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A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation

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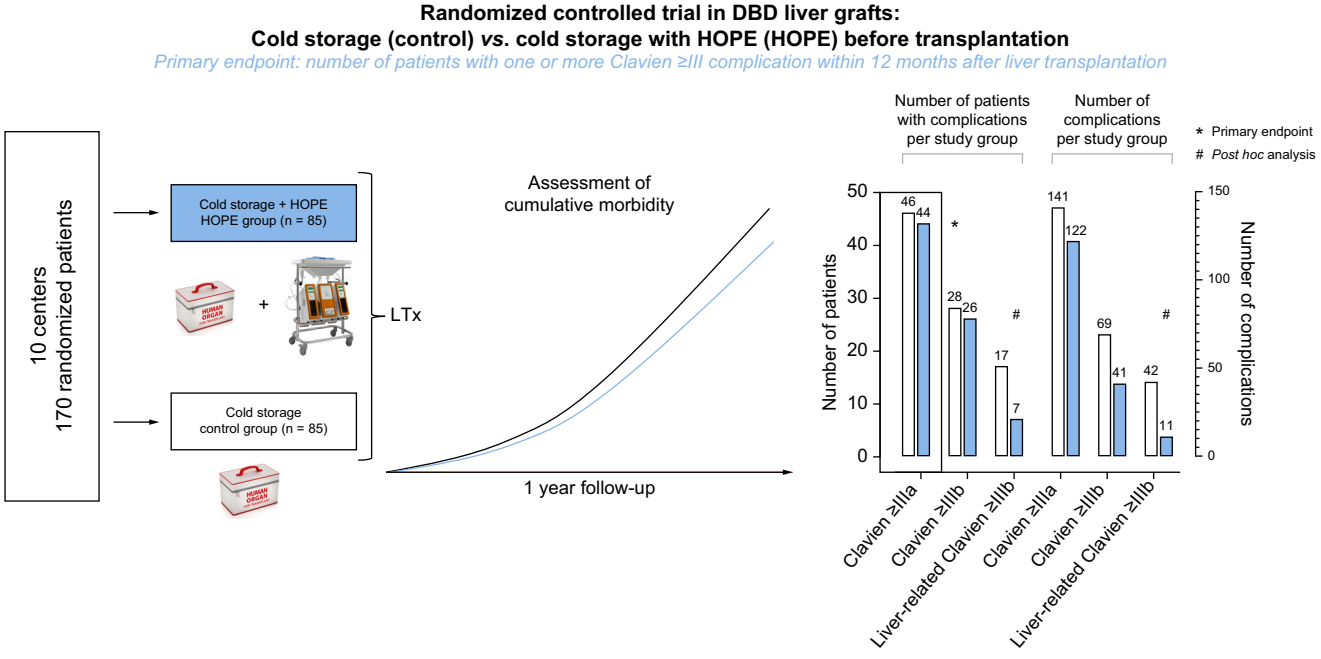
Graphical abstract



Highlights

- The number of patients with at least one Clavien ≥III complication was not significantly different between groups.
- Severe post-transplant complications (Clavien grade IIIb or more), occurred less frequently in the HOPE-group.
- This was caused by a 3.7-fold lower number of liver-related Clavien ≥IIIb complications per patient in the HOPE-group.
- Graft failure due to liver-related complications did not occur in the HOPE-group but occurred in 7% in the control-group.

Impact and implications

This randomized controlled phase III trial is the first to investigate the impact of hypothermic oxygenated perfusion (HOPE) on cumulative complications within a 12-month period after liver transplantation. Compared to conventional cold storage, HOPE did not have a significant effect on the number of patients with at least one Clavien ≥III complication. However, we believe that HOPE may have a beneficial effect on the quantity of complications per patient, based on its application leading to fewer severe liver graft-related complications, and to a lower risk of liver-related graft loss. The HOPE approach can be applied easily after organ transport during recipient hepatectomy. This appears fundamental for wide acceptance since concurring perfusion technologies need either perfusion at donor sites or continuous perfusion during organ transport, which are much costlier and more laborious. We conclude therefore that the *post hoc* findings of this trial should be further validated in future studies.

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A multicenter randomized-controlled trial of hypothermic oxygenated perfusion (HOPE) for human liver grafts before transplantation

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Background & Aims: Machine perfusion is a novel method intended to optimize livers before transplantation. However, its effect on morbidity within a 1-year period after transplantation has remained unclear.

Methods: In this multicenter controlled trial, we randomly assigned livers donated after brain death (DBD) for liver transplantation (LT). Livers were either conventionally cold stored (control group), or cold stored and subsequently treated by 1-2 h hypothermic oxygenated perfusion (HOPE) before implantation (HOPE group). The primary endpoint was the occurrence of at least one post-transplant complication per patient, graded by the Clavien score of ≥III, within 1-year after LT. The comprehensive complication index (CCI), laboratory parameters, as well as duration of hospital and intensive care unit stay, graft survival, patient survival, and biliary complications served as secondary endpoints.

Results: Between April 2015 and August 2019, we randomized 177 livers, resulting in 170 liver transplantations (85 in the HOPE group and 85 in the control group). The number of patients with at least one Clavien ≥III complication was 46/85 (54.1%) in the control group and 44/85 (51.8%) in the HOPE group (odds ratio 0.91; 95% CI 0.50-1.66; $p = 0.76$). Secondary endpoints were also not significantly different between groups. A *post hoc* analysis revealed that liver-related Clavien ≥IIIb complications occurred less frequently in the HOPE group compared to the control group (risk ratio 0.26; 95% CI 0.07-0.77; $p = 0.027$). Likewise, graft failure due to liver-related complications did not occur in the HOPE group, but occurred in 7% (6 of 85) of the control group (log-rank test, $p = 0.004$, Gray test, $p = 0.015$).

Conclusions: HOPE after cold storage of DBD livers resulted in similar proportions of patients with at least one Clavien ≥III complication compared to controls. Exploratory findings suggest that HOPE decreases the risk of severe liver graft-related events.

Introduction

Dynamic preservation strategies are an innovative approach for treatment and assessment of livers before transplantation. This is based on a number of experimental studies demonstrating that liver metabolism can be measured and controlled *ex situ* by either continuous normothermic,[1,2] or short-term hypothermic oxygenated liver perfusion (HOPE),[3] with multiple protective downstream effects, including less Kupffer and endothelial cell activation and subsequently a reduced immune response.[4–6] Such promising results were translated from several preclinical studies and have demonstrated the prevention of liver ischemia reperfusion injury.[7–11] Despite this success, the effect of machine liver perfusion on clinically relevant endpoints, either normothermic or hypothermic, has remained unclear. This is important as liver transplantation (LT) is associated with exceptionally high morbidity even in benchmark cases,[12] despite excellent survival rates, and an observation period of at least one year is mandatory to capture all relevant complications.[10,13] Most published trials or case series underestimate this fact, with primary endpoints consisting of laboratory values of questionable relevance, recorded only within the first week after LT.[10,14,15] In contrast, there is an urgent need to investigate whether machine perfusion techniques also impact on clinically and patient relevant

Keywords: Liver transplantation; Randomised controlled trial; Hypothermic oxygenated machine perfusion; Cumulative complications; Liver-related complications.
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endpoints, the most convincing being complications, and the severity of complications after LT, to justify the additional efforts and costs. The first evidence in this context was presented only very recently with a study on HOPE-treated donation after circulatory death (DCD) livers,[4] which showed less symptomatic cholangiopathies within 6 months after LT. Further recent randomized trials on donation after brain death (DBD) livers, treated by HOPE, showed less liver graft injury as a primary endpoint, and reported complications during a follow-up of 3 and 6 month.[9,10] However, the effect of HOPE on cumulative morbidity within 1 year after LT remains unknown.

Patients and methods

Trial design

The HOPE (hypothermic oxygenated perfusion for human liver grafts) trial is an investigator-initiated multicenter randomized-controlled trial (RCT), which included 10 European transplant centers (Birmingham, Gent, Groningen, Leeds, Leuven, London, Lyon, Paris, Vienna, and Zurich) from six countries (Fig. 1).

Allocated livers were randomly assigned in a 1:1 ratio to be preserved either by conventional cold storage (control group), or by cold storage plus subsequent 1-2 h HOPE. Randomization

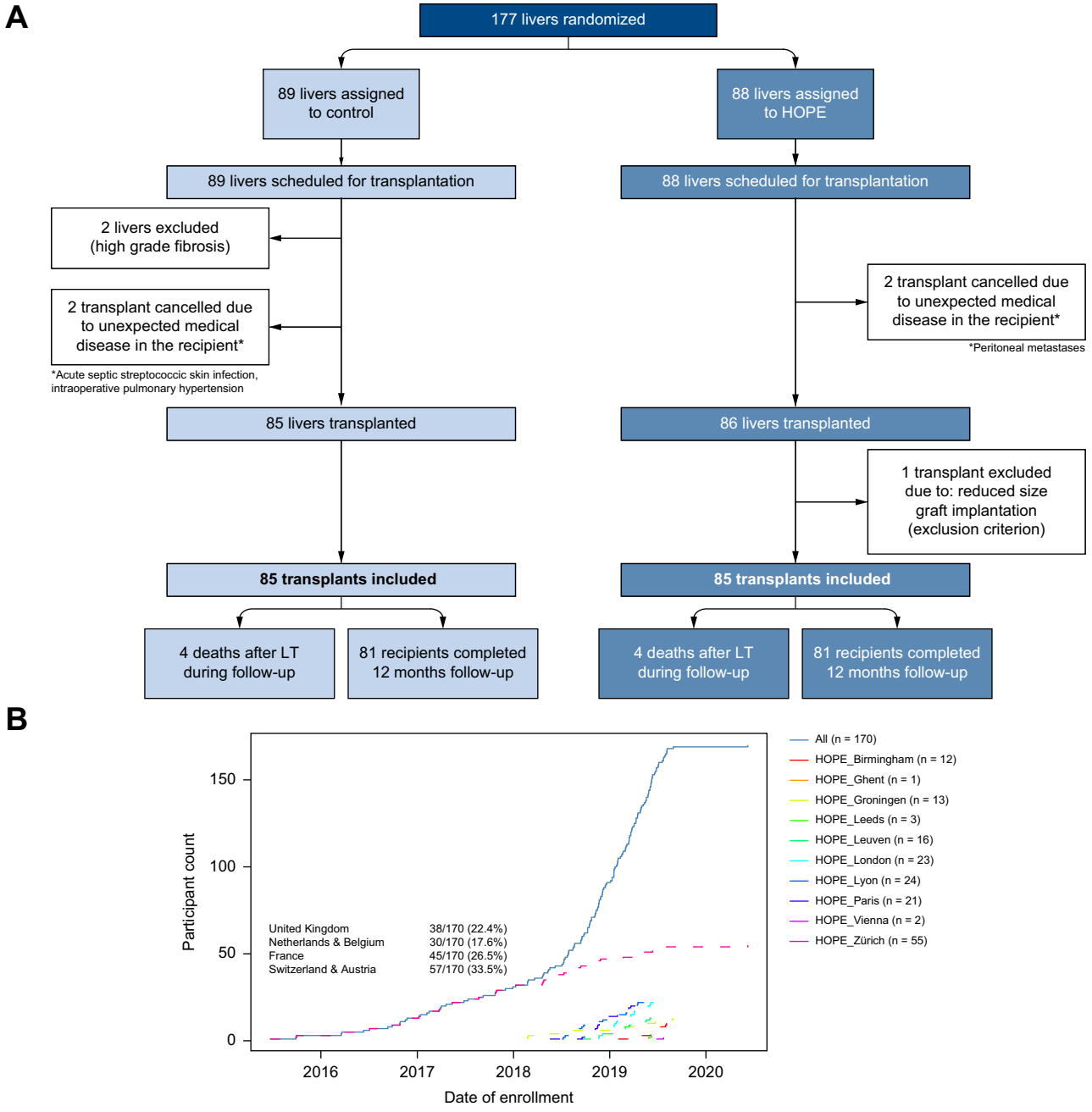

Fig. 1. CONSORT diagram and overall/center-specific recruitment. (A) CONSORT diagram for donor livers enrolled in the trial. 177 livers were randomized and 170 livers were transplanted according to protocol, *e.g.* 85 in the control group and 85 in the HOPE group (dropout rates 4/89, 4.5%; 3/88, 3.4%). (B) Overall and center-specific annual recruitment of cases. HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

was performed after the donor liver was accepted for transplantation. Center-stratified block randomization with a fixed block size of eight was used to generate a randomization list per center. The block size was not communicated to the investigators. A centralized web-based tool (Randomizer Software, Institute for Medical Informatics, Statistics and Documentation of the Medical University of Graz, www.randomizer.at) was used for randomization at the first center (Zurich), and randomization was performed by an independent person using Microsoft Excel for the other centers. Randomization lists were then stored in the electronic case report files (secuTrial®) to ensure allocation concealment.

The trial did not interfere with organ allocation or acceptance; patients, organ procurement teams, and the treating physicians were blinded to the trial group assignments, while surgeons were unblinded due to the perfusion procedure itself. The trial protocol and the amendments have been approved by the ethical committees and the national authorities, and are enclosed in the supplement. Data capturing was performed by electronic case report files using the secuTrial® platform. The trial was entirely funded by the Swiss National Science Foundation (33IC30_166909, 32003B_153012), including the perfusate, the perfusion machine disposables, and the monitoring. The funding party played no role in study design, performance, analysis, or the decision to publish. The participating centers provided the perfusion device (Liver Assist®, Organ Assist, now XVIVO), and the training for machine perfusion for each center was supervised by the study PI.

Trial patients

All patients ≥18 years of age, who were listed for liver only transplantation with a whole DBD graft were eligible for inclusion in the trial. Exclusion criteria were all partial or combined liver transplants, living donor or DCD liver transplantation, cold ischemia times of more than 15 h, and an acute or unexpected medical contraindication for LT. All included patients provided written informed consent.

Perfusion procedure

All study centers used the Liver Assist® device for machine liver perfusion, with a pressure controlled oxygenated hypothermic liver perfusion through the portal vein only (Fig. S1), targeting a flow rate between 150-300 ml/min at a pressure of 3 mmHg, and a perfusate temperature between 8 and 12 °C. The perfusate consisted of 3 L re-circulating Belzer MPS® (Bridge to Life Ltd.) with active oxygenation (70-110 kPa). The minimum perfusion duration was defined as 1 h, while perfusion was generally continued until the recipient hepatectomy was completed.

Endpoint measures

The primary endpoint was the occurrence of one or more major post-transplant complication, defined as a Clavien score of ≥III, per patient (binary) within 1 year after LT.[16] The Clavien score ranges from I (for any deviation from the normal postoperative course without pharmacological treatment or surgical, endoscopic, or radiologic interventions) to V (for death).

Secondary endpoints were the comprehensive complication index (CCI, from 0 for no complication to 100 for death),[18] laboratory measurements (aspartate aminotransferase [AST], alanine aminotransferase [ALT], bilirubin, alkaline phosphatase, gamma glutamyltransferase, international normalized ratio, and Factor V), biliary complications, duration of intensive care unit (ICU) and hospital stay, as well as recipient and graft survival at 1 year after LT. Laboratory measurements were taken at 6 h, 12 h, day 1-7, discharge and 3, 6, 9 and 12 months after LT. Measurements taken on day 1-7 were summarized as area under the curve (AUC), using natural cubic spline interpolation. Measurements taken 3, 6, 9 and 12 months after LT were considered as longitudinal data.

Outcomes analyzed *post hoc*

As many patients developed more than one major complication within the 12-month period after transplantation, we decided *post hoc* to also consider the number of complications per patient. The occurrence and grading of complications were assessed by the local investigators and controlled by the monitors. The final Clavien score was controlled by two independent clinicians, who were blinded to the preservation method (J.E., R.P.). All complications were additionally classified into three groups:

- Recipient-related complications: opportunistic infections, myocardial infarction, lung embolism, lung infections, hypertension, gastric ulcer, colitis, ileus, diabetes, diarrhea, pyelonephritis, seizures, cerebral ischemia, cerebral bleeding, mesenteric ischemia, ascites (without the need to drain), incarcerated umbilical or inguinal hernias (with the need for surgical repair), accidental traumas, recurrence of hepatocellular carcinoma, secondary cancer.
- Liver graft-related complications: primary non-function, biliary necrosis, biliary strictures (anastomotic and non-anastomotic), bile leaks, hepatic artery thrombosis, hepatic artery stenosis, hepatic artery aneurysms, portal vein thrombosis, hepatic vein thrombosis, acute biopsy proven liver rejection, cholangitis, cholangiosepsis, hepatic encephalopathy, elevated liver enzymes (three-fold over normal values), cholestasis, ascites (with the need for drainage).
- Transplant procedure-related complications: post-transplant hematoma in the first week (with the need for lavage), intermittent kidney failure (with the need for renal replacement therapy), wound infections (with the need for wound opening), elective incisional hernias (transplant incision).

In addition, graft survival was analyzed separately for recipient-related and liver graft-related graft loss.

Monitoring and safety

Monitoring and safety were organized and supervised by the GSO (Gesellschaft für Studienmanagement und Onkologie mbH) Hamburg with regular reporting of all serious adverse events to the national authorities. Adverse events were defined according to EN ISO 14155 as any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) whether or not related to the investigational medical device. Serious adverse events were defined as adverse events that:

a) led to death, injury or permanent impairment to a body structure or a body function; b) led to a serious deterioration in health of the patient, that either resulted in: a life-threatening illness or injury, a permanent impairment of a body structure

or a body function, in-patient hospitalization or prolongation of existing hospitalization, or in the requirement for medical or surgical intervention to prevent life-threatening illness.

Statistical analysis

The trial was powered to detect a clinically relevant difference in the incidence of major complications, *e.g*. an absolute risk difference of 25% in the incidence of at least one Clavien ≥III complication by the treatment of liver grafts with HOPE. This calculation was based on initial data from the first clinical series on hypothermic liver perfusion,[17] which showed a significantly decreased hospital stay by machine liver perfusion (10.9 *vs.* 15.3 days, p = 0.006, 29% less), and reduced early graft dysfunction (5 *vs*. 25%). It was assumed that the proportion of patients with at least one ≥grade III complication within 1 year will decrease from 60% in the control group to 35% in the HOPE group. For the sample size calculation, to achieve a power of 90%, a significance level of 0.05 was considered for a two-sided z-test with pooled variance.[18] This resulted in a sample size of 82 per arm, 164 in total. The sample size was then increased to 85 per arm, 170 in total, to account for expected dropouts after transplantation.

Primary and secondary endpoint analyses were prespecified in the protocol (supplementary information) and in the statistical analysis plan (Version 03, December 2020, supplementary information), which was finalized before the database was locked. The primary endpoint was analyzed by a generalized linear model (GLM) with binomial error and logit link and treatment as an explanatory variable to estimate an odds ratio (OR) with 95% CIs for the effect of HOPE *vs.* control. Two pre-specified sensitivity analyses were performed: We fitted a generalized linear mixed-effects model (GLMM), with a random intercept for center, for which the randomization was stratified (sensitivity analysis 1). We then added covariates expected to be associated with the primary outcome, *i.e*. 'recipient lab MELD score', 'cold storage time', 'age of recipient', 'age of donor' and 'previous transplantation', as fixed explanatory variables (sensitivity analysis 2). Due to some missing data in these co-variates, we used multiple imputation with 50 imputations. The imputation model contained the covariates mentioned above, the randomized treatment, the primary outcome and the CCI as well as donor and recipient sex, donor height and weight and treatment before liver transplantation. The results were pooled according to Rubin's rules.

The secondary outcome CCI was compared between groups by a linear regression model with treatment as an explanatory variable. The same two sensitivity analyses were performed as described for the primary outcome but using a linear mixed-effects model (LMM) instead of a GLMM. AUCs of laboratory values (AST and ALT) were log-transformed to better meet the normality assumption and were analyzed using LMMs. Binary secondary outcomes were analyzed by GLM with binomial error and logit link. Length of hospital stay and length of ICU stay were analyzed by cause-specific Cox proportional hazards models on time to discharge alive, accounting for death during hospital or ICU stay as a competing risk.

Number of major complications (per patient) was analyzed *post hoc* using a GLM with log link and quasi-Poisson error. Further, time to graft failure was analyzed *post hoc*, once overall and once separated for liver-related graft failure and participant-related graft failure. Cause-specific Cox proportional hazards models were used for the two subtypes of graft failure (competing risks).

It should be noted that no adjustments were made for type I error rate inflation due to the analysis of multiple outcomes.

All statistical methods and results, including deviations from the original statistical analysis plan are reported in detail in the statistical report (Version 1.5, November 16th, 2022, supplementary information).

Results

Patients

Between April 2015 and August 2019, we randomized 177 livers, accepted for transplantation into eligible recipients. After randomization, six transplants were cancelled before any trial procedure, including four assignments to the control group, and 2 assignments to the perfusion group. The reasons for cancellations were high-grade fibrosis in two liver grafts, an unexpected severe pulmonary hypertension after intubation in one recipient, an acute streptococcic skin infection discovered at recipient hospital entry, and unexpected peritoneal metastasis after recipient laparotomy (n = 2). In one further case, the perfused liver was reduced to a left lobe before transplantation, to compensate for a severe size mismatch, resulting in transplantation of a partial graft. This patient was therefore excluded from the trial, according to the protocol criteria. These early dropouts, *e.g.*, at the day of randomization, were compensated for by additional recruitment (Fig. 1).

Overall, 170 liver transplants were performed within the trial, and 85 patients in each study arm were included in the analysis (Fig. 1). All patients completed the 1-year follow-up, with the exception of deaths during this time (n = 8).

The baseline characteristics of the donors and preservation factors are shown in Table 1; baseline characteristics of recipients are shown in Table 2. Despite randomization, there were some imbalances between groups. For example, there were less cerebral hemorrhages and more other causes of death in the HOPE group. Liver weight was 126 g lower, and cold storage 54 min shorter in the HOPE group (Table 1). In addition, more female recipients (14.1%), less cases with Child-Pugh B/C cirrhosis, and less conservative treatment before LT were recorded in the HOPE group (Table 2).

Machine perfusion parameters were within the range defined in the protocol, *e.g*. median perfusion time 96 min, median perfusion flow 200 ml/min, median perfusion pressure 3 mmHg, median perfusate temperature 10 °C, and median oxygenation 100 kPa (Fig. S2).

Primary endpoint: number of patients with Clavien ≥III complications

A total of 1,190 complications were documented for all study patients during 1 year after LT with no patients lost to follow-up. The proportion of patients with at least one Clavien ≥IIIa complication did not significantly differ between groups – 54.1% (46/85) in the control group and 51.8% (44/85) in the HOPE group. This resulted in an unadjusted OR of 0.91 (95% CI 0.50–1.66, p = 0.76). The absolute risk difference was estimated as -2.35% (95% CI 16.96%–12.40%). In our sensitivity

Table 1. Characteristics of liver donors and liver graft preservation.

Variable	Overall	Control	HOPE	Missing (%)
N	170	85	85	
Before randomization				
Donor age, years	60.5 (47.0–72.0)	62.0 (44.0–71.0)	59.0 (48.0–72.0)	0
Donor sex, female	82 (48.5)	42 (50.0)	40 (47.1)	0.6
Donor height, m – mean (SD)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	1.2
Donor weight, kg – mean (SD)	76.3 (15.8)	77.8 (16.9)	74.9 (14.5)	1.2
Donor cause of death				6.5
Cerebral hemorrhage	73 (45.9)	39 (48.8)	34 (43.0)	
Cerebral trauma	37 (23.3)	18 (22.5)	19 (24.1)	
Anoxia	23 (14.5)	12 (15.0)	11 (13.9)	
Cerebral disease	1 (0.6)	0	1 (1.3)	
Suicide	4 (2.5)	3 (3.8)	1 (1.3)	
Other	21 (13.2)	8 (10.0)	13 (16.5)	
After randomization				
Preservation solution				0.6
Histidin-Tryptophan-Ketoglutarat (HTK)	4 (2.4)	1 (1.2)	3 (3.6)	
University of Wisconsin (UW)	53 (31.4)	27 (31.8)	26 (31.0)	
Institute George Lopez (IGL)-1	112 (66.3)	57 (67.1)	55 (65.5)	
Duration of cold storage, min	393.0 (320.0–482.0)	427.0 (356.0–487.0)	373.0 (299.2–471.8)	7.6
Duration of HOPE, min	95.5 (73.0–137.0)	—	95.5 (73.0–137.0)	57.6
Duration of total preservation time, min	451.0 (371.0–552.5)	427.0 (356.0–487.0)	474.0 (403.5–588.0)	13.5
Liver weight, g – mean (SD)	1517.0 (591.8)	1,583.0 (759.0)	1,457.3 (378.6)	17.1
AST HOPE perfusate, U/L		—	117.6 (60.0- 266.9)	76.1
ALT HOPE perfusate, U/L		—	177.1 (75.0-467.0)	76.1

Continuous variables are presented as median (IQR) and categorical variables as n (%), unless otherwise stated.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HOPE, hypothermic oxygenated perfusion.

Table 2. Characteristics of liver transplant recipients.

Variable	Overall	Control	HOPE	Missing (%)
n	**170**	**85**	**85**	
Recipient age, years	59.0 (50.2–64.0)	57.0 (49.0–64.0)	60.0 (51.0–64.0)	0
Recipient sex, female	48 (28.2)	18 (21.2)	30 (35.3)	0
Underlying disease				0
Acute liver failure	1 (0.6)	1 (1.2)	0 (0)	
Cirrhosis Child-Pugh A	49 (28.8)	23 (27.1)	26 (30.6)	
Cirrhosis Child-Pugh B,C	93 (54.7)	50 (58.8)	43 (50.6)	
Other	27 (15.9)	11 (12.9)	16 (18.8)	
Laboratory MELD	20.0 (11.0–27.0)	19.0 (12.0–26.0)	20.0 (11.0–28.0)	0
Treatment before liver transplant				0
TACE, RFA	41 (24.1)	21 (24.7)	20 (23.5)	
TIPS	10 (5.9)	5 (5.9)	5 (5.9)	
Conservative	38 (22.4)	23 (27.1)	15 (17.6)	
No treatment	61 (35.9)	34 (40.0)	27 (31.8)	
Other	20 (11.8)	2 (2.4)	18 (21.2)	
Previous liver transplant	7 (4.1)	2 (2.4)	5 (5.9)	0
Transplant center				0
Birmingham	12 (7.1)	7 (8.2)	5 (5.9)	
Ghent	1 (0.6)	0 (0)	1 (1.2)	
Groningen	13 (7.6)	7 (8.2)	6 (7.1)	
Leeds	3 (1.8)	2 (2.4)	1 (1.2)	
Leuven	16 (9.4)	8 (9.4)	8 (9.4)	
London	23 (13.5)	11 (12.9)	12 (14.1)	
Lyon	24 (14.1)	12 (14.1)	12 (14.1)	
Paris	21 (12.4)	11 (12.9)	10 (11.8)	
Vienna	2 (1.2)	1 (1.2)	1 (1.2)	
Zürich	55 (32.4)	26 (30.6)	29 (34.1)	

Continuous variables are presented as median (IQR) and categorical variables as n (%).
HOPE, hypothermic oxygenated perfusion; MELD, model for end-stage liver disease; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TIPS, transjugular intrahepatic portosystemic shunt.

analyses using GLMMs with random intercept for center, the ORs were estimated as 0.874 (95% CI 0.46–1.67, $p = 0.68$) and 0.91 (95% CI 0.47–1.78, $p = 0.787$), when adjusted for MELD, donor age, recipient age, duration of cold storage, and previous liver transplantation.

Secondary endpoints

The overall CCI of all complications was not significantly different between study groups, *e.g.* the median 12-month CCI was 49.5 (IQR 29.6–64.5) in the control group and 49.4 (IQR 33.2-63.9) in the HOPE group (Table 3). Laboratory

Table 3. Pre-specified secondary endpoints (recipient outcome within 12 months after LT) and additional outcome parameters.

Variable	Overall	Control	HOPE	*p* value	Effect size (95% CI)	Missing (%)
N	170	85	85			
CCI 12, months	49.4 (29.6–64.4)	49.5 (29.6–64.5)	49.4 (33.2–63.9)	0.89‡	MD 0.685–7.202 to 8.338)‡	0
Peak AST, U/L	825 (430–1,705)	896 (409–2,478)	803 (435–1,303)			0
AST AUC, U/L – day 1-7	1,147 (687–2,171)	1,147 (683–2,752)	1,149 (693–1,856)	0.25*	MD -0.157 (–0.42 to 0.11)*	1.8
Peak ALT, U/L	654 (365–1,188)	695 (379–1,575)	636 (341–1,055)			0
ALT AUC, U/L – day 1-7	2,022 (1,242–3,750)	1,978 (1,232–4,128)	2,048 (1,252–3,475)	0.49*	MD -0.089 (–0.34 to 0.16)*	0
INR AUC, day 1-7	7.1 (6.6–7.8)	7.1 (6.5–8.1)	7.1 (6.6–7.8)			0
Bilirubin AUC, μmol/L – day 1-7	199 (103–438)	202 (95–542)	200 (119–381)			11.2
GGT AUC, U/L – day 1-7	1,653 (806–2,615)	1,774 (761–2,621)	1,531 (918–2,610)			11.8
AP AUC, U/L – day 1-7	846 (622–1,320)	874 (637–1,255)	803 (619–1,323)			0.6
Hospital stay, days	15 (13.0–25.0)	15 (13.0,25.0)	17 (12.0–24.5)	0.79#	HR 0.958 (0.70 to 1.30)#	1.8
ICU stay, days	3.0 (2.0–5.0)	3.0 (2.0–6.0)	3.0 (2.0–5.0)	0.75#	HR 1.051 (0.77 to 1.43)#	0
Any biliary complication	34 (20.0)	19 (22.4)	15 (17.6)	0.44§	OR 0.744 (0.35 to 1.58)§	0
Overall graft loss in 1 year	11 (6.5)	7 (8.2)	4 (4.7)	0.36§	OR 0.550 (0.140 to 1.896)§	0
Recipient death in 1 year	8 (4.7)	4 (4.7)	4 (4.8)	1.00§	OR 1.000 (0.229 to 4.359)§	0
Additional outcome parameters after LT						
Duration of transplantation, min	380 (295–477)	384 (302–464)	371 (284–480)			3.5
Anastomotic biliary complications	32 (19.0)	18 (21.2)	14 (16.5)			0
Non-anastomotic biliary complications (NAS)	4 (2.4)	3 (3.5)	1 (1.2)##			0
Early allograft dysfunction**	53 (31.2)	39 (45.9)	14 (16.5)			0
Hepatic artery thrombosis	2 (1.2)	0	2 (2.4)			0
Hepatic artery stenosis	3 (1.8)	2 (2.4)	1 (1.2)			0
Liver-related graft loss due to:	6 (3.5)	6 (7.1)	0	0.004		0
Primary non function	3 (1.8)	3 (3.5)	0	0.015†		0
NAS	3 (1.8)	3 (3.5)	0			0
Recipient-related graft loss	5 (2.9)	1 (1.2)	4 (4.7)	0.223‡‡	HR 3.90 (0.44 to 34.90)‡‡	0
Primary tumor recurrence	1 (0.6)	0	1 (1.2)			0
Secondary tumor growth	3 (1.8)	1 (1.2)	2 (2.4)			0
Opportunistic infection	1 (0.6)	0	1 (1.2)			0
Retransplantation	3 (1.8)	3 (3.5)	0			0
CCI 3 month	41.8 (23.0–52.6)	42.4 (22.6–52.7)	41.8 (24.2–52.6)			0
CCI 6 month	46.0 (27.3–58.8)	42.4 (22.6–52.7)	46.8 (29.8–60.1)			0
CCI 9 month	48.3 (29.6–63.2)	48.2 (29.6–59.9)	48.9 (29.8–63.7)			0

Continuous variables are presented as median (IQR) and categorical variables as n (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CCI, comprehensive complication index; GGT, gamma glutamyltransferase; HOPE, hypothermic oxygenated perfusion; HR, hazard ratio; INR, international normalized ratio; LT, liver transplantation; MD, mean difference; NAS, non-anastomotic strictures; OR, odds ratio.
‡Linear model, with similar MD estimated in sensitivity analyses by simple and covariate-adjusted linear mixed-effects model.
*Linear mixed-effects model with a random intercept per center. AUCs were log-transformed to better meet the normality assumption.
#Cause-specific Cox proportional hazards models on time to discharge alive from hospital or ICU (accounting for death during hospital or ICU stay as competing risk).
§Generalized linear model with log link function.
†Due to zero events in the HOPE arm, the HR could not be estimated. The p-value was calculated by the log rank test and the Gray test for comparing the two cumulative incidence function curves.
‡‡Cox proportional hazards model.
**Olthoff criteria.
##This graft was not lost, conservative treatment of biliary complication.

values (AST, ALT, international normalized ratio, gamma-glutamyltransferase, bilirubin, alkaline phosphatase) during the first week after liver transplant, assessed by AUC, and peak AST and peak ALT, were not significantly different between study groups (Table 3). Of note, the AUC for factor V was not calculated due to lack of data (missing values 46.5%). The further course of laboratory values at 3, 6, 9, and 12 months is shown in Fig. S3, together with those within the first week. Longitudinal analysis of laboratory measurements at 3-12 months using GLMM did not reveal significant differences between groups (supplementary information). ICU and hospital length of stay were also similar in both groups (Table 3). One-year overall graft survival was 95.3% (81/85) in the HOPE group with three tumor-related graft losses and one graft loss due to candida pneumonia, and 91.8% (78/85) in the control group with six liver graft-related and one tumor-related graft loss (OR 0.550; 95% CI 0.140–1.896, $p = 0.36$, Table 3).

Post hoc analysis: quantity of complications per patient

We recorded 574 complications in the control group and 616 complications in the HOPE group. The vast majority of complications were minor and graded as Clavien I-II in both arms (433/574, 75.4% and 494/616, 80.2%, Fig. 2). Likewise, the number of Clavien IIIa complications, treated under local anesthesia, was not significantly different, with 72/574 (12.5%) in the control group, and 81/616 (13.1%) in the HOPE group (Fig. 2), which underlines a comparable number of minor (anastomotic) biliary complications in both groups within benchmark values (Table 3B).[12]

In contrast, severe complications (Clavien-Grade ≥IIIb), *e.g.* operative re-exposures, single- and multi-organ failures, or death, occurred less frequently in the HOPE group (41 of 616 complications, 6.6%) than the control group (69 of 574 complications, 12.0%) (rate ratio 0.59, 95% CI 0.31–1.11), corresponding to a 41% reduction (Fig. 2). This was caused by a

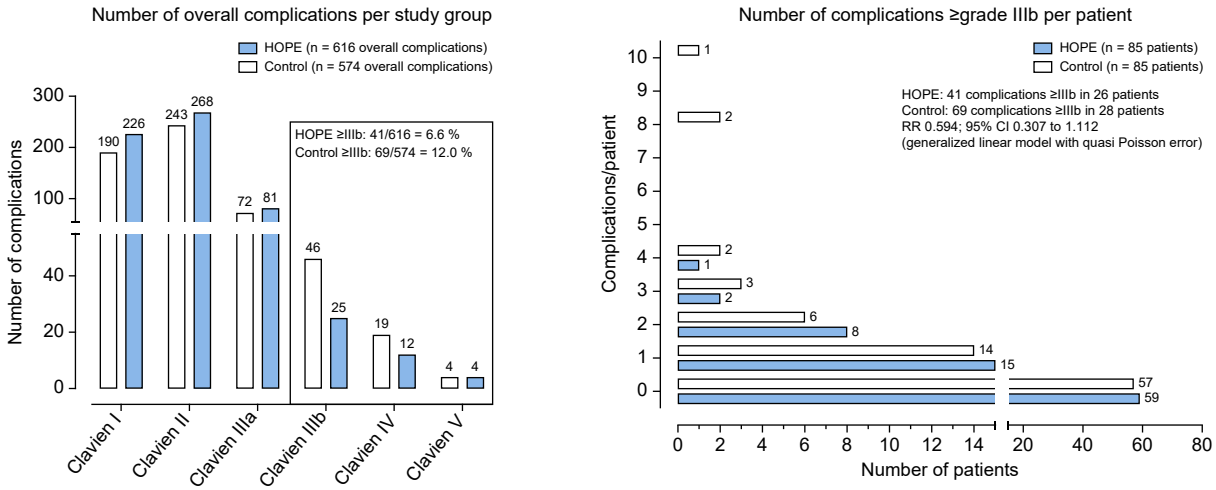

Fig. 2. Number of complications within 1 year after LT (1,190 recorded complications in 170 included patients). Number of complications per Clavien score by randomized treatment and study group (left panel). Number of Clavien >IIIb complications per randomized patient and study group (right panel). HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

74% lower number of liver graft-related Clavien ≥IIIb complications per patient in the perfusion group compared to the control group (11 complications in 7 patients *vs.* 42 complications in 17 patients (rate ratio 0.26; 95% CI 0.07–0.77; $p = 0.027$; Figs 3 and 4). Accordingly, the CCI for patients with liver graft-related complications (n = 83) within 1-year follow-up was lower in the perfusion group (median 30.6; IQR 20.9–37.1), compared to the control group (median 43.6; IQR 29.6–58.6, Table S1). Consistently, liver-related graft failure did not occur in the HOPE group, while six liver grafts were lost in the control group due to severe liver-related complications, *e.g.* primary non function or cholangiopathy (log-rank test, $p = 0.004$, Gray test $p = 0.015$, Table 3B and Fig. 5).

Safety and serious adverse events

The number of reported serious adverse events was comparable in the two study groups (Table S2). There was also no relevant clinical difference between the two groups in the severity of these events. Four device malfunctions occurred in 88 machine liver perfusions (4.5%), which resulted in insufficient perfusion flow through the portal vein in three cases, and in excessive perfusion (>400 ml/min) despite low portal pressure in one case. In one of these cases, an unexpected peritoneal metastasis in the recipient was confirmed through histology, with consecutive cancelled transplantation. This case was therefore excluded from the analysis. The other three device malfunctions were included.

Discussion

Despite benchmark analysis and multiple reports on outcome after LT, quantifying morbidity in a liver transplant population remains a major challenge. We present the first randomized machine liver perfusion trial on cumulative recipient morbidity within a one-year period after transplantation, which is

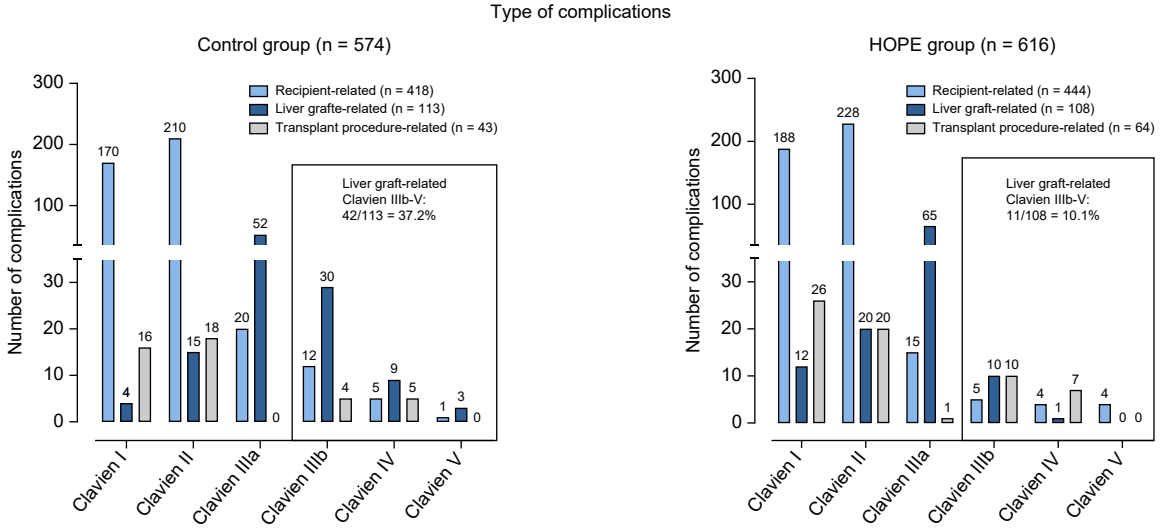

Fig. 3. Specification of complications within 1 year after LT. Number of complications per Clavien grading level by relatedness to recipient, liver graft, or transplant procedure for patients in the control arm (left panel) and in the HOPE arm (right panel). HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

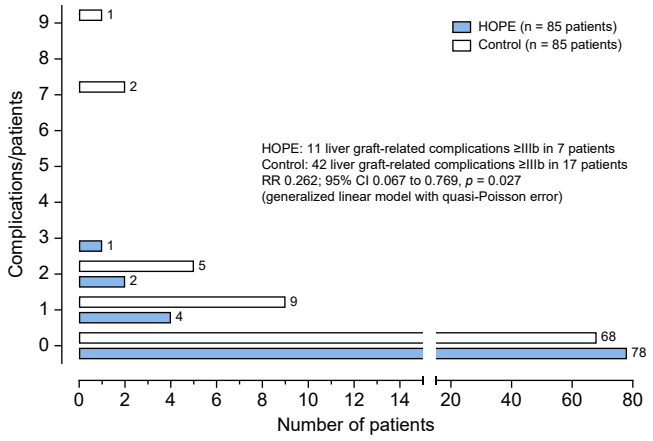

Fig. 4. Number of liver graft-related ≥IIIb complications per patient. Number of liver graft-related ≥IIIb complications per randomized patient and study group with comparison by a generalized linear model with quasi-Poisson error. HOPE, hypothermic oxygenated perfusion; RR, risk ratio.

mandatory for a reliable assessment of complications.[12] The trial shows that HOPE after cold storage of DBD livers did not significantly affect the number of patients with at least one grade ≥III complication within 1 year after LT. There was also no significant difference in all pre-specified secondary endpoints, which focus on laboratory values, initial ICU and hospital stay, and survival.

However, many patients developed more than one major complication within 1-year follow-up, which is ignored by the binary primary endpoint and is likewise not captured by average laboratory values, post-transplant ICU stay, or graft survival, with a subsequent potential underestimation of graft treatment effects.[19] Instead, the extent of post-transplant morbidity was only recognized by the frequency and the severity of complications, with a 74% lower number of liver-related Clavien ≥IIIb complications in the HOPE arm, compared to the control group. Yet, these results were found in a *post hoc* analysis, and are therefore of an exploratory nature. Further studies will be needed to confirm this potentially clinically important effect of HOPE on the most expensive complications after surgery (Clavien ≥IIIb).[20] A similar effect was recently shown for hypothermic oxygenated kidney perfusion,[21] which may serve as a strong argument for reimbursement of this technology by healthcare providers.

Machine liver perfusion has attracted wide attention within the transplantation community over the last 5 years, but is still rarely applied by most transplant surgeons.[22] This probably relies on the perception that this strategy is time consuming and costly, despite current research disclosing several advantages compared to conventional cold storage, including mitochondrial energy restoration or assessment of liver quality before implantation.[23–26] Another reason for such restrictive use of machine liver perfusion is the lack of available convincing RCTs demonstrating clinically relevant benefits as primary endpoints, as opposed to data on recipient laboratory values or early allograft dysfunction within the first week after LT.[10,14,15]

As a first step in this direction, a recently published RCT assessing the impact of D-HOPE (dual-HOPE, *e.g.* perfusion of both, the hepatic artery and the portal vein) on DCD livers, showed a decrease in symptomatic cholangiopathies.[16] However, while intrahepatic cholangiopathies are a frequent and feared complication in DCD liver transplants, DBD liver recipients are rarely affected by this type of injury.[6,27] Our results

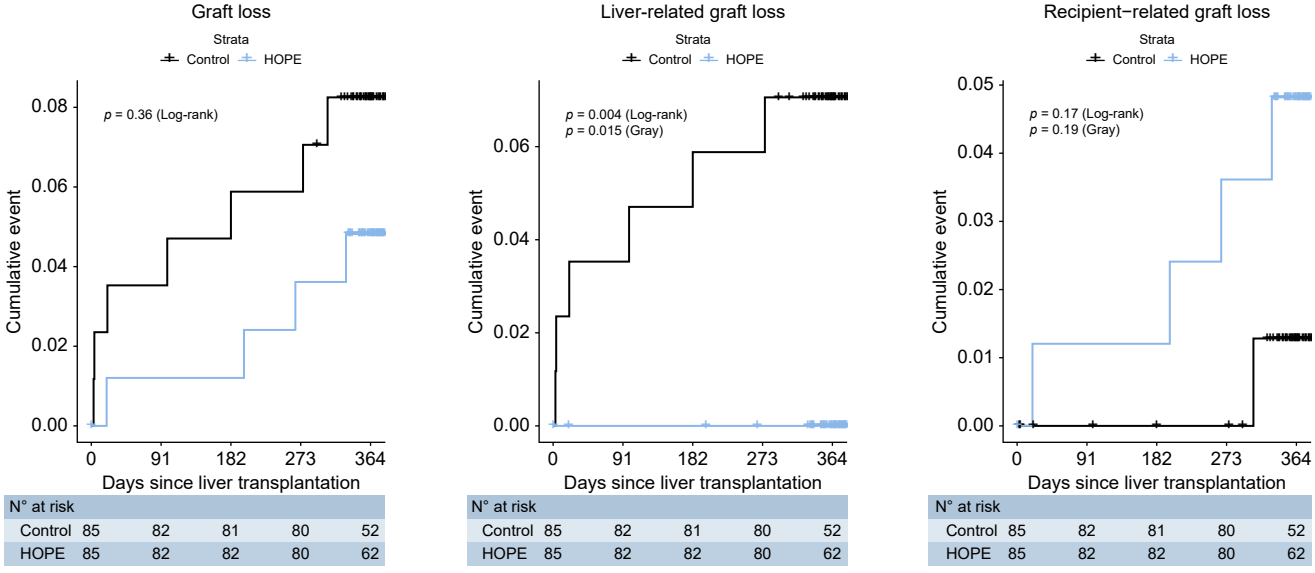

Fig. 5. Graft loss within 1 year after LT. 1-KM curves for graft loss (all types, left) and the two subtypes liver-related (middle) and recipient-related graft loss by treatment (right), which are treated as two competing risks by the Gray test. Liver related graft failure did not occur in the HOPE group, while six liver grafts were lost in the control group due to liver related complications, *e.g.* primary non-function (n = 3) or intrahepatic cholangiopathy (n = 3). The resulting HRs for HOPE *vs*. control, estimated by (cause specific) Cox proportional hazards models, and the log-rank test for the 1-KM curve as well as the Gray test for the cumulative incidence function (CIF) curves (testing the null-hypothesis of no difference between curves) are as follows: • Graft loss: HR = 0.57 (95% CI 0.17–1.94), log-rank test *p* value: 0.36; • Liver-related graft loss: HR = 0.00 (95% CI 0.00–inf), log-rank test *p* value 0.004, Gray test *p* value: 0.015 (due to zero events in the HOPE arm, the HR cannot be estimated); • Patient-related graft loss: HR = 3.90 (95% CI 0.44–34.90), log-rank test *p* value 0.17, Gray test *p* value: 0.19. HOPE, hypothermic oxygenated perfusion; LT, liver transplantation.

indicate that HOPE treatment may be also effective in preventing additional major complications in patients receiving DBD livers, the most common grafts in the West. This result is consistent with another published RCT on HOPE-treated extended criteria donor livers, which reported less Clavien ≥III complications with HOPE treatment.[10] However, this trial focused on first week peak serum ALT levels as a primary endpoint, with only 23 patients included in each study arm, and a 3-month follow-up for complications. In addition, only the highest-graded complication per patient was counted, which limits interpretation regarding overall morbidity. In contrast, we present here a meticulous assessment of numerous complications per patient up to 1 year after LT.

The mechanism of HOPE has been investigated in several experimental studies and has been shown to be dependent on sufficient perfusate oxygenation under hypothermic conditions in livers, kidneys and in hearts.[8,28,29] Oxygenated cold perfusion triggers a mitochondrial metabolic conversion with sufficient reduction of accumulated citric acid metabolites and electron donors, *e.g.*, succinate and NADH, during perfusion, while avoiding reverse electron transfer to mitochondrial complex-I.[25] HOPE-treated livers are therefore uploaded with ATP, without major oxidative stress, and simultaneously present low lactate and low succinate levels, and a well-preserved complex I-IV function, which enables immediate graft function after implantation.[8,25,28–30] Based on this, the benefit of HOPE should increase with increasing graft injury.[3]

Despite these well described biochemical effects, end-ischemic HOPE liver treatment has failed to prevent anastomotic biliary complications, *e.g.* IIIa complications, even when applied additionally through the hepatic artery (D-HOPE).[4] Accordingly, the extrahepatic bile duct epithelium, *e.g.* the common bile duct, appears more difficult to protect, compared to intrahepatic cholangiocytes and hepatocytes, and further research is needed, for example to investigate the effect of changes in perfusate compositions.

This study has limitations. First, the restrictions of using a binary primary endpoint are well known, and should have been anticipated when designing the study, which was in 2011 (first registration in clinicalTrials.gov.). At that time, very limited data on morbidity after LT was available and there was only scarce information on the effect of machine liver perfusion. Second, despite randomization, we noted imbalances between groups in terms of liver weight, cold storage time, sex distribution, underlying disease of recipient, and donor cause of death.

Third, the analysis is based on a modified intention to treat population, given only 170/177 recipients were actually transplanted. Due to the small number of exclusions (7/177, 3.9%) and similar number of exclusions in both groups, a relationship with the intervention is unlikely and the consequences in terms of selection bias should be minor. Fourth, composite endpoints, such as the CCI, need to be adjusted in terms of complications caused by liver graft injury and those caused by the inherently high recipient morbidity in a liver transplant population. This should be carefully considered in future trial designs on LT. Lastly, given the high number of secondary endpoints and times of analysis, as well as the *post hoc* analyses, it is likely that some false findings could have occurred, given that no adjustment for multiplicity was performed.

One strength of our trial is the low and almost equal discard rate in both study arms. This is caused by late randomization, *e.g.* after arrival of procured livers in the transplant centers, in contrast to published normothermic perfusion trials, reporting high discard rates in the control group.[14,15] Secondly, our trial also shows a difference in graft survival by machine liver perfusion technique, when looking at liver-related graft failure. This is important, as the study design was not selective, with no exclusion of sick recipients, marginal liver grafts, or retransplants, documented by a cumulative 12-month CCI clearly above the benchmark value in both groups.[12] Third, the trial illustrates that frequently used endpoints in previous studies, including CCI, serious adverse event counts, length of hospital stay, or liver function parameters, are insufficient for the assessment of liver-related morbidity after LT. The trial is furthermore representative of the real world, owing to the participation of 10 well-established European liver transplant centers from six countries, with a homogeneous case distribution per country and region. Finally, the applied machine liver perfusion technique appears safe with no graft loss due to pump malfunctions.

In summary, we demonstrate that the HOPE approach has no effect on the number of patients with one or more post-transplant Clavien ≥III complication. We believe however that morbidity after LT can only be captured by quantifying and specifying complications per patient. HOPE may be beneficial in this respect, by reducing the number of severe liver-related complications per patient. As it is a simple and quick perfusion technique, it can be applied easily after organ transport during recipient hepatectomy. This appears fundamental since concurring perfusion technologies need either perfusion at donor sites or continuous perfusion during organ transport, which are much costlier and more laborious. We conclude therefore that the *post hoc* findings of this trial should be further validated in future studies.

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Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; CCI, comprehensive complication index; DBD, donated after brain death; DCD, donation after circulatory death; GLM, generalized linear model; GLMM, generalized linear mixed-effects model; HOPE, hypothermic oxygenated perfusion; ICU, intensive care unit; LMM, linear mixed-effects model; LT, liver transplantation; OR, odds ratio; RCT, randomized-controlled trial.

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Conflict of interest

This is an investigator-initiated trial (IICT) with no financial involvement of any perfusion or industrial companies.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

AS: Conceptualization, Methodology, Investigation, Resources, Writing original draft, Writing review & editing, Supervision; MM: Validation, writing review & editing, Supervision; XM: Resources, Investigation, Supervision; JE: Validation, Writing review & editing, Supervision; RP: Validation, Writing review & editing; SvF: Software, Formal analysis; KS: Software, Formal analysis; RXSDS: Validation, Writing review & editing; OdR: Validation, Writing review & editing; JYM, ML, MCC, NDH, MAA, RA, DM, IJ, MPDH, RJP, AP, PM, PK, MA, DK, GB, XR: Resources, Investigation; KP: Validation, Project administration, Data Curation, Supervision; ALK, SA: Validation, Project administration, Data Curation, Supervision; BM: Resources, Conceptualization, writing & editing; PAC: Conceptualization, Methodology, Investigation, Resources, Writing review & editing; PD: Conceptualization, Methodology, Investigation, Resources, Writing Original draft, Writing review & editing, Visualization, Supervision, Funding Acquisition.

Data availability statement

The data used to support the findings of this study are included and available within the article.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.12.030.

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Author names in bold designate shared co-first authorship

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