104 (75.4)	64 (71.9)	40 (81.6)			

MASLD	5 (3.6)	4 (4.5)	1 (2.0)			
Other	29 (21.0)	21 (23.6)	8 (16.3)			
Ascites before LT *	83 (60.1)	54 (60.7)	29 (59.2)	0.03 (-0.32, 0.38)	0 (0)	0.999
Portal hypertension *				0.30 (-0.05, 0.65)	2 (1.4)	0.261
Absent	23 (16.9)	13 (14.9)	10 (20.4)			
Moderate	65 (47.8)	39 (44.8)	26 (53.1)			
Severe	48 (35.3)	35 (40.2)	13 (26.5)			
TIPS *	10 (7.2)	5 (5.6)	5 (10.2)	-0.17 (-0.52, 0.18)	0 (0)	0.326
Bridge treatment to LT *				0.44 (0.08, 0.79)	0 (0)	0.180
No treatment	86 (62.3)	55 (61.8)	31 (63.3)			
Surgery	10 (7.2)	4 (4.5)	6 (12.2)			
Ablation (radiofrequency, microwave)	7 (5.1)	6 (6.7)	1 (2.0)			
TACE	34 (24.6)	24 (27.0)	10 (20.4)			
SIRT	1 (0.7)	0 (0.0)	1 (2.0)			
ABO Blood Group				0.29 (-0.06, 0.64)	0 (0)	0.504
A	60 (43.5)	39 (43.8)	21 (42.9)			
AB	7 (5.1)	4 (4.5)	3 (6.1)			

B	12 (8.7)	10 (11.2)	2 (4.1)			
O	59 (42.8)	36 (40.4)	23 (46.9)			
Child-Pugh score *				0.16 (-0.19, 0.51)	0 (0)	0.679
A	48 (34.8)	33 (37.1)	15 (30.6)			
B	24 (17.4)	14 (15.7)	10 (20.4)			
C	66 (47.8)	42 (47.2)	24 (49.0)			
MELD score †*	18 (9.3, 27)	18 (9.0, 28)	18 (11.0, 25)	0.10 (-0.25, 0.45)	0 (0)	0.924‡
Donor patient characteristics						
Age, years †*	71 (61.5, 79)	71 (63.0, 79)	70 (61.0, 80)	0.02 (-0.33, 0.37)	0 (0)	0.988 ‡
Age > 65 years *	92 (66.7%)	61 (68.5%)	31 (63.3%)	0.11 (-0.24, 0.46)	0 (0)	0.574
BMI †*	31 (26.6, 34)	30 (25.1, 34)	32 (28.0, 35)	-0.29 (-0.64, 0.06)	0 (0)	0.121 ‡
Liver macrovesicular steatosis > 30% *	6 (5.8)	3 (5.4)	3 (6.3)	-0.04 (-0.42, 0.35)	28 (20.2)	0.999
ASAT > 150 IU/ml*	24 (17.4)	18 (20.2)	6 (12.2)	0.22 (-0.13, 0.57)	0 (0)	0.348
ALAT > 170 IU/ml*	23 (16.7)	16 (18.0)	7 (14.3)	0.10 (-0.25, 0.45)	0 (0)	0.640
Sodium (serum) >155 mmol/L *	21 (15.2)	9 (10.1)	12 (24.5)	-0.39 (-0.74, -0.04)	0 (0)	0.055

Occurrence of cardiac arrest before liver procurement *	35 (25.4)	22 (24.7)	13 (26.5)	-0.04 (-0.39, 0.31)	0 (0)	0.840
ICU stay prior to liver procurement >7 days *	8 (5.8)	5 (5.6)	3 (6.1)	-0.02 (-0.37, 0.33)	0 (0)	0.999
Catecholamine hemodynamic support *	123 (89.1)	81 (91.0)	42 (85.7)	0.17 (-0.18, 0.51)	0 (0)	0.396

[1]Values are expressed with percentages in parentheses unless indicated otherwise; P values are from a Pearson's chi-square test unless indicated otherwise; † values are median (IQR); ‡ Mann-Whitney; [2]Standardized Mean Difference, [3]CI = Confidence Interval; *Indicating variables used for the propensity score patient matching, [4]Missing values in parentheses are percentages.

Balance of matching variables after inverse probability of treatment weighting was assessed using the standard mean difference (SMD). SMD value ≤ 0.1 indicates very small differences; value between 0.101 and 0.300 indicates small differences; value between 0.301 and 0.500 indicates moderate differences; value above 0.500 indicates considerable differences. **Abbreviations**: BMI, body mass index; HOPE, hypothermic oxygenated machine perfusion; HCC, hepatocellular carcinoma; TIPS, transjugular intrahepatic portosystemic shunt; MASLD, metabolic dysfunction-associated steatotic liver disease; TACE, transarterial chemoembolization; SIRT, selective internal radiation therapy; LT, liver transplantation; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase

Table S2: Multivariable Cox-proportionnal hazards of overall survival for patients with extended criteria donor undergoing orthotopic liver transplantation.

Characteristics	Missing values[1]	Univariable analysis[2]		Multivariable analysis[2]	
		HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Liver conservation					
Liver conservation, HOPE (vs static cold storage)	0 (0)	0.34 (0.13, 0.88)	**0.013**	0.30 (0.10, 0.91)	**0.045**
Liver cold ischemia time, minutes	0 (0)	1.00 (1.00, 1.00)	0.33	--	--
Patient characteristics					
Age, years	0 (0)	1.03 (0.98, 1.08)	0.22	2.09 (0.81, 5.39)	0.139
Sex, male	0 (0)	1.47 (0.57, 3.77)	0.25	1.38 (0.44, 4.29)	0.573
Indication for LT, HCC (vs cirrhosis)		1.56 (0.82, 2.96)	0.18	1.08 (0.48, 2.39)	0.846
Bridge treatment to LT*	0 (0)		0.64	--	--
No treatment		--	--		
Surgery		0.38 (0.05, 2.83)			
Ablation (Radiofrequency, microwaves)		1.56 (0.47, 5.24)			
TACE		1.10 (0.53, 2.28)			
MELD score	0 (0)	1.00 (0.97, 1.03)	0.93	--	--
Child-Pugh score	0 (0)		0.65	--	--

A		--	--		
B		1.03 (0.42, 2.54)			
C		0.74 (0.37, 1.51)			
Liver cold ischemia time	0 (0)	1.00 (1.00, 1.00)	0.33	--	--
Extended criteria donor characteristics					
Liver macrovesicular steatosis > 30% †	0 (0)	1.43 (0.55, 3.7)	0.55	--	--
BMI > 30	0 (0)	0.90 (0.42, 1.93)	0.78	--	--
ASAT >150 IU/mL	0 (0)	0.70 (0.27, 1.79)	0.43	--	--
ALAT >170 IU/mL	0 (0)	0.77 (0.30, 1.98)	0.58	--	--
Sodium (serum) >155 mmol/L	0 (0)	0.28 (0.07, 2.00)	0.38	--	--
Occurrence of cardiac arrest before liver procurement	0 (0)	0.49 (0.21, 1.18)	**0.088**	0.50 (0.17, 1.46)	0.21
ICU stay prior to liver procurement >7 days	0 (0)	0.93 (0.22, 3.87)	0.92	--	--

[1]Missing values in parentheses are percentages.

[2]Risks were computed using multivariable Cox proportional hazards models performed after multiple imputation of liver steatosis missing data. Both univariable and multivariable Cox analyses were performed in each imputed data set (n=20) and the estimates and standard errors were pooled into a final point estimate with robust standard errors according to Rubin's rule.

***** SIRT coefficients were infinite due to absence of event in one treatment group and was thus removed to avoid instability of the model

Abbreviations: LT, liver transplantation; HR: Hazards Ratio, CI: confidence interval;

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