

Dual hypothermic machine perfusion mitigates early allograft injury of liver grafts regardless of prolonged total preservation times – a retrospective study with propensity score matching analysis

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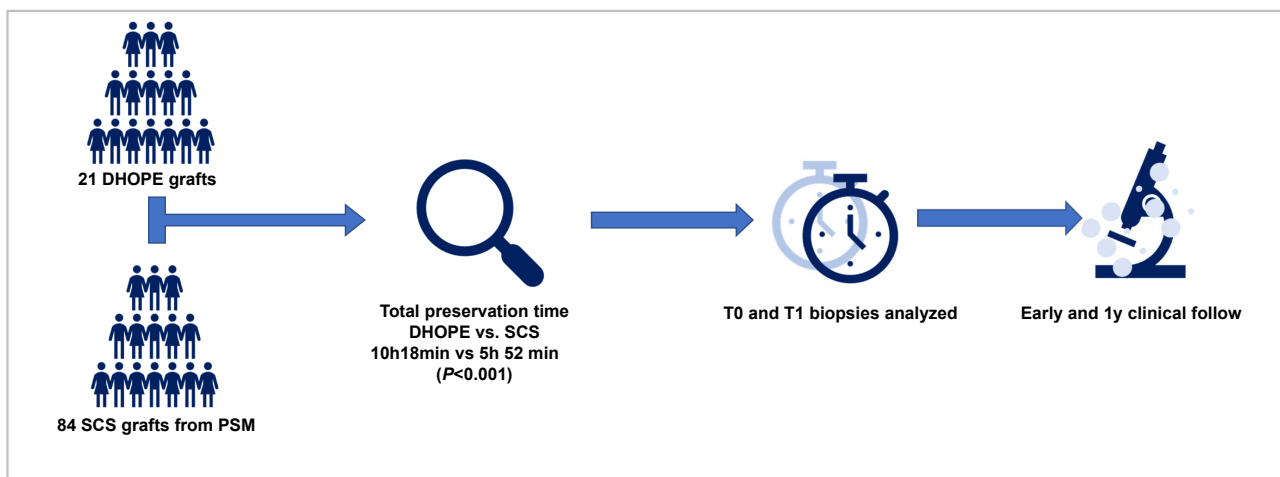
Aims. Transplant centers worldwide face the need to extend the donor acceptance criteria. To preserve the safety of transplantation, we have implemented a dual hypothermic oxygenated machine perfusion (DHOPE) program. This, in some cases, enabled a prolonged total preservation time (TPT), raising worries about the impact on graft outcomes. This study aimed to compare outcomes between DHOPE-treated grafts and conventionally stored grafts.

Methods. We analyzed outcomes of 21 DHOPE-preserved grafts and performed a propensity score-matched comparison with static cold storage (SCS) grafts. In addition, biopsies from 20 liver grafts were examined for histological evidence of preservation-related injury. All patients were followed for 1 year to assess graft and patient survival.

Results. The median TPT was significantly longer in the DHOPE group (10 hours 18 minutes) compared with the SCS group (5 hours 52 minutes; $P < 0.001$). Despite this difference, no significant variation was observed between groups in terms of patient and graft survival, primary nonfunction, early allograft dysfunction, or major complications. Histological evaluation also showed no statistically significant differences in ischemia-reperfusion injury.

Conclusion. Our findings demonstrate that extending TPT during DHOPE is feasible and does not compromise safety. Both early postoperative outcomes and 1-year graft survival show no significant difference from conventional preservation.

Dual Hypothermic Machine Perfusion Mitigates Early Allograft Injury Of Liver Grafts Regardless Of Prolonged Total Preservation Times – A Study With Propensity Score Matching Analysis



Significantly lower ALT and bilirubin on day 7 in DHOPE group while no difference in survival or ischemia-reperfusion injury. DHOPE is a practical and safe method for liver grafts preservation even in prolonged preservation time scenarios.

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Graphical Abstract

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Key words: liver transplantation, hypothermic machine perfusion, ischemia-reperfusion injury, liver biopsy, prolonged cold ischemia time, DHOPE

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INTRODUCTION

Over the history of liver transplantation, a significant shift in donor demographics has occurred¹. Donors are now typically older and often present with more comorbidities, which increases the risk of postoperative complications. To preserve safety while expanding the donor pool through extended-criteria donors (ECDs), several ex vivo machine perfusion methods have been established^{2,3}.

Dual hypothermic oxygenated perfusion (DHOPE) has been shown to reduce ischemia-reperfusion injury (IRI), restore cellular bioenergetics, and lower the rate of biliary complications^{4,5}. In clinical use, DHOPE may also improve operating room logistics. Case reports have described preservation times of up to 20 hours using DHOPE, with satisfactory postoperative results⁶.

Recent evidence suggests that prolonging total preservation time (TPT) in liver grafts is possibly both feasible and safe⁷. Extended preservation may benefit scenarios such as long-haul organ transport, donor-recipient immunologic matching, or possible future genetic manipulation. It may also reduce night-time surgeries, limiting staff fatigue and potentially lowering costs. However, prolonged cold ischemia time (CIT) and TPT remain associated with increased risk of early allograft dysfunction (EAD) and other adverse outcomes⁸.

Based on published evidence, we hypothesized that extended DHOPE is clinically feasible. We applied it in situations involving expected prolonged preservation times or complex operating room (OR) logistics. The aim of the study was to find any difference in early and 1-year outcomes between the SCS and DHOPE groups with prolonged total preservation time.

MATERIALS AND METHODS

Study design and inclusion criteria

We present a single-institution, retrospective cohort study supplemented with a propensity score matching (PSM)-based analysis. All patients were considered eligible for enrolment if they were ≥ 18 years old and undergoing their first liver-only transplantation. Patients with fulminant liver failure were excluded. All included transplantations were performed at the Center of Cardiovascular Surgery and Transplantation, Brno, between April 2021 and December 2023.

Patients were allocated to either the DHOPE arm (group A) or the static cold storage (SCS) arm (group B). Allocation was based on the surgeon's clinical judgment and expected OR logistics, such as a complicated hepatectomy or a currently unavailable OR due to ongoing surgery. DHOPE was the preferred method for ECD or donation after circulatory death (DCD) grafts. To minimize selection bias, a pseudo-randomized PSM control group (group C) was created from patients trans-

planted in our institution who were not included in the study.

All participants provided written consent previously approved by the institutional ethics committee for study participation and biological samples collection. All consecutive patients during the enrolment period were provided a choice to participate in the study. Only patients providing consent were included in groups A or B; those who did not consent to biopsy harvest remained eligible for the PSM pool. Donors meeting at least one of the following criteria were classified as ECD: age > 65 years, ICU stay > 7 days, BMI > 30 , or DCD status⁹.

Group A and B patients

Patients were enrolled into either the DHOPE group (group A) or the conventional SCS group (group B) to assess histological changes during preservation. In 33 cases, sequential biopsies were obtained: one after procurement and another after reperfusion. These were used to evaluate preexisting donor pathology and subsequent IRI. A complete laboratory workup was performed for 7 days postoperatively.

Of 33 biopsy pairs, 25 were diagnostic: 16 from DHOPE and 9 from SCS. The remainder were excluded due to poor tissue quality. Biopsies were blindly evaluated by an experienced pathologist for signs of IRI. Follow-up data at 30 days and 1 year were collected, including imaging and cholangiography when clinically indicated, as in cases of suspicion for anastomotic or non-anastomotic biliary stricture (NAS).

Group C patients

After preliminary analysis revealed significant differences in TPT, we have created a retrospective control group. Propensity scores were calculated for all patients undergoing elective first liver-only transplantation with SCS grafts at our center from 1/2010 to 12/2023. Matching was performed at a 1:4 ratio against the DHOPE cohort, ensuring balance across donor variables. Outcomes were then compared as specified in the study endpoints.

Study endpoints

Graft survival in 30 days and incidence of EAD in groups A and C were set as the primary endpoints¹⁰. Secondary endpoints included histologic comparison of IRI between groups A and B, patient survival at 30 days, graft and patient survival at 1 year, Clavien-Dindo grade ≥ 3 complications within 30 days, postoperative day 7 ALT, AST, and bilirubin levels, and symptomatic non-anastomotic biliary stricture incidence within 1 year in groups A and C.

Procurement and preservation

TPT was defined as the time span from cessation of spontaneous organ perfusion in the donor until the graft

reperfusion in the recipient. Donors were evaluated as suitable for allocation using standard institutional criteria. All DCD donors were Maastricht type III. Following circulatory arrest, organs were flushed with Custodiol solution, the abdominal cavity was packed with ice and retrieved conventionally. Grafts arrived either from local or outlying hospitals, which influenced SCS duration prior to commencement of DHOPE.

The LiverAssist device (XVIVO, Groningen) was primed with 3L of PumpProtect solution (Carnamedica, Warsaw) and precooled. Upon arrival to our institution, livers expecting the DHOPE were cannulated (portal vein and hepatic artery) and perfused using the LiverAssist device. Perfusion protocol: portal pressure 2–5 mmHg, arterial pressure 25 mmHg, pO₂ >100 kPa, temperature 10–12 °C. Perfusion was terminated shortly before hepatectomy completion at the surgeon's request.

Biopsy acquirement and analysis

Immediately after procurement a core-cut biopsy from liver segments V or VIII was taken. Another pair of biopsies from the same liver segment was acquired after graft reperfusion. All biopsies have been immediately formalin-fixed, subsequently paraffin-embedded, stained with hematoxylin and eosin (H&E), histochemical stains used in routine liver diagnostic practice (Gömöri silver reticulin stain, Sirius red elastic stain, Iron Prussian blue reaction, Fouchet-van Gieson stain for bilirubin and collagen, Periodic acid-Schiff histochemical stain, Schmorl's stain for lipopigments and metalloproteins, Acid fuchsin Orange G stain for fibrin, red blood cells, muscle) and a study-designed set of immunostaining for cytokeratin 7 (CK7), cytokeratin 8/18 (CK8/18), cluster of differentiation antigens CD3, CD31, CD34, CD45 has been performed according to standard procedures. Biopsies were reviewed and evaluated by an experienced pathologist for preexisting donor liver pathological changes, which commonly may overlap successive IRI, namely hepatocellular steatosis and/or vacuolisation/ballooning of steatohepatitis, hepatocellular regressive changes of active hepatitis, cholestasis in cholestatic lesions, sinusoidal congestion in cardiac diseases, which form a significant part of histopathological features in IRI and IRI grading as well. Based on histopathologic and immunohistologic examination, semiquantitative grading for IRI severity according to published criteria with Suzuki scores on scale of 0–12 and IRI scores on scale of 0–24 were stated for both zero and post-reperfusion biopsies to exclude the preexisting donor liver changes^{11–13}. Suzuki classification grading scale incorporates the presence and the degree of congestion, vacuolization and necrosis (scale 0–4); IRI grading system includes neutrophilic sinusoidal infiltrates, necrosis, congestion, ballooning, large droplet macrovesicular steatosis and cholestasis (scale 0–4).

Statistical analysis and data processing

Analysis was performed using Statistica 14 (Tibco, Palo Alto) and R 4.3.1 (R Foundation, Vienna) software. Propensity score variables included donor age, BMI, cause of death (trauma, anoxia, cerebrovascular, other),

and ICU stay¹⁴. We were unable to include DCD status in the propensity score calculation due to the historical scarcity of these donors in our institution.

Binary variables were reported as absolute and percentage frequencies. Continuous variables were presented as median with ranges of absolute minimum and maximum values. Normality was tested using the Kolmogorov-Smirnov test. All variables except the AST on day 7 were normally distributed. Differences between groups were tested by a parametric Welch Two Sample t-test or Mann-Whitney test as appropriate. For binary variables, the groups were analyzed by applying the Chi-square test or the Fisher exact test. Statistical significance was defined as *P*-value <0.05 and confidence intervals (CI) were at 95%. Kaplan-Meier analysis was used to determine 30-day and 1-year overall and graft survival. There were no missing data in the dataset. Participants lost to follow-up were treated as censored observations in the Kaplan-Meier survival analysis. Their data were included up to the time of last contact. Reverse Kaplan-Meier analysis was used to calculate the median follow-up time for the primary endpoint.

Propensity score matching

Propensity scores were calculated for 568 eligible donors. Genetic matching at a 1:4 ratio yielded 84 controls, balanced across variables¹⁵. Group balance was confirmed using variance ratio, pseudo R² reduction, standard mean differences, and raw binary differences¹⁶.

RESULTS

Donor and recipient characteristics

Patients were divided into three groups: DHOPE grafts with biopsies (A), SCS grafts with biopsies (B), and PSM historical controls (C). (Table 1)

Age

Median donor age was 51 years (26–73) in group A, 51 (37–63) in group B, and 52 (10–76) in group C. (A:B *P*=0.623; A:C *P*=0.936)

BMI

Median donor BMI was 25.77 (19–48) in group A; 25.92 (21.15–30.73) in group B, and 25.03 (18–50) in group C. (A:B *P*=0.927; A:C *P*=0.755)

ECD status

Based on the previously stated characteristics, 14 donors were considered ECD (66.66%) in group A, 4 (33.33%) in group B, and 36 (42.85%) in group C. (A:B *P*=0.302; A:C *P*=0.087)

MELD score

Median of calculated MELD was 16 (9–29) in group A, 19.5 (7–27) in group B, and 15.5 (7–27) in group C. (A:B *P*=0.750; A:C *P*=0.662)

Table 1. Prospectively and retrospectively enrolled graft and recipient characteristics.

	Group A DHOPE (n=21)	Group B SCS (n=12)	<i>P</i> A vs. B	Group C SCS PSM (n=84)	<i>P</i> A vs. C
ECD	14 (66.66%)	5 (41.66%)	0.302	36 (42.85%)	0.087
Recipient MELD	16 (9–29)	19.5 (7–27)	0.750	15.5 (7–32)	0.662
Recipient age	55 (32–68)	47 (44–65)	0.623	57 (20–69)	0.936
BMI	25.77 (19–48)	25.92 (21.15–30.73)	0.927	25.03 (18–50)	0.755
Diagnoses					
ALD	13 (61.90%)	6 (50.00%)	-	39 (46.42%)	-
AIH	2 (9.52%)	1 (8.33%)	-	1 (1.19%)	-
PBC	2 (9.52%)	0	-	9 (10.71%)	-
PSC	2 (9.52%)	2 (16.66%)	-	8 (9.52%)	-
HCC	0	1 (8.33%)	-	0	-
HBV	1 (4.76%)	0	-	1 (1.19%)	-
Budd-Chiari syndrome	0	1 (8.33%)	-	0	-
Cryptogenic	1 (4.76%)	1 (8.33%)	-	17 (20.23%)	-
Other	0	0	-	9 (10.71%)	-

SCS, static cold storage; ECD, extended criteria donor; MELD, model of end-stage liver disease; ALD, alcoholic liver disease; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; HCC, hepatic carcinoma; HBV, hepatitis B virus infection. All values are presented as median, with min – max in brackets (min – max) or absolute value with relative value reported as percentage (relative value).

Primary diagnosis

Alcoholic liver disease was the leading indication for transplantation: 61.9% in group A, 50% in group B, and 50% in group C.

Preservation time

Median of pre-DHOPE SCS was 2h45m (0:30–6:40) and median TPT including DHOPE was 10h18m (4:28–13:50) with perfusion duration 6h25m (3:20–10:00). Median TPT for group B was 4h37m (3:14–7:58). Median TPT for group C was 6h25m (2:30–13:54). TPT differed significantly between A and both group B and C ($P<0.001$). (Table 2)

Operative course and postoperative results

All liver transplantations in all groups were performed in standard fashion, performing caval anastomosis with piggy-back technique, and end-to-end hepatic, portal, and biliary anastomosis¹⁷. In patients unsuitable for common biliary duct end-to-end anastomosis, a Roux-en-Y reconstruction technique was performed.

Severe postoperative complications

In group A, we have documented 6 (28.57%) major complications ($CD\geq 3$), two of them requiring re-transplantation (PNF leading to MOF; severe mixed-type rejection). Other complications included stress cardiomyopathy, wound infection, surgical revision for bleeding, and biliary stenosis with the need for stenting.

In group B, 7 (58.33%) patients experienced complications of $CD\geq 3$, none of them leading to patient death or early graft loss (<30 days since transplantation). There were two cases of surgical revision due to biliary leakage. In one case, there was documented hepatic and biliary stenosis with reoperation. Additional cases included spleen rupture warranting a splenectomy, pleural effusion with

drainage, hepatic anastomosis stenosis with stent implantation, and two cases of early rejection.

In group C, there were 23 (27.38%) major complications, with two leading to early mortality. The most common were reoperations for bleeding (8 cases, 9.5%). Additional cases included reintubation, stroke, abdominal compartment syndrome, renal failure with renal replacement therapy, pleural effusion with drainage, hepatic vein thrombosis, hematemesis, wound infection, bile leak, ileus, and vegetative state.

Primary outcomes and follow-up

For group A, patient survival was 100%, and graft survival was 95.2% in 30 days. The patient and graft survival after 1 year were 95.2% and 90.5%, respectively. For group C, the patient and graft survival were both 97.6% in 30 days, and both were 93.9% in 1 year (Fig. 1). Median follow-up time was 4.42 years (1.78–8.20).

Fourteen cases of EAD were reported in group C (16.66%), while no EAD occurred in group A. A trend toward higher EAD incidence in group C ($P=0.067$) was documented.

There was no documented case of symptomatic NAS in a 1-year follow-up in the DHOPE group.

Secondary endpoints

Biopsy analysis

We have analyzed 16 valid biopsy pairs in group A and 9 valid biopsy pairs in group B. Median of baseline Suzuki score was 2 points out of 12 in group A and 1 point out of 12 in group B. The IRI score baseline was 3 points out of 24 of IRI score for both groups. Post-reperfusion Suzuki score median was 4/12 for group A and 3/12 for group B. Post-reperfusion IRI score median was 7/24 for both groups. (Table 2, Fig. 2)

Table 2. Biopsy analysis.

n=29	DHOPE (n=20)	SCS (n=13)	<i>P</i>
No. of valid	16 (80%)	9 (69.23%)	-
Suzuki score T ₀ (0–12 points)	2 (0–5)	1 (0–2)	0.204
Suzuki score T ₁ (0–12 points)	4 (0–7)	3 (1–4)	0.164
Suzuki score Δ	2 (0–6)	2 (1–3)	0.952
IRI score T ₀ (0–24 points)	3 (0–8)	3 (1–5)	0.652
IRI score T ₁ (0–24 points)	7 (2–12)	7 (6–9)	0.756
IRI score Δ	3.5 (0–8)	4 (3–6)	0.214

T0, biopsy taken immediately after procurement; T1, biopsy taken after complete graft reperfusion.
All values are presented as median, with min – max in brackets (min – max).

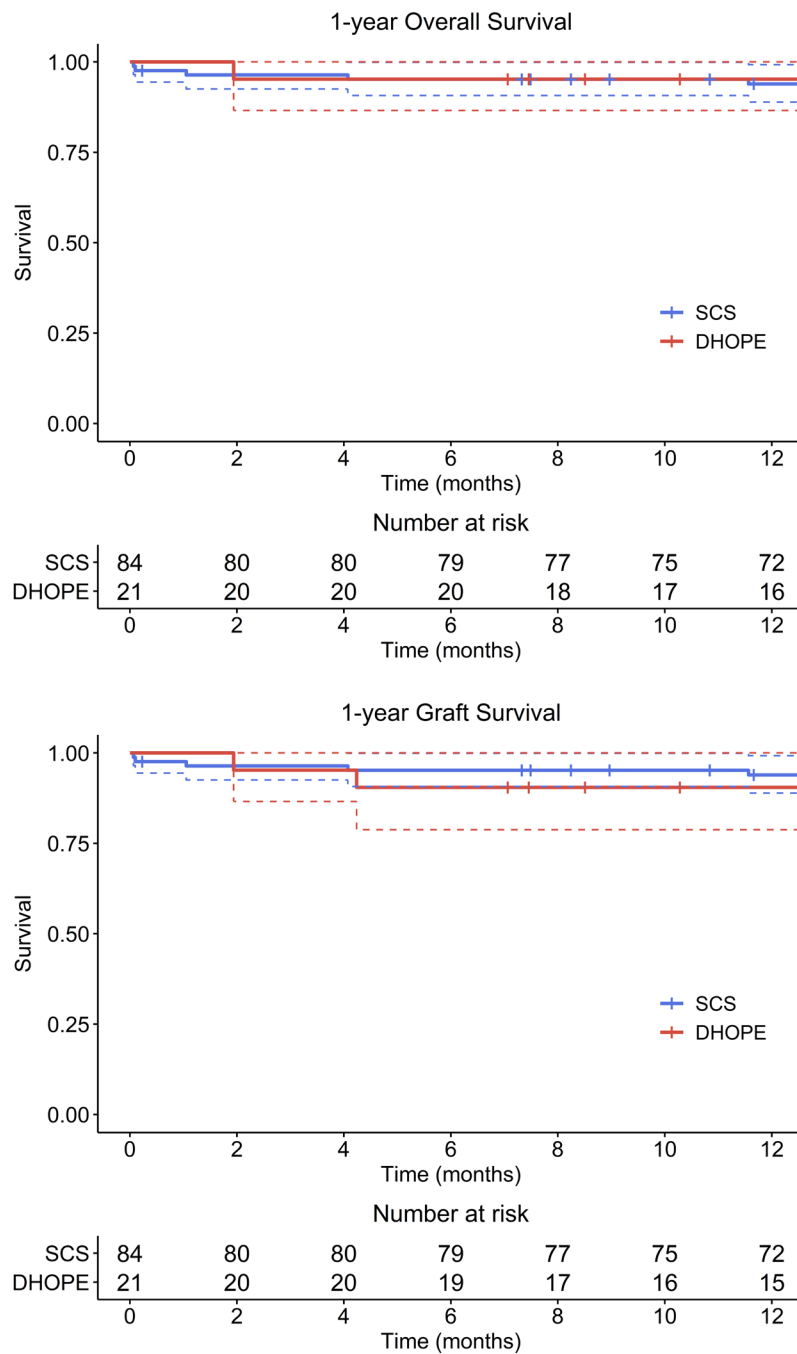


Fig. 1. Kaplan-Meier plots of 1-year graft and overall survival.
SCS, static cold storage; DHOPE, dual hypothermic oxygenated perfusion.

Table 3. Procedural and postoperative values – propensity matched control.

(N=105)	DHOPE (n=21)	SCS (n=84)	P
SCS (hh:mm)	02:45 (00:30–06:40)	05:52 (02:30–13:54)	<0.001
Perfusion time (hh:mm)	06:25 (03:20–10:00)	-	-
TPT (hh:mm)	10:18 (04:28–13:50)	05:52 (2:30–13:54)	<0.001
RBC (units)	3 (0–9)	3 (0–10)	0.747
FFP (units)	4 (2–6)	4.5 (2–11)	0.014
TC (units)	2 (0–3)	2 (0–4)	0.765
AST day 0 (μkat/L)	9.39 (2.04–25.34)	10.82 (0.91–233.5)	0.217
AST day 7 (μkat/L)	0.45 (0.15–2.12)	0.68 (0.13–128.25)	0.072
ALT day 0 (μkat/L)	6.27 (2.17–16.55)	7.08 (0.95–124.51)	0.217
ALT day 7 (μkat/L)	1.3 (0.44–5.85)	2.06 (0.23–50.83)	0.048
Bilirubin day 0 (μmol/L)	45.6 (12.7–296.6)	53.55 (11.1–240.5)	0.965
Bilirubin day 7 (μmol/L)	12.7 (1.61–77.8)	21.45 (5.8–157.1)	0.048
EAD	0	14 (16.66%)	0.067
CD gr≥3	6 (28.57%)	23 (27.38%)	> 0.999
30-day mortality	0	2 (2.38%)	-
30-day retransplantation	1 (4.76%)	0	-
1-year mortality	1 (4.76%)	5 (6%)	-
1-year retransplantation	1 (4.76%)	0	-
Length of stay (days)	19 (11–60)	19 (7–101)	-

hh:mm, hours : minutes; SCS, static cold storage; TPT, total preservation time; RBC, packed red blood cells; FFP, fresh frozen plasma; TC, thrombocyte concentrates; EAD, early allograft dysfunction; CD gr≥3, Clavien-Dindo grade 3 or higher.

All values are presented as median, with min – max in brackets (min – max) or absolute value with relative value reported as percentage (relative value).

PSM analysis

At 30 days, mortality and re-transplantation did not differ between group A and group C. No significant differences in Clavien–Dindo grade ≥3 complications were observed. No group differences in blood loss, RBC, or platelet transfusion were reported; however, group C received significantly more FFPs. Day 7 laboratory results showed significantly lower ALT and bilirubin in group A ($P=0.048$). (Table 3)

DISCUSSION

Liver transplantation faces increasing logistical and technical challenges due to limited donor quality and constantly rising demand. With rising numbers of waiting patients, redo transplantations and decreasing quality of available donor organs, we need to utilize novel methods of organ reconditioning and viability testing. Novel preservation methods are equally essential to safely extend storage times. DHOPE, in contrast to NMP, is a relatively easy method to store liver grafts using only oxygenated acellular perfusate without the need for an oxygen carrier¹⁸. While multiple-day preservation was achieved using NMP, recent reports support prolonged preservation with DHOPE as well^{19,7}.

The primary aim of this study was to compare outcomes of DHOPE and SCS grafts despite prolonged TPT. We selected 30-day graft survival and incidence of EAD for the primary outcome as these metrics are both considered solid measures of early outcome. There was no documented difference in the survival between the groups.

A trend towards lower incidence of EAD was documented in the DHOPE group.

Median TPT in group A was 10h18m, which is 6h25m longer than controls ($P<0.001$). Despite prolonged TPT—typically a risk factor for EAD and biliary complications—outcomes did not differ. Shorter pre-DHOPE SCS in group A (2h45m vs. 4h37m in group B vs. 6h25m in group C) may have contributed to the results by restoring oxygen supply to graft tissue earlier.

Biopsy analysis using semi-quantitative Suzuki and IRI scales revealed no significant differences in IRI between groups. However, single-point biopsies carry an inherent risk of sampling bias, underscoring the need for standardized biomarkers. Promising markers such as perfusate FMN could provide better graft viability assessment^{20,22}.

We have provided PSM analysis to provide an assessment of results with balanced entry-point variables, which confirmed comparable outcomes. DHOPE grafts results were comparable in survival, complications, or transfusion use, and showed favorable trends in liver function (lower ALT, bilirubin) and EAD incidence.

Brüggenwirth has published a multicenter observational study of prolonged hypothermic oxygenated perfusion of 93 patients, with a low incidence of PNF (1%) and a relatively higher number of EAD (36%), but with a high percentage of DCD donors (46%). Also, the median pre-perfusion SCS duration in this group was higher than in our group (5h32m vs. 2h45m in our group), which could also influence the EAD incidence and overall outcome. These short times of pre-DHOPE SCS (0h30m–6h40m) were achieved in donors being hospitalized in the adjacent hospital.

Globally, in the field of solid organ transplantation, there is increasing interest in safe preservation extension across organ types, including lungs and hearts^{23,24}. This allows transplant teams to prepare better for the procedure, optimize the recipient's physical state, and perform complicated hepatectomies or combined organ transplantation. These novel methods enable reducing the night-time procedural volume and shift organ transplantation from urgent to semi-elective surgery. Notably, in the DHOPE group, only 2 procedures (9.52%) started between 9 PM and 6 AM, while 37 (44.04%) started during this time in group C.

A key concern with prolonged storage is NAS, with a variable onset of symptom presentation. Buis et al. published a group with a median onset of symptoms of 4.1 months, and over 50% of symptomatic NAS presented in the first-year post transplantation²⁵. In the paper of Eden et al., specifically reporting 5-year outcomes of HOPE-treated grafts, there was a cumulative incidence of NAS after 1 year of 2.5% for DBD and 11.5% for DCD grafts²⁶. Encouragingly, we observed no symptomatic NAS in DHOPE patients during follow-up.

Machine perfusion not only enables safer utilization of marginal donors but also offers a platform for diagnostic and therapeutic interventions, including online metabolic monitoring and potential future tissue modification²⁷.

STUDY LIMITATIONS

Since the extended times of DHOPE were due to logistical reasons, the protocol-based randomization with extended SCS cases was not considered feasible for this study. We seek to overcome this issue with a retrospective PSM-based control group in a 1:4 ratio, which was balanced in all variables compared with our prospective cohort.

Biopsy-based IRI assessment may suffer from sampling bias. Future approaches, such as liquid biopsy biomarkers from perfusate, may provide a more comprehensive evaluation.

CONCLUSION

DHOPE is a practical and safe method for liver graft preservation. Our data demonstrate the feasibility of prolonging preservation times without compromising early or 1-year outcomes. In settings requiring longer preservation or complex logistics, DHOPE provides a reliable solution, supporting safer scheduling and reducing the burden of nighttime transplantation.

ABBREVIATIONS

ALT, Alanine-aminotransferase; AST, Aspartate-aminotransferase; BMI, Body mass index; CIT, Cold ischemia time; DCD, Donation after circulatory death;

DHOPE, Dual hypothermic oxygenated machine perfusion; EAD, Early allograft dysfunction; ECD, Extended criteria donor; IRI, Ischemia-reperfusion injury; NAS, Non-anastomotic biliary stenosis; PSM, Propensity score matching; SCS, Static cold storage; TPT, Total preservation time.

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