First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants

An International-matched Case Analysis

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Background: Exposure of donor liver grafts to prolonged periods of warm ischemia before procurement causes injuries including intrahepatic cholangiopathy, which may lead to graft loss. Due to unavoidable prolonged ischemic time before procurement in donation after cardiac death (DCD) donation in 1 participating center, each liver graft of this center was pretreated with the new machine perfusion "Hypothermic Oxygenated PErfusion" (HOPE) in an attempt to improve graft quality before implantation.

Methods: HOPE-treated DCD livers (n = 25) were matched and compared with normally preserved (static cold preservation) DCD liver grafts (n = 50) from 2 well-established European programs. Criteria for matching included duration of warm ischemia and key confounders summarized in the balance of risk score. In a second step, perfused and unperfused DCD livers were compared with liver grafts from standard brain dead donors (n = 50), also matched to the balance of risk score, serving as baseline controls.

Results: HOPE treatment of DCD livers significantly decreased graft injury compared with matched cold-stored DCD livers regarding peak alanine-aminotransferase (1239 vs 2065 U/L, $P = 0.02$), intrahepatic cholangiopathy (0% vs 22%, $P = 0.015$), biliary complications (20% vs 46%, $P = 0.042$), and 1-year graft survival (90% vs 69%, $P = 0.035$). No graft failure due to intrahepatic cholangiopathy or nonfunction occurred in HOPE-treated livers, whereas 18% of unperfused DCD livers needed retransplantation. In addition, HOPE-perfused DCD livers achieved similar results as control donation after brain death livers in all investigated endpoints.

Conclusions: HOPE seems to offer important benefits in preserving higher-risk DCD liver grafts.

Keywords: donation after cardiac death, Hypothermic Oxygenated PErfusion, ischemic cholangiopathy

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Before the introduction of the currently widely accepted brain death criteria in 1968, donation after cardiac death (DCD) was the only source of cadaveric grafts for orthotopic liver transplantation (OLT). Although subsequently in the last 3 decades, donation after brain death (DBD) has been preferentially used in most countries, the worldwide increasing shortage of brain death donors reestablished the interest for DCD donors, as an additional potential pool of organs. Several reports, however,[1,2] suggest inferior graft survival and increased biliary complications in DCD livers with most concerns for an ischemic cholangiopathy (IC), typically developing within the first 3 to 6 months after OLT.[1] The majority of transplant physicians agree that long periods of donor warm ischemia in DCD donation are responsible for intrahepatic cholangiopathy and graft loss,[2–10] besides additional risk factors including donor age,[5–7,11] duration of graft-cold ischemia,[4–6] previous liver transplant,[4,9] recipient age,[9] and recipient body-mass-index.[4,12]

Dynamic liver preservation techniques, using perfusion of a variety of solutions at different temperatures, have been proposed to protect or rescue marginal liver grafts, including DCD livers.[13,14] The Zurich group has developed a hypothermic oxygenated perfusion system (HOPE) of liver grafts, initially on basis of experimental research in various animal models,[15–21] followed by clinical application in grafts obtained from DCD donors earlier this year.[22] DCD donation in Switzerland was possible only at the price of prolonged normothermic ischemia times due to local legislative regulations, and justified the routine use of HOPE in an attempt to improve graft quality before implantation. The aim of the current study is to test the impact of the HOPE protocol in the first worldwide-perfused 25 human DCD grafts with subsequent transplantation. Thus, we compared standard procurement of DCD grafts, that is, without dynamic perfusion techniques, with HOPE-treated DCD grafts. Short of a randomized controlled trial (RCT), we matched both approaches for the duration of donor warm ischemia and additional key risk factors.

METHODS

Study Design

The study was designed to analyze conventional cold-stored controlled DCD livers (Maastricht category III) and DCD livers, treated by HOPE. For this purpose, all HOPE-treated DCD livers from Zurich (n = 25) were matched (1:2) with DCD liver grafts (n = 50) from 2 European DCD liver transplant programs (Rotterdam, The Netherlands, n = 40, and Birmingham, UK, n = 10) (Supplementary Table 1, Supplementary Figure 1, http://links.lww.com/

TABLE 1. Key Confounders

	Unperfused DCD, N = 50	HOPE-treated DCD, N = 25	DBD, N = 50	$P^{1,2,3}$
BAR score	5.5 (3–8)	4 (2–4.5)	3.5 (2–6)	ns/ns/ns
Donor age	48 y (33–51)	54 y (36–63)	59 y (46–70)	0.0001/ns/0.04
Donor asystolic WI	17.5 min (16–20)	18 (17–21)	—	–/ns/–
Donor functional WI	23 min (20–29)	31 min (26–36)	—	*–/<0.0001/–*
Donor total WI	33 min (27–40)	36 min (31–40)	—	*–/ns/–*
Total preservation time	395 min (349–447)	317 min (280–391)	350 min (286–425)	*0.01/0.002/ns*
Cold storage (excl. HOPE)	395 min (349–447)	188 min (141–264)	350 min (286–425)	*0.01/<0.0001/<0.0001*
Recipient age	56 y (49–59)	60 y (57–64)	54 y (50–62)	ns/*0.0008*/ 0.005
Recipient MELD score	16 (10–21)	13 (9–15)	11 (9–17)	*0.02/ns/ns*
Recipient sex	Male 35/50 (70%)	Male 20/25 (80%)	Male 37/50 (74%)	*ns/ns/ns*
Underlying disease				
Alcoholic cirrhosis	15/50 (30%)	4/25 (16%)	5/50 (10%)	*0.02/ns/ns*
Hepatitis C cirrhosis	11/50 (22%)	8/25 (32%)	19/50 (38%)	*ns/ns/ns*
Hepatitis B cirrhosis	2/50 (4%)	1/25 (4%)	6/50 (12%)	*ns/ns/ns*
Autoimmunhepatitis	4/50 (8%)	—	—	–
Cryptogenic cirrhosis	5/50 (10%)	—	9/50 (18%)	*ns/–/–*
PBC	3/50 (6%)	1/25 (4%)	1/50 (2%)	*ns/ns/ns*
PSC	3/50 (6%)	0/25	2/50 (4%)	*ns/ns/ns*
NASH	3/50 (6%)	6/25 (24%)	1/50 (2%)	*ns/0.05/0.005*
Other*	4/50 (8%)	5/25 (20%)	7 (14%)	*ns/ns/ns*
Hepatocellular carcinoma	*5/50 (10%)*	*18/25 (72%)*	14/50 (28%)	*0.04/0.0001/0.0004*

MELD indicates model of end-stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.
*Other underlying diseases: e.g. Polycystic liver disease, Hepatitis of unknown origin or Cholangiocarcinoma.

SLA/A881). The collection of data was approved by the Swiss Cohort study (KEK-ZH-Nr. 2013-0504). The study periods were January 2012 to December 2014 for HOPE-treated DCD livers, and January 2005 to January 2014 for unperfused DCD livers.

Criteria for matching included donor warm ischemia and key confounders summarized in the balance of risk (BAR) score (donor age, recipient age, model of end-stage liver disease (MELD) score, cold storage, retransplantation, preoperative recipient life support).[23] Matching was performed retrospectively and anonymously using SPSS software (version 21, IBM Corp, Armonk, NY). Matched patients were identified by applying hierarchically the following criteria: primary liver transplant, graft type (controlled DCD), donor asystolic warm ischemia (cardiac arrest to cold flush) within ±4 minutes, and BAR score within ±5 points, but below a threshold of BAR ≤9, cold storage ≤8 hours. Matching criteria limits were extended in the unperfused group for 2 cases with shorter asystolic warm ischemia (10 and 11 minutes), in 9 cases for cold storage >8 hours, and in 3 of those cases for BAR 10, due to the lack of suitable matches (Supplementary Table 1, http://links.lww.com/SLA/A881).

The transplant procedure was performed by classic liver implantation technique in HOPE-treated livers, and by cava preserving technique (piggyback) in unperfused DCD livers (Rotterdam and Birmingham). Liver reperfusion was performed in all cases by portal perfusion first and subsequent arterial perfusion. Bile duct anastomoses were performed by duct-to-duct technique without stent in most cases. Hepaticojejunostomy was used in 6% (3/50) of unperfused DCD livers. The most frequent underlying diseases in recipients were hepatitis C, alcoholic, or non-alcoholic steatohepatitis cirrhosis (Table 1). Standard immunosuppression was tacrolimus, azathioprine or mycofenolate mofetil, and reducing dose steroids. All HOPE-treated patients and the majority of unperfused DCD recipients (40/50) received additional basiliximab on day 0 and day 4. The primary endpoint was the incidence and severity of biliary complications within 1 year after transplantation. Secondary endpoints included liver ischemia reperfusion injury and function as well as graft survival.

In a last step, we performed a comparison of HOPE-treated and unperfused DCD livers with conventional cold-stored DBD livers, serving as negative controls. For this purpose, 50 liver grafts from standard brain dead donors from 2 centers (Zurich, n = 40, and Birmingham, n = 10) were also matched according to the BAR score[23] (study period February 2004 to May 2014) (Supplementary Figure 1, http://links.lww.com/SLA/A881).

Key Confounders in Matched Patients

The matching process resulted in comparable asystolic donor warm ischemia (18 vs 17.5 minutes, ns) and BAR scores (4 vs 5.5, ns) between DCD groups (HOPE-treated DCD vs unperfused DCD) (Table 1, Supplementary Figure 1, http://links.lww.com/SLA/A881). Consistently, donor age and recipient MELD score were also not different (54 vs 48 years, ns; MELD 13 vs MELD 16, ns) (Table 1).

Significant variations due to less suitable cases seemed in terms of the following parameters; first, cold storage was generally shorter in HOPE-treated compared with unperfused livers, because of in-house donors in all HOPE-treated cases, and also because of significant reduction of cold ischemia by the length of the perfusion time (188 vs 395 minutes, $P < 0.0001$) (Table 1). Secondly, recipient age was higher in HOPE-treated DCD livers (60 vs 56 years, $P = 0.008$) (Table 1). Third, despite comparable total donor warm ischemia time (withdraw to cold flush) in both DCD groups (36 vs 33 minutes, ns), unperfused DCD livers exhibited a shorter period of relevant hypotension before cardiac arrest, resulting in significant shorter functional donor warm ischemia (systolic pressure <50 mm Hg to cold flush) in unperfused DCD livers, as compared to HOPE-treated DCD livers (23 vs 31 minutes, $P < 0.0001$) (Table 1).

The control group (DBD patients) was comparable with HOPE-treated and unperfused DCD patients in terms of BAR and MELD scores (Table 1, Supplementary Figure 1, http://links.lww.com/SLA/A881).

Hypothermic Oxygenated Perfusion

Machine perfusion of DCD livers was performed, as reported earlier.[22] Briefly, hypothermic oxygenated perfusion was done in all cases after cold flush and cold storage during recipient hepatectomy, exclusively through the portal vein for 1 to 2 hours [median 118 minutes, interquartile range (IQR) 101–149 minutes]. As perfusate, we used recirculated University of Wiscon-sin (UW) gluconate solution (KPS-1) at low flow rates (120–180 mL/min), which was

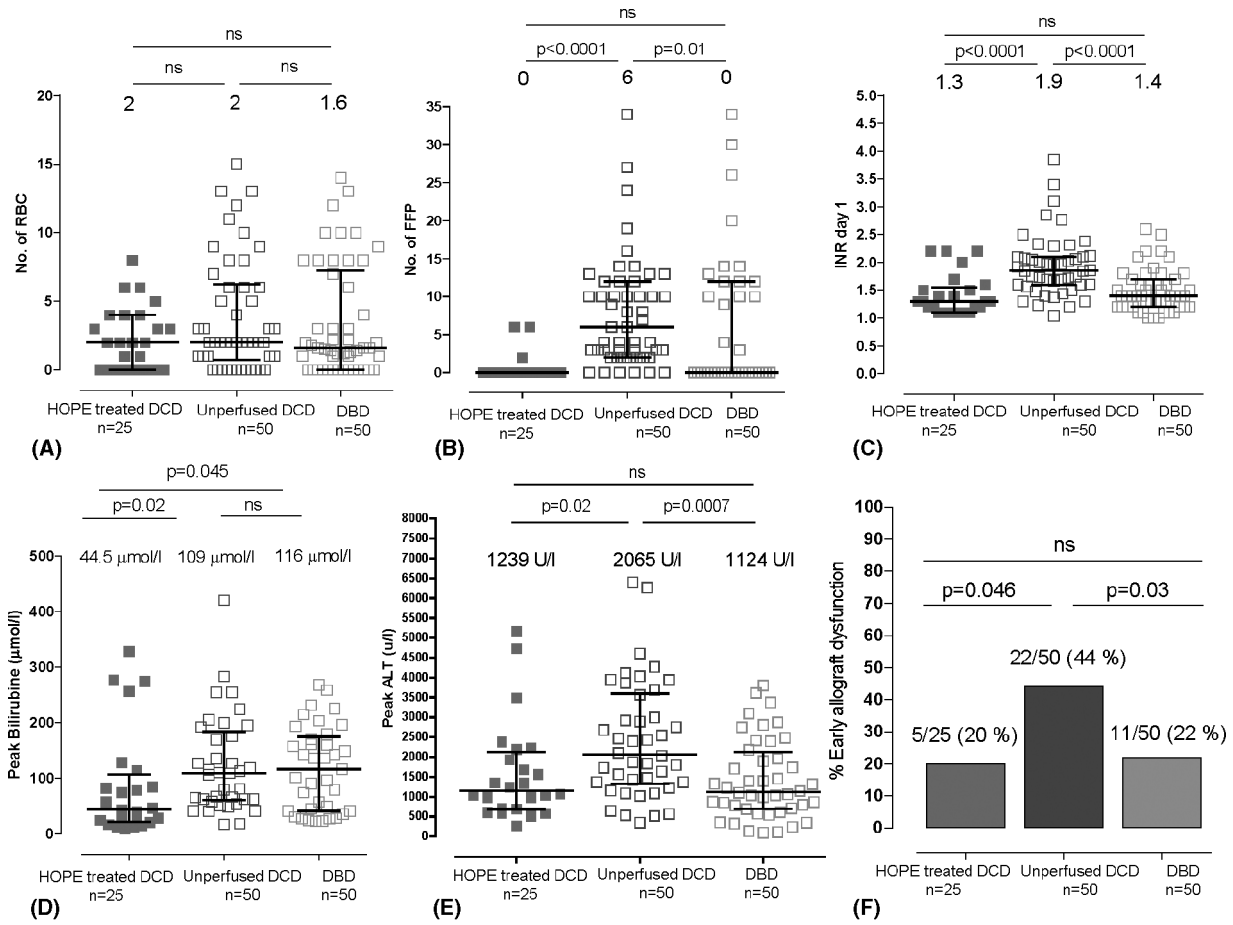

FIGURE 1. HOPE-treated DCD livers showed significant less need for transfusions (A, B), improved liver graft function (C, F), and decreased peak liver enzymes (D, E) as compared to unperfused DCD livers. In addition, HOPE-treated DCD livers achieved similar results as matched DBD livers (A-F).

oxygenated (pO_2 80–100 kPa) and cooled (10°C) by an ECOPS device (Organ Assist).[22]

Procurement and Definitions

Super rapid en bloc multiorgan retrieval was carried out in all DCD livers with heparinized flush of abdominal organs by cannulation of the iliac artery after abdominal incision according to earlier reports.[24] No premedication was given to DCD donors before withdraw of support. The bile duct was flushed in situ and ex situ with preservation solution. All HOPE-treated DCD liver grafts were stored after procurement in Institute George Lopez-1 solution until machine perfusion, whereas unperfused DCD liver grafts were stored in UW solution. No DCD graft was treated with fibrinolytic agents.

Early allograft dysfunction was defined by the occurrence of the following: bilirubin > 170 µmol/L on day 7 after OLT, or international normalized ratio (INR) > 1.6 on day 7 after OLT, or peak alanine-aminotransferase (ALT) > 2000 U/L within the first 7 days after OLT.[25] IC was defined and classified as either multifocal or unifocal intrahepatic strictures without the presence of concomitant hepatic artery thrombosis or arterial complications.[1,26] Each patients chart was reviewed retrospectively for clinical data, liver function tests, and imaging. IC was detected clinically and confirmed by images (endoscopic, percutaneous, or magnetic resonance cholangiography). Median follow-up was 448, 528, and 1530 days for HOPE-treated DCD livers, unperfused DCD livers, and DBD liver, respectively.

Statistical Analysis

The results are expressed in median and IQR for metric parameters and in percentages for nominal parameters. Continuous and categorical parameters were compared with the 2-tailed Mann-Whitney-Wilcoxon nonparametric test; dichotomous parameters were compared with the Fisher exact test. Survival analysis was adjusted to cold ischemia in DCD groups (Cox regression). SPSS version 21 (IBM Corp., Armonk, NY) and GraphPad Prism version 5 (GraphPad Software, Inc, La Jolla, CA) were used for statistical analysis.

RESULTS

Comparison of HOPE Treated and Unperfused (Cold-stored) DCD Livers

HOPE treatment of DCD liver grafts improved significantly several biochemical and clinical parameters during OLT and after 1-year follow-up (Table 2). First, HOPE-treated DCD livers demonstrated less liver enzyme release after reperfusion, as compared to unperfused DCD livers (1239 vs 2065 U/L peak ALT, 1808 vs 2848 U/L peak aspartate-aminotransferase, 44 vs 109 µmol/L peak bilirubin) (Table 2, Figs. 1 and 2). Secondly, HOPE-treated DCD livers showed less early allograft dysfunction, as expressed by INR at day 1 (1.3 vs 1.9, $P < 0.0001$), or by increase of either ALT, bilirubin,

TABLE 2. Outcome After OLT

	Unperfused DCD, N = 50	HOPE-treated DCD, N = 25	DBD, N = 50	$P^{1/2/3}$
Intraoperative RBC	2 U (0.8–6.3)	2 U (0–4)	1.6 U (0–7.3)	*ns/ns/ns*
Intraoperative FFP	6 U (2–12)	0 U (0)	0 U (0–12)	*0.01/< 0.0001/ns*
Duration of transplant	390 min (330–477)	345 min (300–413)	350 min (300–420)	*ns/ns/ns*
INR day 1	1.9 (1.1–1.6)	1.3 (1.6–2.1)	1.4 (1.2–1.7)	*<0.0001/<0.0001/ns*
Peak ALT	2065 U/L (1331–3596)	1239 U/L (689–2126)	1124 U/L (693–2126)	*0.007/0.02/ns*
Peak AST	2848 U (1485–6724)	1808 U (1133–3547)	1473 U (762–3764	*0.005/0.04/ns*
Peak creatinine	158 μmol/L (108–218)	154 μmol/L (105–313)	159 μmol/L (117–248)	*ns/ns/ns*
Renal replacement	5/50 (10%)	7/25 (28%)	11/50 (22%)	*ns/ns/ns*
Peak bilirubine	109 μmol/L (60–183)	44 μmol/L (21–106)	116 (41–174)	*ns/0.016/0.046*
Early graft dysfunction*	22/50 (44%)	5/25 (20%)	11/50 (22%)	*0.03/0.046/ns*
PNF	3/50 (6%)	0/25	0/50	*ns/ns/ns*
HAT	3/50 (6%)	1/25 (4%)	1/50 (2%)	*ns/ns/ns*
Acute rejection (>RAI 4)	8/50 (16%)	3/25 (12%)	6/50 (12%)	*ns/ns/ns*
ICU stay	3 d (2–6)	3 d (1.3–5.7)	3 d (2–5.7)	*ns/ns/ns*
Hospital stay	18 d (15–29)	20 d (14–23)	17.5 d (13–26)	*ns/ns/ns*
3-month alkaline phosphatase	178 U/L (77–415)	109.5 U/L (63–740)	100 U/L (79–193)	*0.05/0.04/ns*
6-month alkaline phosphatase	172.5 U/L (97–327)	92 U/L (71–220)	131 U/L (96.327)	*ns/0.02/ns*
IC	11/50 (22%)			
Multifocal†	8/50 (16%)	0/25	2/50 (4%)	*0.015/0.013/ns*
Unifocal†	3/50 (6%)			
Anastomotic strictures or leaks	12/50 (24%)	5/25 (20%)	10/50 (20%)	*ns/ns/ns*
Total biliary complication	23/50 (46%)	5/25 (20%)	12/50 (24%)	*0.035/0.042/ns*
Retransplant for IC or PNF	9/50 (18%)	0/25	1/50 (2%)	*0.031/0.025/ns*
Graft loss total	15/50 (30%)	2/25 (8%)	2/50 (4%)	*0.009/0.041/ns*
1-year graft survival	69%	90%	96%	*0.002/0.035/ns*

ALT indicates alanine-aminotransferase; AST, aspartate-aminotransferase; FFP, fresh frozen plasma; HAT, hepatic artery thrombosis; ICU, intensive care unit; RAI, rejection activity index; WI, warm ischemia.

*Definition of Early Allograft Dysfunction (EAD) according to Olthoff et al, Liver Transpl. 2010.[25]

†Definition of ischemic cholangiopathy (IC) according to Lee et al, Liver Transpl. 2007 and Buis et al, Liver Transpl. 2007.[26,32]

or INR during the first week after OLT[25] (20% vs 44% early allograft dysfunction, $P = 0.046$, Table 2). Of note, liver function was delayed despite significant more substitution of coagulation factors during OLT in unperfused versus HOPE-treated DCD livers (6 vs 0 U fresh frozen plasma, Table 2; Fig. 1). Six percent of unperfused DCD liver grafts (3/50) showed primary nonfunction (PNF) after OLT compared with no PNF in HOPE-treated DCD livers (Table 2). Third, although HOPE treatment did not decrease the rate of extrahepatic biliary complications (5/25 vs 12/50), the percentage of intrahepatic cholangiopathy >1-year follow-up was significantly less compared with unperfused DCD livers (0/25 vs 11/50, $P = 0.013$; Table 2, Fig. 3). Consistently, 3- and 6-month serum levels of alkaline phosphates increased in unperfused as compared to HOPE-treated DCD livers (Table 2, Fig. 3). Eight of 50 unperfused DCD liver grafts developed

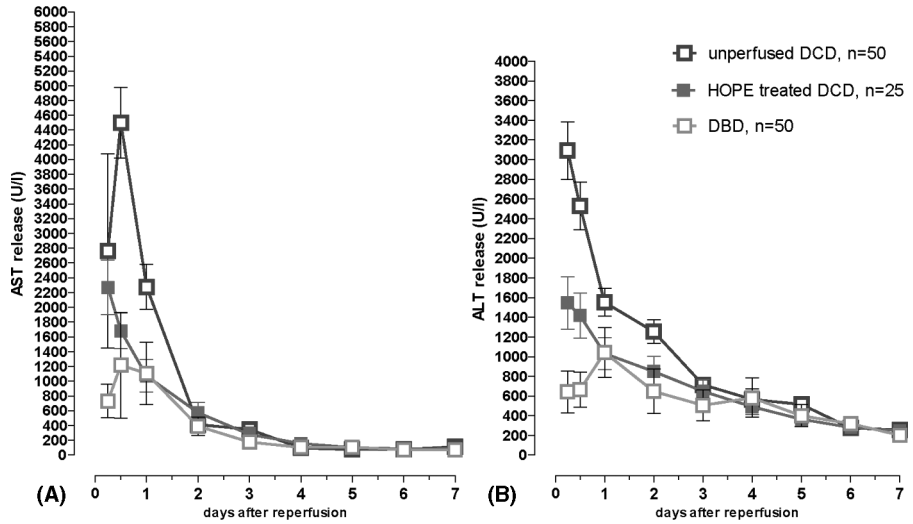

FIGURE 2. Cumulative ALT and AST release during the first week after OLT demonstrated significant higher reperfusion injury in unperfused DCD livers as compared to HOPE-treated DCD and DBD livers (A, B). ALT, alanine-aminotransferase; AST, aspartate-aminotransferase.

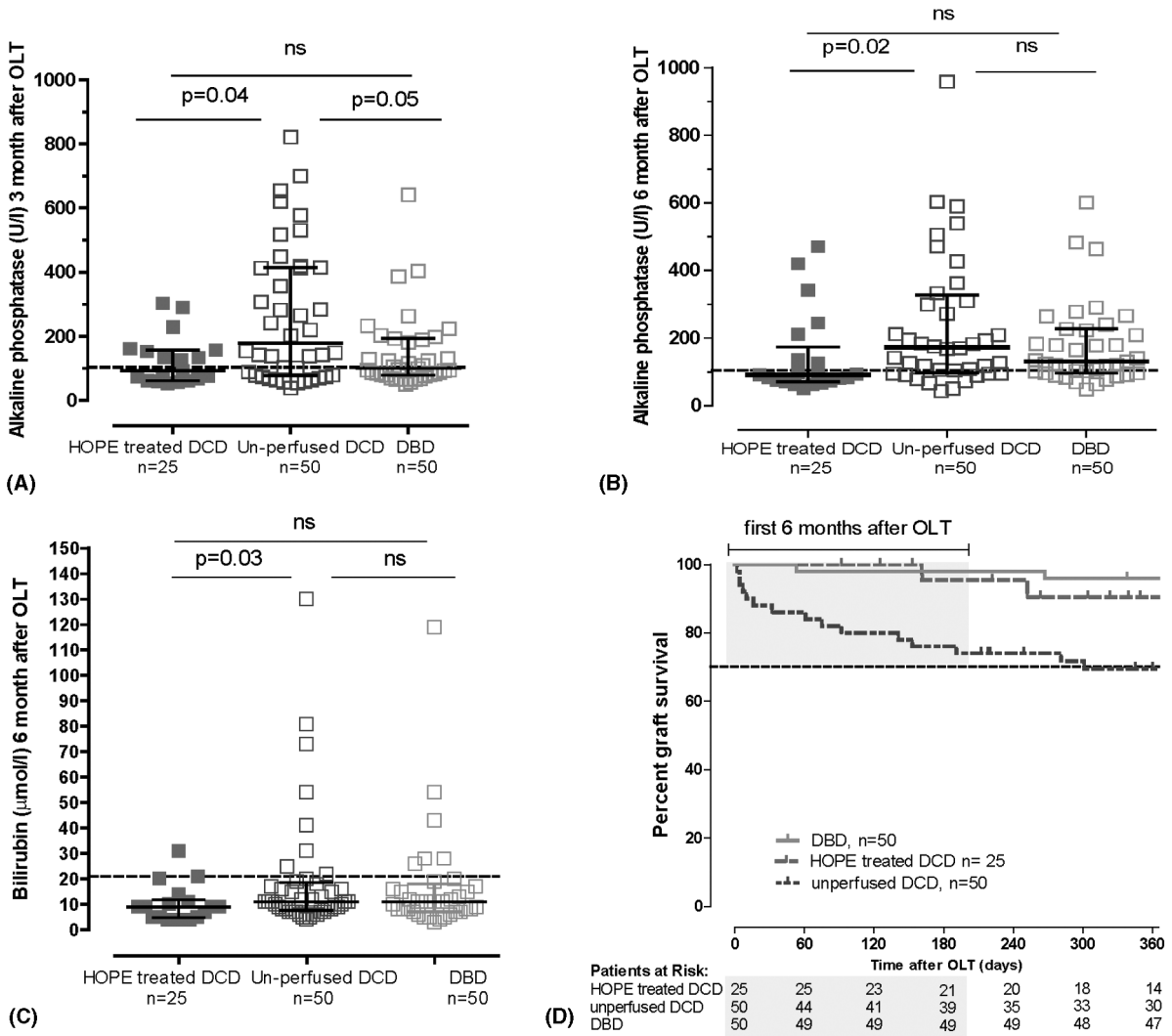

FIGURE 3. Parameters of cholestasis (bilirubin, alkaline phosphatase) 3 and 6 months after OLT were significantly less in HOPE-treated DCD livers as compared to unperfused DCD livers (A-C). Actuarial 1-year graft survival was 90% in HOPE-treated DCD livers as compared to 69% in unperfused DCD livers (D).

multifocal IC with a median time to retransplantation of 153 days (IQR 75–301 days) (Supplementary Figure 2, http://links.lww.com/SLA/A881). HOPE treatment resulted furthermore in no graft loss due to PNF or intrahepatic biliary complications during the observation period in contrast to 18% graft losses in unperfused DCD livers ($P = 0.025$, Table 2). Overall and cholangiopathy-free graft survival after 1 year was 90% in HOPE-treated DCD livers compared with 69% in unperfused DCD livers ($P = 0.035$; Table 2, Fig. 3). The effect of HOPE was independent from the length of cold storage as tested by regression analysis (hazard ratio 4.19; 95% CI, 0.96–18.2).

Comparison of HOPE-treated and Unperfused (Cold-stored) DCD Livers With DBD Livers

To quantify the effect of HOPE, we compared all DCD livers with conventional cold-stored DBD livers, matched for key confounders by the BAR score.

Although the difference between matched unperfused DCD livers and DBD livers was high in terms of reperfusion injury, graft function, later bile duct complications, and graft survival, no significant differences were observed in all analyzed endpoints between HOPE-treated DCD livers and matched DBD livers (Figs. 1–3, Table 2).

DISCUSSION

This is the first comparison between standard-preserved and HOPE-treated DCD liver transplantation disclosing important benefits in favor of the HOPE approach. The study shows that DCD livers treated by HOPE developed less reperfusion injury and better graft function with a lower incidence of later intrahepatic biliary complications. The most relevant observation is an improved graft survival with the use of HOPE. In fact, HOPE-treated DCD livers achieved similar results in all investigated endpoints comparable with low-risk DBD liver transplants.[27] The benefits of HOPE treatment could be documented from the initial phase of reperfusion until the later follow-up at 1 year after OLT.

Randomized trials to compare human machine liver perfusion techniques with conventional cold storage are not yet available. First reports on normothermic or hypothermic perfusion are currently restricted to feasibility and practical aspects in standard or extended liver grafts.[22,28–30] Our recent experimental and clinical observations have suggested significant advantages for the HOPE technique on reperfusion injury in DCD livers,[18,21] with further downstream impact on graft immune responses.[20] HOPE also conferred protection against biliary injury in a rodent liver transplant model.[19] The mechanisms seem to be related to changes in mitochondrial respiration rates during HOPE in addition to perfusion effects on the sinusoidal glycocalix.[18,31] It is currently unclear, how much oxygen is needed under cold perfusion conditions in human livers. We, however, believe that the effects of HOPE depend on oxygenation of the perfusate, as recent experimental studies in pig livers with deoxygenated perfusates point to this fact.[18]

Due to the severe shortage of organs in Switzerland, a DCD liver transplant program was initiated in 2012 in Zurich, but with strict ethical regulations (confirmation of brain death after cardiac arrest), resulting in long period of asystolic donor warm ischemia. Due to this unavoidable and unsuitable graft warm ischemia, and the knowledge gathered in animal models, DCD liver transplants program in Zurich included HOPE treatment before implantation. Candidates for such perfusion approach were selected from the waiting list in accordance to low BAR score, a long expected waiting time, and to the presence of hepatocellular carcinoma in most of the cases (18/25).

Conclusive recommendations must also focus on later biliary complications occurring within the first year after OLT.[1,7,32] Short of an unperfused DCD liver group in Zurich, we searched for a comparable group of DCD livers in 2 well-established European DCD liver transplant centers. To further optimize comparability between the groups, we choose an anonymously computerized matching program offering equal distribution of several key parameters, as for example asystolic cross clamp time, total donor warm ischemia time, the presence of hepatitis C virus, BAR and MELD scores, donor age, and sex of the recipient (Table 1). Of note, although constitutional variations in surgical technique, preservation solution, and also time periods remained between centers, we would like to emphasize that, despite all center-related differences, outcome for DBD liver grafts are identical in Zurich, Rotterdam, and Birmingham, as for example duration of transplant procedure (5.8 vs 6.2 vs 5 hours), intensive care unit (3 vs 4 vs 4 days) and hospital stay (18.5 vs 18 vs 11 days), and also 1-year graft survival (93% vs 90% vs 90%). We assume therefore that HOPE significantly contributes to the observed effect of decreased injury in HOPE-treated DCD livers in contrast to unperfused DCD livers.

An appealing feature of the HOPE technique is its easy application after conventional cold flush and organ transport (endischemic perfusion) obviating the need for the cumbersome transport of perfusion equipment. In contrast to the concept of normothermic perfusion strategies, which intend to avoid any cold storage periods by upfront delivering oxygenated blood under physiologic conditions, the HOPE technique reversibly suppresses mitochondrial oxidative metabolism after cold preservation decreasing the mitochondrial release of reactive oxygen species upon reperfusion with several-fold deactivation of numerous intracellular and extracellular pathways, including the host inflammatory response.[31] It seems that concomitant perfusion of the hepatic artery, in addition to oxygenated perfusion through the portal vein, is unnecessary (data not shown).

The results of this study highlight an incidence of intrahepatic biliary complications in 22% of unperfused DCD liver recipients and a 1-year graft survival <70%. These findings are consistent with most previous reports,[2,7,33–35] although some groups have reported better outcome.[3,12,24,36,37] More important, those later studies have focused on highly selected donor population, not qualifying as extended DCD, that is, short donor asystolic warm ischemia time (5–10 minutes), and donor age ≤50 years[24,36] or even ≤40 years.[3,37] In fact, almost no centers currently implant DCD livers with periods of asystolic donor warm ischemia >20 minutes or donor age >60 years. A recent study from the Netherlands reported on 97 DCD liver recipients with a median asystolic donor warm ischemia of 17 minutes and a donor age of 44 years. The incidence of nonanastomotic biliary strictures cumulated in this analysis to 31%,[38] with a strong correlation between the degree of initial reperfusion injury (peak ALT) and subsequent biliary injury. This observation underlines the importance of the initial ischemia-reperfusion injury before implantation, which, for example, can be prevented by machine perfusion techniques.

In conclusion, this study provides strong evidence that applying HOPE protects extended DCD livers from initial reperfusion injury leading to better graft function and the prevention of intrahepatic biliary complications. HOPE may therefore offer optimization of liver grafts before implantation by a simple and practical perfusion technique with a high impact on enlarging the donor pool. To further test the HOPE strategy, we have initiated a multicentric phase III RCT in DBD liver transplantation, which may establish the protective effects of machine perfusion in liver transplantation.

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DISCUSSANTS

A. Pinna (Bologna, Italy):

The study performed by the group from Professor Clavien shows that extracorporeal perfusion with oxygenated hypothermic solution reduces graft injury in DCD liver grafts compared to static cold preservation. The decrease of the postreperfusion liver injury of such treated grafts is dramatically evident with better graft survival at 1 year of the treated DCD liver grafts compared with the DCD liver grafts not treated with the HOPE technique. The study, however, raised several questions. First, a possible major limitation of the study is the comparison made among transplants performed in different centers at different time periods. Is this a concern in the evaluation of the results? Second, can the authors better explain whether the advantage of HOPE was due to the liver perfusion or from the oxygenation of the perfusate? Third, do the authors think that there is a difference according to the use of different solutions among the 3 centers? Finally, can the authors clarify if they used or not thrombolytic treatment of the liver grafts after retrieval in any arm of the study? This is a well-conducted study full of HOPE for the future.

Response From P. Dutkowski (Zurich, Switzerland):

Thank you Professor Pinna for your valuable comments and questions. We agree that there are differences among the centers in terms of the transplant technique, surgeons, preservation solutions, and time periods. Despite that, outcome in the brain dead (DBD) liver grafts, that is, control groups, was identical in Zurich, Rotterdam, and Birmingham. Therefore, we assume that constitutional variations are rather unlikely to have a major effect on graft outcome. Centers have differences in terms of warm ischemia periods and biliary complications, for example, the rate of intrahepatic cholangiopathy is low in Birmingham with 85% 1-year graft survival, but asystolic warm ischemia time is generally short. Although a center comparison was not the aim of this study, we opted to search for best matches in terms of key confounders including warm ischemia periods. Based on this analysis, we believe that HOPE significantly contributes to the observed effects of decreased injury in HOPE-treated DCD livers in contrast to unperfused DCD livers.

Next, we are convinced that the effects of HOPE depends on the oxygenation of the perfusate, as demonstrated in recent experimental studies in pig livers published recently by our group. Furthermore, experimental studies with gaseous oxygenation without any perfusate also showed a protective effect. Based on this, we currently regard hyperbaric oxygen as the key compound in the HOPE procedure. Finally, no fibrinolytic agents have been used in this study in DCD patients.

T. van Gulik (Amsterdam, The Netherlands):

It is very important that you could show that, contrarily to many beliefs, rescue of the biliary system does not need separate perfusion of the hepatic artery. You succeeded to obtain sufficiently high oxygen saturation by perfusion of the portal vein only. We can now get rid of the technical problems of dual perfusion including the hepatic artery. There are also interesting reports showing that subnormothermic perfusion of the liver is also protective probably through the same pathway. If you combine hyperoxygenation with slightly higher temperature than hypothermic, then the effects might even be greater although difficult to show because your results are already very impressive.

Response From P. Dutkowski (Zurich, Switzerland):

The ideal temperature for machine liver perfusion remains currently unclear. It might be 10°C, 15°C, or even 20°C, as you suggested. We believe, however, that the protective key mechanism relies on a reversible downregulation of mitochondrial electron transfer, which best occurs at low temperatures. Future studies are needed to unravel this issue.

R. Adam (Paris, France):

We want to go a step forward and see now what may happen using HOPE for DBD donors. This is the case in my country because we still have few DCD donors. I know that you are now proposing a prospective randomized study and we will be very keen to participate in it. Do you think that we should be open to this study to all liver grafts, including those without any risk factor, or should we reserve our focus to risky grafts in a way to have a higher chance to demonstrate differences between HOPE and no HOPE treatment?

Response From P. Dutkowski (Zurich, Switzerland):

The benefit of HOPE or other machine perfusion techniques is probably higher in preinjured liver grafts. Therefore, inclusion of too many standard livers in the randomized trial may show less beneficial effects. As the number of accepted and implanted extended criteria liver grafts is increasing everywhere, we would, however, keep at the moment the trial design omitting any selection.