Research Article
Transplantation

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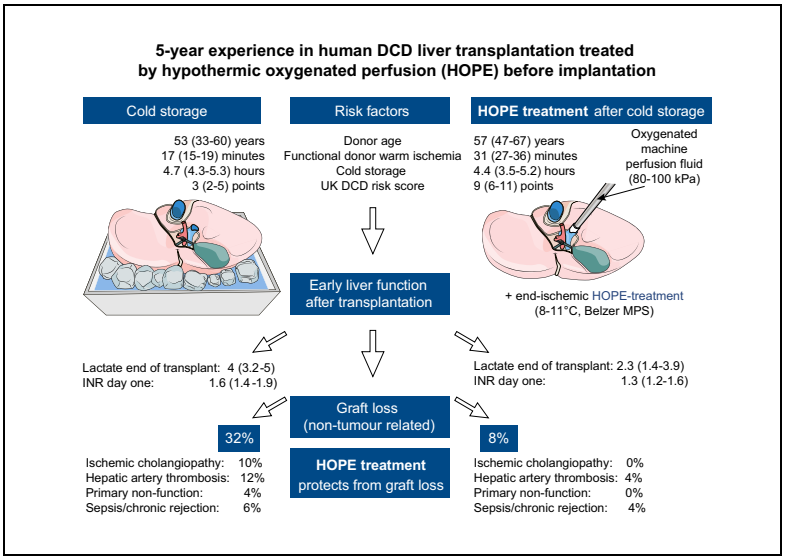
Outcomes of DCD liver transplantation using organs treated by hypothermic oxygenated perfusion before implantation

Graphical abstract



Highlights

- End-ischemic HOPE protected against arterial and biliary complications, resulting in significantly less graft loss.
- Equivalent outcomes were achieved with HOPE as with primary DBD liver transplants.
- HOPE after cold storage is a simple and effective method to treat high-risk DCD livers prior to implantation.

Authors

Andrea Schlegel, Xavier Muller, Marit Kalisvaart, ..., Pierre-Alain Clavien, Paolo Muiesan, Philipp Dutkowski

Correspondence

clavien@access.uzh.ch
(Pierre-Alain Clavien)

Lay summary

Machine perfusion techniques are currently being introduced into the clinic, with the aim of optimising injured grafts prior to implantation. While short-term effects of machine liver perfusion have been frequently reported in terms of hepatocellular enzyme release and early graft function, the long-term benefit on irreversible graft loss has been unclear. Herein, we report on 5-year graft survival in donation after cardiac death livers, treated either by conventional cold storage, or by 1–2 h of hypothermic oxygenated perfusion (HOPE) after cold storage. Graft loss was significantly less in HOPE-treated livers, despite longer donor warm ischaemia times. Therefore, HOPE after cold storage appears to be a simple and effective method to treat high-risk livers before implantation.

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Outcomes of DCD liver transplantation using organs treated by hypothermic oxygenated perfusion before implantation

Andrea Schlegel[1,2,†], Xavier Muller[2,†], Marit Kalisvaart[1], Beat Muellhaupt[3], M. Thamara P.R. Perera[1], John R. Isaac[1], Pierre-Alain Clavien[2,‡,*], Paolo Muiesan[1,‡], Philipp Dutkowski[2,‡]

[1]*NIHR Birmingham Biomedical Research Centre, University Hospitals Birmingham NHS Foundation Trust and University of Birmingham, United Kingdom;* [2]*Department of Surgery and Transplantation, Swiss HPB Centre, University Hospital Zurich, Zurich, Switzerland;* [3]*Department of Gastroenterology and Hepatology, University Hospital Zurich, Zurich, Switzerland*

Background & Aims: Donation after circulatory death (DCD) liver transplantation is known for potentially worse outcomes because of higher rates of graft non-function or irreversible cholangiopathy. The impact of machine liver perfusion techniques on these complications remains elusive. We aimed to provide data on 5-year outcomes in patients receiving DCD liver transplants, after donor organs had been treated by hypothermic oxygenated perfusion (HOPE).

Methods: Fifty HOPE-treated DCD liver transplants performed in Zurich between 2012 and 3/2017 were matched with 50 primary donation after brain death (DBD) liver transplants, and with 50 untreated DCD liver transplants in Birmingham. Match factors focussed on short cold ischaemia, comparable recipient age and low recipient laboratory model for end-stage liver disease scores. Primary endpoints were post-transplant complications, and non-tumour-related patient death or graft loss.

Results: Despite extended donor warm ischaemia, HOPE-treated DCD liver transplants achieved similar overall graft survival, compared to standard DBD liver transplants. Particularly, graft loss due to any non-tumour-related causes occurred in 8% (4/50) of cases. In contrast, untreated DCD livers resulted in non-tumour-related graft failure in one-third (16/50) of cases ($p = 0.005$), despite significantly ($p < 0.001$) shorter functional donor warm ischaemia. Five-year graft survival, censored for tumour death, was 94% for HOPE-treated DCD liver transplants *vs.* 78% in untreated DCD liver transplants ($p = 0.024$).

Conclusions: The 5-year outcomes of HOPE-treated DCD liver transplants were similar to those of DBD primary transplants and superior to those of untreated DCD liver transplants, despite much higher risk. These results suggest that a simple end-ischaemic perfusion approach is very effective and may open the field for safe utilisation of extended DCD liver grafts.

Lay summary: Machine perfusion techniques are currently being introduced into the clinic, with the aim of optimising injured grafts prior to implantation. While short-term effects of machine liver perfusion have been frequently reported in terms of hepatocellular enzyme release and early graft function, the long-term benefit on irreversible graft loss has been unclear. Herein, we report on 5-year graft survival in donation after cardiac death livers, treated either by conventional cold storage, or by 1–2 h of hypothermic oxygenated perfusion (HOPE) after cold storage. Graft loss was significantly less in HOPE-treated livers, despite longer donor warm ischaemia times. Therefore, HOPE after cold storage appears to be a simple and effective method to treat high-risk livers before implantation.

Introduction

Donation after circulatory death (DCD) organs are increasingly used for liver transplantation, due to the persisting organ shortage and waiting list mortality.[1] However, several reports suggest inferior graft survival, increased risk of primary non-function (PNF), and biliary complications in DCD livers, with irreversible ischaemic cholangiopathy (IC) being a major concern.[2] Severe forms, requiring retransplantation, typically develop within the first 3–6 months after liver transplant.[3] While the majority of transplant physicians agree that prolonged periods of donor warm ischaemia contribute significantly to this aggressive biliary complication, others argue that other factors including donor and recipient age, cold ischaemia, donor body mass index (BMI) and hepatic steatosis, or technical issues are equally important.[4,5] Therefore, various dynamic preservation techniques designed to optimise liver grafts before implantation are currently under evaluation.[6–9] In 2012, a novel machine perfusion concept was introduced in Zurich for DCD liver transplantation, hypothermic oxygenated perfusion (HOPE), applied only for 1–2 h after conventional procurement and cold storage.[10,11] While the initial clinical experience with this new technique has already been presented, including the first 25 human DCD livers,[9] our study aimed to document a longer follow-up of 5 years after HOPE treatment in human DCD livers. Secondly, we intended to unravel the efficacy of the HOPE perfusion approach. Therefore, we compared HOPE-perfused DCD livers with the best available not machine perfused alternative *e.g.* untreated DCD livers from a highly experienced transplant unit, in cases where DCD livers were exposed to short cold ischaemia and recipients had low model

Keywords: Donation after cardiac death; Hypothermic oxygenated perfusion; Ischaemic cholangiopathy.
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* Corresponding author. Address: Department of Surgery, Swiss HPB and Transplantation Centre, University Hospital Zurich, Rämistrasse 100, CH-8091 Zurich, Switzerland; Tel.: +41 44 255 3300; fax: +41 44 255 4999.
E-mail address: clavien@access.uzh.ch (P.-A. Clavien).
† Contributed equally as first authors.
‡ Contributed equally as senior authors.

for end-stage liver disease (MELD) scores. Thirdly, this analysis focussed on cumulative post-transplant complications within the first year of post-transplant follow-up, quantified by the comprehensive complications index.[12] Finally, we compared non-tumour-related death or graft loss in HOPE-treated or untreated DCD livers with outcomes in primary donation after brain death (DBD) liver transplants.

Patients and methods

Patient cohort and data collection

This study refers to the first 50 HOPE-treated human livers transplanted from DCD donors at the University Hospital in Zurich, Switzerland between 2012 and 3/2017 with at least 1 year of follow-up. We matched this machine perfused DCD cohort with an untreated DCD cohort (n = 50) from the liver unit at Queen Elizabeth Hospital Birmingham, UK. Primary, adult DBD liver transplants from both centres were also matched and served as a baseline control group (n = 50). Paediatric transplantations, combined transplants, domino, split grafts and living donor liver transplantations were excluded. Livers procured following initial assessment through normothermic regional perfusion in the donor and other machine perfused livers were also excluded.

Centre practice in liver procurement, preservation, transplantation surgery and postoperative management

In Zurich, all DCD liver retrievals between 2012 and 3/2017 were performed by the super rapid procurement technique. Following cannulation of the iliac artery, 2 litres of heparinised saline (20 °C) were used as flush solution, followed by 3–5 litres of precooled (4 °C) Institute-George-Lopez-1-solution. All livers were retrieved within 30 min and received additional cold flush on the bench through the portal vein and the hepatic artery. The biliary system was also flushed prior to packing. After the cold storage period, DCD livers for Zurich underwent HOPE for 1–2 h during recipient hepatectomy. Before implantation, HOPE-treated DCD livers received also a blood flush (200–250 ml) prior to reperfusion in the recipient. The standard implantation technique in Zurich was the cava replacement technique without use of veno-venous bypass. Reperfusion was always initiated by the portal vein, followed by the hepatic artery.

In Birmingham, DCD livers were also procured by the super rapid cannulation technique. However additional *in situ* perfusion of the portal or mesenteric vein was also performed. The flush solution in the UK was cold University of Wisconsin-solution (4 °C). Bench perfusion included the hepatic artery, portal vein and biliary system prior to liver packing. Before reperfusion in the recipient, livers were flushed with 2 litres of cooled saline (or ringers) during anastomosis. Some livers received additional blood flush. The standard implantation technique was a piggyback technique without veno-venous bypass. A porto-caval shunt was used in selected cases, and reperfusion was initiated either through the portal vein or the hepatic artery first.

The "stand-off" period after cardiac arrest in the donor was 5 min in Birmingham, compared to 10 min in Zurich, prior to super rapid laparotomy, cannulation and cold flush. In both groups, the functional donor warm ischaemia time (fDWIT) was defined as duration from systolic blood pressure below 50 mmHg to cold aortic perfusion in the donor. Importantly, none of the donors received heparin before withdrawal in both countries, and DCD livers were not treated with tissue-plasminogen activator.

The immunosuppression protocol was different between both centres: In Zurich DCD liver recipients received prednisolone (500 mg), and induction by basiliximab intraoperatively. Tacrolimus was added at day 3–4 in parallel with ongoing steroids and another dose of basiliximab was administered on day 4. In contrast, in Birmingham, the immunosuppressive regimen consisted of prednisolone (100 mg), tacrolimus and azathioprine or mycophenolate mofetil, all introduced at day 0–1. Basiliximab induction and late introduction of tacrolimus were used in selected cases. The trough level of tacrolimus was adjusted to kidney function in both centres.

Hypothermic oxygenated perfusion

At the end of standard cold storage and transport, DCD livers in Zurich were cleaned on the bench and connected to the Liver Assist device (Organ Assist®) to perform HOPE. For this purpose, a curved catheter was inserted into the portal vein and secured with silk. During recipient hepatectomy, 1–2 h of HOPE perfusion was performed at 10–12 °C with 3 litres of Belzer Machine Perfusion Solution (Belzer MPS®) through the portal vein. The hepatic artery remained untouched. Free outflow of the perfusate was allowed in keeping both ends of the vena cava open. Importantly, perfusion pressure was limited to maximal 3 mmHg and the oxygen concentration in the perfusate was high with a pO_2 of 80–100 kPa in order to recondition liver mitochondria (Fig. 1).[10] At the end of HOPE perfusion, DCD grafts were disconnected and directly implanted.

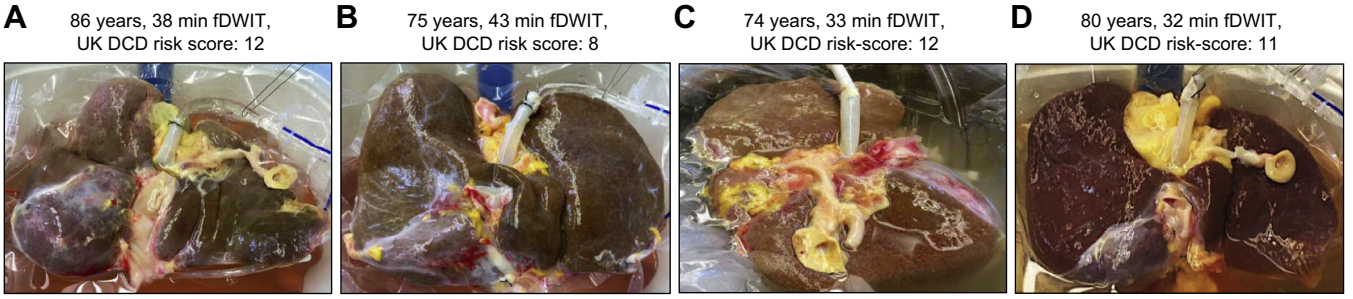

Fig. 1. HOPE treatment of human DCD liver grafts. Four examples of HOPE-treated human DCD livers from the Zurich cohort are shown here. Importantly, the hepatic artery remains untouched throughout cold perfusion. DCD, donation after cardiac death; fDWIT, functional donor warm ischaemia time; HOPE, hypothermic oxygenated perfusion. (This figure appears in colour on the web.)

Risk analysis and matching of the 3 transplant cohorts

In a first step, we analysed several donor, graft and recipient risk parameters (Table 1).

Important risk factors, *e.g.* donor age, donor BMI, donor warm ischaemia, cold storage, recipient age and BMI, MELD score and underlying disease were assessed. Risk stratification was performed according to the recently developed UK DCD risk score.[4] A cohort of 50 patients from Birmingham receiving cold stored DCD livers, which were not machine perfused, were matched to the HOPE-treated DCD cohort from Zurich. In addition, a group of primary DBD liver transplants from both centres was also matched to serve as a baseline control. A computerised case-control matching analysis was done to correct for potential differences in baseline donor and recipient characteristics amongst the groups, separately for the untreated DCD and DBD liver cohort. Based on the overall number of DCD transplantations performed in Birmingham between 2007 and 2017 (total n = 439), a 1:1 case-control matching of each HOPE-treated DCD liver with one untreated DCD liver without replacement was performed. The matching process involved the following parameters with tolerance highlighted in brackets to correct for potential key confounders: cold ischaemia time (±1 h), recipient age (±1 year) and MELD score (±1 point). For each HOPE-treated DCD liver in Zurich, 1 appropriate untreated DCD liver (simply cold stored) from Birmingham and 1 low-risk DBD liver, selected from a combined DBD cohort between 2007 and 2017 (n = 921) from Zurich and Birmingham were matched according to the aforementioned parameters (HOPE-treated DCD: 50 *vs.* untreated DCD: 50 *vs.* low-risk primary DBD: 50). The low-risk DBD matching cohort represents adult, primary liver transplantations into low-risk recipients (laboratory MELD <21 points), in accordance with recent definition of benchmarking in liver transplantation.[13] Any acute liver failures, combined transplants, live donors and split grafts, and auxiliary liver transplants were excluded.

Endpoints

We documented several intraoperative parameters, including transfusions and duration of surgery. We also assessed lactate clearance at the end of liver transplant. Further analysis includes liver function (international normalized ratio [INR] day 1) and injury (peak alanine aminotransferase during the first week after liver transplant). Intensive care unit (ICU) and hospital stay served as surrogate markers for complications. Biliary and vascular complications and rejections are displayed in detail. Overall complications were assessed using the Clavien-Dindo-Classification and the comprehensive complication index.[12,14] Five-year survival rates are shown with a focus on non-tumour-related graft loss, including the rate of PNF, IC and hepatic artery thrombosis (HAT). IC was defined

Table 1. Donor, graft and recipient characteristics.

	DCD + HOPE (n = 50)	DCD untreated (n = 50)	DBD (n = 50)	*p value* DCD + HOPE *vs.* DCD untreated	*p value* DCD + HOPE *vs.* DBD
Donor age (years)	57 (47–67)	53 (33–60)	50 (43–62)	0.05	0.103
No. >60 years	21 (42%)	12 (24%)	13 (26%)	0.088	0.1389
Total donor WIT (min)	36 (31–40)	25.5 (21–31)	–	<0.0001	–
No. >40 min	12 (24%)	3 (6%)		0.0226	
Functional donor WIT (min)	31 (27–36)	17 (15–19)	–	<0.0001	–
No. >30 min	28 (56%)	0		0.0001	
Asystolic donor WIT (min)	19 (17–21)	12.5 (10–15)	–	<0.0001	–
No. >15 min	47 (94%)	6 (12%)		0.0001	
Graft steatosis					
Macrosteatosis >20% (n/%)	4 (8%)	0	0	0.118	0.118
Microsteatosis >20% (n/%)	14 (28%)	2 (4%)	0	0.0019	0.0001
Total cold preservation (hours) (= total out of body time)	6 (5–7)	4.7(4.3–5.3)	5 (4–5)	0.0002	<0.0001
Cold storage (hours)	4.4 (3.5–5.2)	4.7 (4.3–5.3)	5 (4–5)	0.062	0.072
Duration of HOPE (hours)	2 (1.6–2.4)	–	–	–	–
Recipient age (years)	58 (56–62)	57 (51–61)	57 (48–63)	0.072	0.063
Recipient lab MELD (points)	11 (8–14)	11.8 (8.5–15.8)	15 (9–17)	0.504	0.078
Underlying disease/indication (n/%):					
Hepatitis C	16 (32%)	10 (20%)	15 (30%)	0.254	1.0
Hepatitis B	3 (6%)	4 (8%)	2 (4%)	1.0	1.0
Primary sclerosing cholangitis	1 (2%)	7 (14%)	5 (10%)	0.059	0.204
Primary biliary cirrhosis	2 (4%)	9 (18%)	8 (16%)	0.051	0.0916
Alcohol-related liver disease	12 (24%)	12 (24%)	9 (18%)	1.0	0.624
Non-alcoholic steatohepatitis	5 (10%)	3 (6%)	5 (10%)	0.715	1.0
Hepatocellular carcinoma alone	3 (6%)	0	0	0.242	0.242
Retransplantation for IC after LDLT	1 (2%)	0	0	1.0	1.0
Other	7 (14%)	5 (10%)	6 (12%)	0.759	1.0
Hepatocellular carcinoma (n/%)	35 (70%)	10 (20%)	11 (22%)	<0.0001	<0.0001
BAR score (points)	3 (2–4)	3 (2–6)	4 (3–6.75)	0.9028	0.1311
UK DCD risk score (points):	9 (6–11)	3 (2–5)	–	<0.001	–
Low risk (0–5 points) (n/%)	5 (10%)	42 (84%)		<0.0001	
High risk (6–10 points) (n/%)	23 (46%)	8 (16%)		0.0022	
Futile (11–27 points) (n/%)	22 (44%)	0		<0.0001	

Continuous variables are presented as median and IQR; comparisons of continuous variables were made using the Mann-Whitney U test. Categorical variables are expressed in quantities and percentages. To compare categorical variables, the chi-square test or the Fisher's exact test were used. BAR, balance of risk score; DBD, donation after brain death; DCD, donation after circulatory death; IC, ischaemic cholangiopathy; LDLT, Living donor liver transplantation.

radiologically, as intrahepatic or hilar biliary strictures and dilatations, occurring in the absence of hepatic artery stenosis or thrombosis, portal thrombosis, chronic ductopenic rejection, and recurrent primary sclerosing cholangitis (PSC).[15]

Statistical analysis

Data were analysed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, New York, USA) and Prism 7. Median and interquartile range were used to analyse continuous variables and comparisons were made using the Mann-Whitney U test. Categorical variables were expressed in quantities and percentages. To compare categorical variables, the chi-square test or the Fisher's exact test were used. p values <0.05 were considered statistically significant. Long-term survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests. The end of observation period was March 31st 2018.

Ethical approval and quality control

Completeness, plausibility and validity of the data were independently verified (by AS, XM, MK, PM and PD), including objective review of all historical medical charts. The local regulatory board approval was obtained prior to study initiation and database/chart review (CARMS-02246, KEK-No. 2015-0200).

For further details regarding the materials and methods used, please refer to the CTAT table and supplementary information.

Results

Characteristics of the 3 different transplant cohorts

Fifty human DCD livers were transplanted at University Hospital Zurich with previous end-ischaemic HOPE treatment between January 2012 and March 2017. Detailed donor, graft and recipient parameters are highlighted (Table 1). Given the upper donor age limit of 90 years for DCD transplantation in Switzerland, the median donor age in the HOPE cohort was significantly higher ($p = 0.05$) compared to both control groups, untreated DCD and DBD livers in Birmingham, with more than 40% of donors older than 60 years (Table 1, Fig. S1A). All types of donor warm ischaemia were significantly longer in Zurich compared to Birmingham with a median fDWIT of 31 min in the HOPE group *vs.* 17 min in the untreated DCD control group (p <0.0001) (Table 1). In addition, human DCD livers accepted for transplantation in Zurich were more often macro- and micro-steatotic compared to untreated DCD livers in Birmingham with 8% *vs.* 0% and 28% *vs.* 4% ($p = 0.0019$), respectively (Table 1). Consistently, the overall donor-recipient risk,

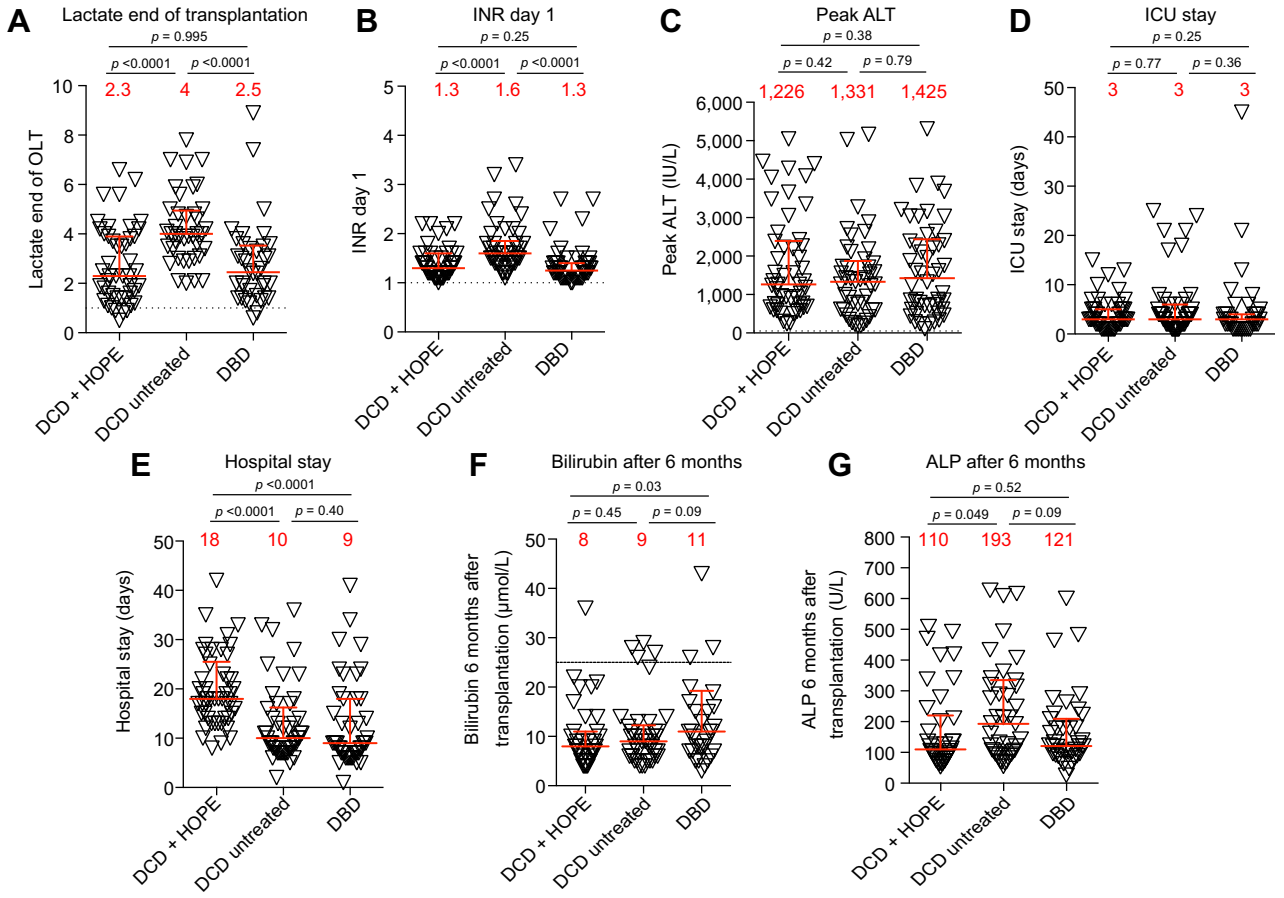

Fig. 2. Liver function and injury after liver transplantation. (A–D) Despite significantly better immediate function following HOPE treatment, livers demonstrate a similar liver enzyme release, as untreated DCD livers. Due to centre-specific recipient management, patients remain in hospital until their special spot at the clinic for rehabilitation becomes available. (E) This led to a similar hospital stay in both DCD cohorts. (F, G) After 6 months, biliary parameters appear similar, with higher median alkaline phosphatase in untreated DCD liver recipients compared to HOPE-treated DCDs, though this did not reach significance. Median and interquartile range were used to present continuous variables, comparisons were made using the Mann-Whitney U test. ALP, alkaline phosphatase; ALT, alanine aminotransferase; DCD, donation after cardiac death; ICU, intensive care unit; INR, international normalized ratio; HOPE, hypothermic oxygenated perfusion. (This figure appears in colour on the web.)

expressed by the UK DCD risk score was significantly higher in the HOPE group with a median of 9 score points *vs.* 3 points in untreated DCD controls (p <0.0001) (Table 1, Fig. S1D), mainly driven by the longer fDWIT and older donor age. Because of the additional 2 h HOPE treatment in the perfusion group, the total out of body time appeared longer in HOPE-treated DCD livers compared to the other 2 cohorts (p = 0.0002, p <0.0001) (Table 1). Based on our matching process, all other parameters, including donor BMI, cold ischaemia time, recipient age, recipient MELD and balance of risk score were similar in all 3 transplant groups (Table 1, Fig. S1B). Importantly, more transplant candidates with hepatocellular carcinoma received a DCD liver in Zurich, compared to Birmingham (p <0.0001). In contrast, in Birmingham there were generally more candidates with PSC and primary biliary cirrhosis, although this did not reach significance (Table 1).

Impact of HOPE treatment on haemodynamic recipient stability and early graft function

Despite the similar overall duration of surgery, untreated DCD livers required significantly more transfusion of fresh frozen plasma during transplantation (median 6 *vs.* 0; p <0.0001) (Fig. S2A-C). HOPE treatment also significantly improved lactate clearance and liver function directly after liver transplant, as demonstrated by lower lactate at the end of transplant surgery (2.3 *vs.* 4, p <0.0001), and lower INR at day one after liver transplant (INR 1.3 *vs.* 1.6; p <0.0001) (Fig. 2A, B). However, notably, we recorded no difference in peak 7-day alanine aminotransferase release after liver transplant between HOPE-treated and untreated DCD livers (Fig. 2C). HOPE-treated DCD liver transplants required similar renal replacement therapy (8/50 *vs.* 11/50, p = 0.611), and ICU stay was also comparably short in both groups (Fig. 2D). While hospital stay was significantly longer in HOPE-treated DCD liver transplants (Fig. 2E), this did not correlate with increased complications (Table 2), but rather reflected different discharge policies between centres. Importantly, liver transplant recipients in Switzerland undergo an obligatory rehabilitation as inpatients, while recipients in the UK are discharged and simply followed up as outpatients.

Impact of HOPE treatment on post-transplant complications and graft loss

Patients receiving untreated DCD livers experienced significantly more acute rejections (p = 0.0019), compared to HOPE-treated livers (Table 2). Six months after liver transplant, the median alkaline phosphatase also appeared to be significantly

Table 2. Outcome parameters and complications.

Outcome Parameter	DCD + HOPE n = 50	DCD untreated n = 50	DBD n = 50	*p* value DCD HOPE *vs.* DCD untreated	*p* value DCD HOPE *vs.* DBD
Non-anastomotic strictures	4 (8%)	11 (22%)	1 (2%)	0.09	0.362
Anastomotic strictures:	12 (24%)	9 (18%)	4 (8%)	0.624	0.0538
Treated conservative/ERCP	11 (22%)	6 (12%)	4 (8%)	0.287	0.0905
Treated with hepaticojejunostomy	1 (2%)	3 (6%)	0	0.617	1.0
Temporary anastomotic stent	8 (16%)	8 (16%)	4 (8%)	1.0	0.3567
Temporary PTCD	3 (6%)	5 (10%)	0	0.715	0.242
Biliary cast	3 (6%)	2 (4%)	0	1.0	0.242
Bile leak	1 (2%)	1 (2%)	2 (4%)	1.0	1.0
Arterial complication	4 (8%)	6 (12%)	3 (6%)	0.741	1.0
Primary non-function	0	2 (4%)	1 (2%)	0.494	1.0
Total graft loss	7 (14%)	18 (36%)	3 (6%)	0.0198	0.3178
Cause of graft loss:					
Ischaemic cholangiopathy	0	5 (10%)	0	0.0125	1.0
Primary non-function	0	2 (4%)	0		
Hepatic artery thrombosis	2 (1x conduit, 1x HAT) (4%)	6 (1× conduit, 2× HAS, 1× pseu–doaneurysm, 2× HAT) (12%)	3 (6%)	0.268	1.0
Sepsis	1 (2%)	3 (6%)	2 (4%)	0.617	1.0
Chronic rejection	1 (2%)	0	0	1.0	1.0
Overall non-tumour related graft loss	4 (8%)	16 (32%)	5 (10%)	0.005	1.0
Secondary tumour	3 (6%)	2 (4%)	0	1.0	0.242
Cause of patient death:					
Ischaemic cholangiopathy	0	3 (6%)	0	0.118	1.0
Primary non-function	0	1 (2%)	0		
Hepatic artery thrombosis	0	3 (6%)	2 (4%)	0.242	0.494
Secondary tumour	3 (6%)	2 (4%)	0	1.0	0.242
Sepsis	2 (4%)	3 (6%)	2 (4%)	1.0	1.0
Renal replacement therapy	8 (16%)	11 (22%)	4 (8%)	0.611	0.3567
Tumour recurrence	3 (6%)	2 (4%)	0	1.0	0.242
Secondary tumour	3 (6%)	1 (2%)	1 (2%)	0.617	0.617
Treated acute rejection	2 (4%)	14 (28%)	5 (10%)	0.0019	0.436
Comprehensive complication index 1 y (points)	47.15	44.0	33.7	0.8982	0.08
Clinically fit and well	40 (80%)	33 (66%)	40 (80%)	0.176	1.0

Continuous variables are presented as median and IQR; comparisons of continuous variables were made using the Mann-Whitney U test. Categorical variables were expressed in quantities and percentages. To compare categorical variables, the chi-square test or the Fisher's exact test were used. ERCP, endoscopic retrograde cholangiopancreatography; PTCD, percutaneous transhepatic cholangiography and drainage; HAS, hepatic artery stenosis; HAT, hepatic artery thrombosis.
This p value refers to graft loss by ischemic cholangiopathy and PNF.

lower ($p = 0.049$) following HOPE treatment, while bilirubin was similar across the 3 groups (Fig. 2F, G). Two cases of PNF occurred in the untreated DCD liver group *vs.* none in the HOPE group. Because of the high number of hepatocellular carcinoma recipients in the HOPE cohort, 6 recipients experienced recurrence of their tumour disease (6/50 = 12%; 3 deaths and 3 alive), compared to 4 in the untreated DCD liver group (4/50 = 8%) (Table 2), while 3 patients and 1 patient developed a secondary tumour, respectively.

Anastomotic biliary strictures were not different between both groups (12/50 *vs.* 9/50, $p = 0.624$). However, the number of non-anastomotic biliary strictures was more than twice in untreated DCD livers compared to HOPE-treated livers (4/50 *vs.* 11/50, $p = 0.09$). Overall, 7 grafts were lost in the untreated DCD liver group by IC or PNF compared to none in the HOPE group ($p = 0.0125$). In contrast, none of the mostly hilar cholangiopathies in the HOPE-treated group led to graft loss within 5 years under conservative treatment (repeated ballooning) (Table 2).

In summary, graft loss due to any non-tumour-related causes (arterial thrombosis, sepsis, chronic rejection, cholangiopathy) cumulated to one-third (16/50) of cases in untreated DCD livers compared to only 8% (4/50) of cases in the HOPE group ($p = 0.005$) (Table 2). Accordingly, 5-year graft survival, censored for tumour recurrence, was 94% in the HOPE-treated DCD group, compared to 78% in the untreated DCD group ($p = 0.024$) (Fig. 3).

Of note, only one recipient with PSC developed a non-anastomotic stricture, which occurred 58 days after the DCD liver transplantation. This contrasts with the more common later recurrence of PSC.[16] All other recipients with a non-anastomotic stricture were not in the PSC or primary biliary cirrhosis group.

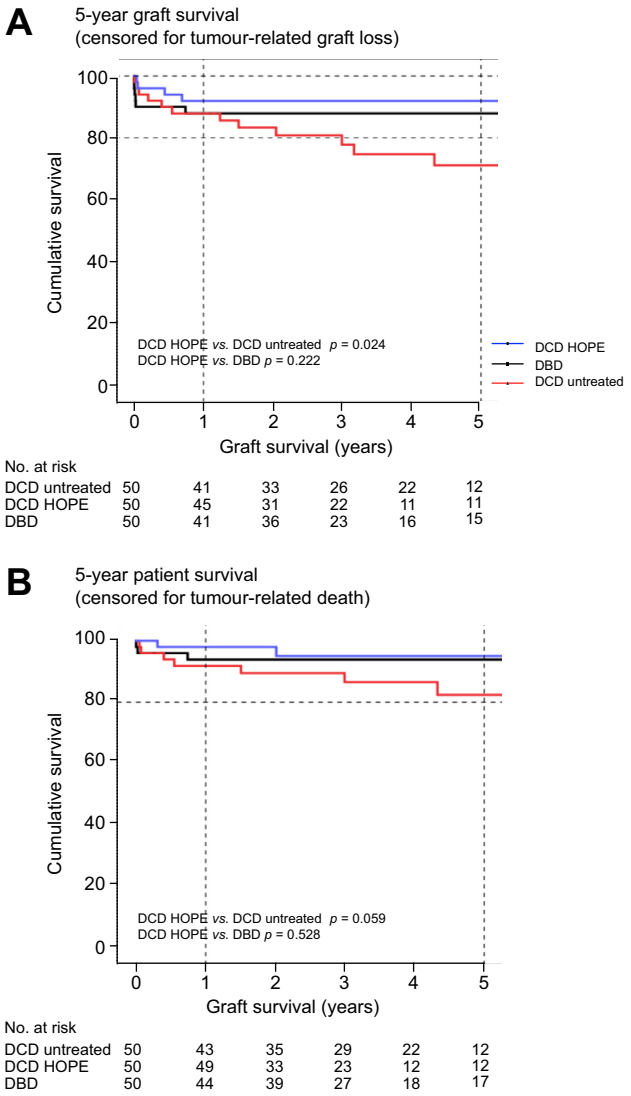

Fig. 3. Five-year graft and patient survival after liver transplantation. Despite the significantly higher risk in the DCD cohort from Switzerland, (A) the overall 5-year graft survival rate appeared excellent, ranging between 70 and 80% in all 3 cohorts. HOPE treatment protects the recipient from development of severe complications including the requirement for retransplantation because of PNF, IC or HAT. Additionally, 5-year graft and patient survival, censored for tumour-related death were significantly improved by HOPE. Long-term survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests. DCD, donation after cardiac death; IC, ischaemic cholangiopathy; HAT, hepatic artery thrombosis; HOPE, hypothermic oxygenated perfusion. (This figure appears in colour on the web.)

Discussion

This is the first outcome report following implantation of machine perfused human DCD liver grafts with a 5-year follow-up. We present several clinically relevant findings. First, HOPE-treated DCD liver recipients showed similar reperfusion injury and improved haemodynamic stability after graft implantation as untreated DCD recipients, despite higher risk. Second, HOPE-treated grafts displayed better function in terms of improved lactate clearance and a significantly lower INR on day one. Third, HOPE liver recipients experienced less non-tumour-related graft loss, including IC, vascular complications and PNF. Finally, this protection contributed to a significantly better 5-year graft survival following HOPE treatment.

In Switzerland, stand-off periods were 5 min longer (10 min), compared to the UK and The Netherlands (5 min), and contributed to extended functional donor warm ischaemia time. The policy in Zurich was therefore to apply HOPE in all DCD liver grafts before implantation, together with short cold ischaemia (≤6 h) and implantation in recipients with low MELD scores. Consistently, to evaluate the impact of the HOPE perfusion approach, we intended to compare HOPE-treated liver grafts with untreated DCD livers with similar short cold ischaemia times and comparable low MELD recipients. Birmingham currently has outstanding experience in controlled (Maastricht III) DCD liver transplants in Europe, having performed 439 DCD liver transplants during the last 10 years, which enabled us to match our patients in terms of short cold ischaemia, low MELD score, and comparable recipient age. However, donors in Zurich were generally older than the Birmingham cohort and also had significantly longer fDWIT, which led to an overall higher risk in the Zurich DCD cohort.[4,17] Despite this match limitation, HOPE treatment effectively protected DCD liver recipients from complications and graft loss.[9]

Three groups worldwide have significant experience with clinical cold liver perfusion. While Guarrera *et al.* from New York have previously demonstrated the impact in extended DBD liver grafts, the team from Groningen recently presented their results after dual-HOPE through hepatic artery and portal vein in DCD livers, providing short-term outcome data.[18,19] In contrast, we provide here the first 5-year outcome analysis with

assessment of cumulative complications within 1 year.[9,11] Based on our experimental data, we suggest that the benefit of HOPE treatment is related to a primary antioxidative mitochondrial effect, subsequently leading to less reperfusion injury and improved early function.[20–22] HOPE treatment after initial cold storage may therefore protect human DCD liver recipients from severe IC and other fatal complications.[11,21,23] The results are consistent with experiences with hypothermic oxygen persufflation in human liver transplantation and controlled oxygenated rewarming, which showed similar protective effects in livers and other organs.[8,24] Our findings are also underlined by several experimental studies in small and large animal models of liver transplantation.[20,21,25] showing a link between early graft inflammation and later cholangiopathy.[23,26]

Machine perfusion has become a hot topic nowadays, based on the idea of optimising marginal grafts before implantation, and enabling prediction of organ function. In contrast to applying machine liver perfusion at hypothermic conditions, warm perfusion strategies currently aim to replace cold ischaemia as much as possible. Normothermic machine perfusion (NMP) is therefore applied directly after cold flush[7,27–29] or even before procurement, *e.g.* by normothermic regional perfusion or ischaemia free organ transplantation.[6,30] Of note, NMP instead of cold storage has been recently reported in a randomised trial of human DBD and DCD livers.[31] The results show lower peak serum aspartate aminotransferase (488 U/L *vs.* 965 U/L), as well as less early allograft dysfunction[30] (10% *vs.* 29.8%) in the NMP group after transplantation, while graft and patient survival after 1 year were similar and excellent (≥95%) in both groups.[31,32] However, it appears unclear, whether normothermic perfusion can be successfully applied after cold ischaemia, and whether NMP prevents severe cholangiopathy. Recent data from the UK rather suggest that end-ischaemic normothermic perfusion of DCD livers fails to protect from irreversible biliary injury.[27] Together with experimental data in discarded human DCD livers, these findings point more to an activation instead of prevention of inflammatory pathways during normothermic perfusion.[27,33] In contrast, applying HOPE after warm and cold ischaemia, is well-known to trigger a substantial change in mitochondrial metabolism similar to hibernating animals, with consecutive reload of the adenine nucleotide pool within 1–2 h of cold oxygenated machine perfusion.[34,35] We believe therefore, that a short-term cold oxygenated perfusion is necessary prior to any reperfusion at normothermic conditions.[9,19,27,36] This approach has been recently tested in a model of discarded human livers, where authors demonstrated improved viability during normothermic evaluation of high-risk human livers following previous HOPE treatment.[37]

A clear limitation of this study is its retrospective design. Based on this, there are necessarily differences in implantation techniques and in terms of the immune suppression used between HOPE-treated DCD livers in Zurich and untreated DCD livers in Birmingham. In addition, the cold ischaemia period before HOPE was short, and it remains unclear whether the same results can be expected with for example more than 10 h cold storage prior to HOPE. However, we would like to emphasise that we found a clear improvement in most endpoints in HOPE-perfused DCD livers, despite longer donor warm ischaemia times, compared to untreated DCD livers. This is also the first report on longer graft survival after a newly established and easily performed perfusion approach in the field of liver transplantation.

The results of ongoing randomised clinical trials to assess the impact of HOPE in DBD and DCD liver transplantation are eagerly awaited (NCT 01317342, NCT 02584283).

We conclude that outcomes in HOPE-treated human DCD liver transplants maintained over a period of 5 years comparable to primary low-risk DBD transplants and were superior to untreated DCD livers, performed at a highly experienced centre. These results suggest that a simple, end-ischaemic perfusion approach is highly effective and may open the field for safe utilisation of extended DCD liver grafts.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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

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Supplementary data

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Author names in bold designate shared co-first authorship

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