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

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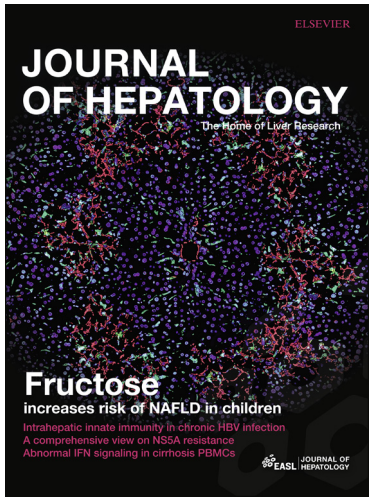

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Outcomes of liver transplantations from donation after circulatory death (DCD) treated by hypothermic oxygenated perfusion (HOPE) before implantation

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Key words: Donation after cardiac death, Hypothermic oxygenated perfusion, ischemic cholangiopathy

Abbreviations

AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; BAR: Balance of risk score; BMI: Body mass index; COR: controlled oxygenated rewarming; DBD: Donation after brain death; DCD: Donation after circulatory death; FFP: Fresh frozen plasma; fDWIT: functional donor warm ischemia time; HAT: Hepatic artery thrombosis; HCC: Hepatocellular carcinoma; HOPE: Hypothermic oxygenated perfusion; IC: Ischemic cholangiopathy; ICU: Intensive care unit; IFOT: ischemia free organ transplantation; INR: International normalized ratio; IQR: Interquartile range; kPa: Kilopascal; KPS-1: Kidney-Perfusion-Solution-1; LT: Liver transplantation; MELD: Model of end stage liver disease; NASH: Non-alcoholic steatohepatitis; NRP: Normothermic regional perfusion; OLT: Orthotopic liver transplantation, PNF: Primary non function; RBC: Red blood cells; RCT: Randomized controlled trial; ROS: Reactive oxygen species; UK: United Kingdom; UW: University of Wisconsin; WI: Warm ischemia

Abstract

Objectives: To provide 5-year outcome in human livers donated after circulatory death (DCD), treated by hypothermic oxygenated perfusion (HOPE).

Background: DCD liver transplantation is known for potential worse outcome due to higher rates of graft non-function or irreversible cholangiopathy. The impact of machine liver perfusion techniques on these complications remains elusive.

Methods: Fifty HOPE treated DCD liver transplants in Zurich between 2012 and 3/2017 were matched with 50 primary DBD liver transplants, and also with 50 un-treated DCD livers in Birmingham. Match factors focused on short cold ischemia, comparable recipient age and low recipient lab MELD. Primary endpoints were post-transplant complications, and non-tumor related patient death or graft loss.

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

Conclusions: Outcome of HOPE treated human DCD liver transplants maintained over a period of five years, was comparable with DBD primary transplants, and also superior to un-treated DCD livers, despite much higher risk. These results suggest strong effectivity of a simple end-ischemic perfusion approach and may open the field for safe utilization of extended DCD liver grafts.

Lay summary:

Machine perfusion techniques are currently introduced in the clinic, with the aim to optimize 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 have been unclear. We report here, for the first time, on five-year graft survival in DCD livers, treated either by conventional cold storage, or by 1-2 hour hypothermic oxygenated perfusion (HOPE) after cold storage. Graft loss was significantly less in HOPE treated livers, and also comparable to DBD primary transplants, despite long donor warm ischemia times. HOPE after cold storage appears therefore as a simple and effective method to treat high risk DCD livers before implantation.

Donation after circulatory death organs (DCD) are increasingly used for liver transplantation (LT), due to the persisting organ shortage and waiting list mortality[1]. Several reports, however, suggest inferior graft survival, increased risk for primary non-function (PNF), and biliary complications in DCD livers, with most concerns for an irreversible ischemic cholangiopathy (IC)[2]. Severe forms, requiring retransplantation, typically develop within the first 3 to 6 months after LT[3]. While the majority of transplant physicians agree, that prolonged periods of donor warm ischemia contribute significantly to this aggressive biliary complication, others argue that also factors including donor and recipient age, cold ischemia, donor body-mass-index (BMI) and hepatic steatosis, or technical issues are equally important[4,5]. Various dynamic preservation techniques are therefore currently under evaluation to optimize liver grafts before implantation[6–9]. Since 2012, a novel machine perfusion concept has been introduced in Zurich for DCD liver transplantation, hypothermic oxygenated perfusion (HOPE), applied only for 1-2 hours after conventional procurement and cold storage [10,11]. While initial clinical experiences of this new technique has been earlier presented including the first 25 human DCD livers[9], our study aimed now, first, to document a longer follow-up of five years after HOPE treatment in human DCD livers. Secondly, we intended to unravel the efficacy of the HOPE perfusion approach and compared therefore HOPE perfused DCD livers with the best available not machine perfused e.g. untreated DCD livers from a highly experienced transplant unit, e.g. DCD livers exposed to short cold ischemia in low MELD recipients. Third, this analysis focussed on cumulative post-transplant complications within the first year of post-transplant follow up, quantified by the comprehensive complications index (CCI)[12]. Fourth, we compared non-tumour related death or graft loss in HOPE treated or un-treated DCD livers with outcomes in primary DBD liver transplants.

Patients and Methods

1. Patient cohort and data collection

This study refers to the first fifty HOPE-treated human livers transplanted from DCD donors at the University Hospital in Zurich, Switzerland between 2012-3/2017 with at least 1 year follow up. We matched this machine perfused DCD cohort with an un-treated DCD cohort (n=50) from the liver unit at Queen Elizabeth Hospital Birmingham, United Kingdom (UK). Primary, adult DBD liver transplants from both centres were also matched and served as 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 (NRP) in the donor and other machine perfused livers were also excluded.

2. 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 heparinized saline (20 °C) were used as flush solution, followed by 3-5 litres of precooled (4°C) Institute-George-Lopez-1-solution (IGL-1). All livers were retrieved within 30 minutes 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 hypothermic oxygenated perfusion (HOPE) for 1-2 hours 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 with however additional in situ perfusion of the portal or mesenteric vein besides the Aorta. The flush solution in UK was cold University of Wisconsin-solution (UW) (4°C). Bench perfusion included 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 minutes in Zurich, prior to super rapid laparotomy, cannulation and cold flush. In both groups, the functional donor warm ischemia (fDWIT) time 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 (tPA).

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

3. Hypothermic Oxygenated Perfusion (HOPE)

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 hours 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 3mmHg and the oxygen concentration in the perfusate was high with a pO_2 of 80-100kPa in order to recondition liver mitochondria (**Figure 1**)[10]. At the end of HOPE perfusion, DCD grafts were disconnected and directly implanted.

4. Risk analysis and matching of the three 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 ischemia, 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]. Fifty not machine perfused, simply cold stored DCD livers from Birmingham 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 a baseline control. A computerized 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 ischemia time (±1hrs), recipient age (±1year) and MELD score (±1point). For each HOPE-treated DCD liver in Zurich, one appropriate untreated DCD liver (simply cold stored) from Birmingham and one low risk DBD liver, selected from a combined DBD cohort between 2007-2017 (n=921) from Zurich and Birmingham was matched according to above mentioned parameters (*HOPE treated DCD: 50 vs. un-treated DCD:50 vs. low risk primary DBD:50*). The low risk DBD matching cohort

represents adult, primary liver transplantations into low risk recipients (lab MELD <21points), in accordance with recent definition of benchmarking in liver transplantation[13]. Any acute liver failures, combined transplantations, live donors and split grafts, auxiliary liver transplantations were excluded.

5. Endpoints

We documented several intraoperative parameters, including transfusions and duration of surgery. We also assessed lactate clearance at the end of LT. Further analysis includes liver function (INR day 1) and injury (Peak Alanine Aminotransferase (ALT) during first week after LT. 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 (CCI)[12,14]. Five-year survivals are shown with a focus on non-tumour-related graft loss, including the rate of primary-non-function (PNF), ischemic cholangiopathy (IC) and hepatic artery thrombosis (HAT). Ischemic cholangiopathy (IC) was defined radiologically, as intrahepatic or hilar biliary strictures and dilatations, occurring in the absence of hepatic artery stenosis (HAS) or thrombosis (HAT), portal thrombosis, chronic ductopenic rejection, and recurrent PSC[15].

6. 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. End of observation period was March 31st , 2018.

7. 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).

Results

1. Characteristics of the three different transplant cohorts

Fifty human DCD livers were transplanted at University Hospital Zurich with previous endischemic HOPE treatment between January 2012 and March 2017. Detailed donor, graft and recipient parameters are highlighted in **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) when compared to both control groups, un-treated DCD and DBD livers in Birmingham, with more than 40% of donors older than 60 years (**Table 1, Supplementary Figure 1A**). All types of donor warm ischemia were significantly longer in Zurich compared to Birmingham with a median functional donor warm ischemia (fDWIT) of 31 min in the HOPE group *vs.* 17 min in the un-treated 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 un-treated DCD livers in Birmingham with 8% vs. 0% and 28% vs. 4% (*p=0.0019*), respectively (**Table 1**). Consistently, the overall donor-recipient risk, 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, Supplementary Figure 1D**), mainly driven by the longer fDWIT and older donor age. Due to the additional 2h HOPE treatment in the perfusion group, the total out of body time appeared longer in HOPE treated DCD livers compared to the other two cohorts (*p=0.0002, p<0.0001*) (**Table 1**). Based on our matching process, all

other parameters, including donor Body-Mass-Index (BMI), cold ischemia time, recipient age, recipient MELD and Balance of risk (BAR) score were similar in all three transplant groups (**Table 1, Supplementary Figure 1B**). Importantly, more transplant candidates with Hepatocellular Carcinoma (HCC) received a DCD liver in Zurich, compared to UK (*p<0.0001*). In contrast, in Birmingham there were generally more candidates with primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC), although this did not reach significance (**Table 1**).

2. Impact of HOPE treatment on hemodynamic recipient stability and early graft function

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

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

Un-treated DCD livers experienced significantly more acute rejections (*p=0.0019*), compared to HOPE treated livers (**Table 2**). Six months after LT, the median alkaline phosphatase (ALP) appeared also significantly lower (*p=0.049*) following HOPE treatment, while bilirubin was similar across the three groups (**Figure 2F&G**). Two PNF occurred in the un-treated DCD liver group vs. none in the HOPE group. Due to the high number of HCC recipients in the HOPE cohort, six recipients experienced recurrence of their tumour disease (6/50=12%; 3 deaths and 3 alive), compared to four in the un-treated DCD liver group (4/50=8%) (**Table 2**). Three and one patients 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 un-treated DCD livers compared to HOPE treated livers (4/50 vs 11/50, *p=0.09*). Overall, seven grafts were lost in the un-treated DCD liver group by ischemic cholangiopathy 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-tumor related causes (arterial thrombosis, sepsis, chronic rejection, cholangiopathy) cumulated to one-third (16/50) of cases in un-treated DCD livers compared to only 8 % (4/50) of cases in the HOPE group (*p=0.005*) **(Table 2).** Accordingly, five–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)*(**Figure 3**).

Of note, only one recipient with PSC developed a non-anastomotic stricture already 58 days after the DCD liver transplantation, which is in contrast to a usually much later recurrence of PSC[16]. All other recipients with a non-anastomotic stricture were not in the PSC or PBC group.

Discussion

This is the first outcome report following implantation of machine perfused human DCD liver grafts with a five-year follow up. We present several clinically relevant findings. First, HOPE-treated DCD liver recipients showed similar reperfusion injury and improved hemodynamic stability after graft implantation compared to 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 ischaemic cholangiopathy, vascular complications and primary non-function. Finally, such protections accumulated to a significantly better five-year graft survival following HOPE treatment.

In Switzerland, stand-off periods were five minutes longer (10 minutes), compared to the UK and The Netherlands (5 minutes), and contributed to extended functional donor warm ischemia time. The policy in Zurich was therefore to apply HOPE in all DCD liver grafts before implantation, together with short cold ischemia (≤ 6hrs) and implantation in low MELD recipients. Consistently, to evaluate the impact of the HOPE perfusion approach, we intended to compare in this study HOPE-treated liver grafts with un-treated DCD livers with similar short cold ischemia times and comparable low MELD recipients. Birmingham has currently an outstanding experience in controlled (Maastricht III) DCD liver transplants in Europe, including 439 DCD liver transplants during the last 10 years, which enabled us to match our patients in terms of short cold ischemia, low MELD score, and comparable recipient age. However, donors in Zurich were generally older than the Birmingham cohort with also significantly longer functional donor warm ischemia (fDWIT) time, 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 with however short term outcome[18,19]. In contrast, we provide here the first five-year outcome analysis with assessment of cumulative complications within one year [9,20]. Based on our experimental data, we suggest that the benefit of HOPE treatment is related to a primary antioxidative mitochondrial effect, with subsequent less reperfusion injury and improved early function[21–23]. HOPE treatment after initial cold storage may therefore protect human DCD liver recipients from severe IC and other fatal complications[20,22,24]. The results are consistent to experiences with hypothermic oxygen persufflation in human liver transplantation and controlled oxygenated rewarming (COR), which showed similar protective effects in livers and other organs[8,25]. Our findings are also underlined by several experimental studies in small and large animal models of liver transplantation[21,22,26], showing a link between early graft inflammation and later cholangiopathy [24,27].

Machine perfusion has become a hot topic nowadays, based on the idea to optimize marginal grafts before implantation, and to provide prediction of organ function. In contrast to applying machine liver perfusion at hypothermic conditions, warm perfusion strategies aim currently to replace as much as possible cold ischemia. Normothermic machine perfusion (NMP) is therefore applied directly after cold flush[7,28–30] or even before procurement, e.g. by normothermic regional perfusion (NRP) or IFOT (ischemia free organ transplantation)[6,31]. Of note, NMP instead of cold storage has been recently reported in a randomized trial of human DBD and DCD livers[32]. The results show lower peak serum aspartate aminotransferase (AST) (488 U/l *vs.* 965 U/I), as well as less early allograft dysfunction [31] (10% vs 29.8%) in the NMP group after transplantation, while graft and patient survival after one year were similar and excellent (≥ 95%) in both groups[32,33]. It appears however unclear, whether normothermic perfusion can be successfully applied also after cold ischemia, and whether NMP prevents from severe cholangiopathy. Recent data from UK rather suggest that endischemic normothermic perfusion of DCD livers fails to protect from irreversible biliary injury[28]. 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[28,34]. In contrast, applying HOPE after warm and cold ischemia, 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 hours of cold oxygenated machine perfusion[35,36]. We believe therefore, that a short term cold oxygenated perfusion is necessary prior to any reperfusion at normothermic conditions[9,19,28,37]. 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[38].

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

Ongoing randomized clinical trials to assess the impact of hypothermic oxygenated perfusion in DBD and DCD liver transplantation currently recruit participants and results are awaited (NCT 01317342, NCT 02584283).

We conclude that outcome of HOPE treated human DCD liver transplants maintained over a period of five years comparable with primary low risk DBD transplants, and also superior to un-treated DCD livers, performed at a highly experienced center. These results suggest strong effectivity of a simple, end-ischemic perfusion approach and may open the field for safe utilization of extended DCD liver grafts.

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Figure legends

Figure 1: HOPE treatment of human DCD liver grafts (4 examples): Four examples of HOPE treated human DCD livers from the Zurich cohort are shown here. Importantly, the hepatic artery remains untouched throughout cold perfusion.

Figure 2: Liver function and Injury after Liver transplantation: Despite significantly better immediate function following HOPE treatment, livers demonstrate a similar liver enzyme release, as untreated DCD livers (A-D). Due to centre-specific recipient management, patients remain in hospital until their special spot at the clinic for rehabilitation becomes available. This lead to a similar hospital stay on both DCD cohorts (F). After 6-month, biliary parameter appear similar, with a higher median ALP in untreated DCD liver recipients compared to HOPE treated DCDs, though this did not reach significance (G&H). Median and interquartile range were used to present continuous variables, comparisons were made using the Mann-Whitney U test.

Figure 3: Five-year graft and patient survival after liver transplantation: Despite the significant higher risk in the DCD cohort from Switzerland, the overall 5-year graft survival appear excellent, ranging between 70 and 80% in all three cohorts (A). HOPE treatment protects the recipient from development of severe complications including the requirement for retransplantation for PNF, IC or HAT and 5-year graft and survival, censored for tumour related death was significantly improved by HOPE. Long-term survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests.

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)	4.8 (4.2-5.4)	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. DCD: Donation after circulatory death; DBD: Donation after brain death; IC: Ischaemic Cholangiopathy; LDLT: Live-donor-liver transplantation; BAR: Balance of risk score; UK: United Kingdom;

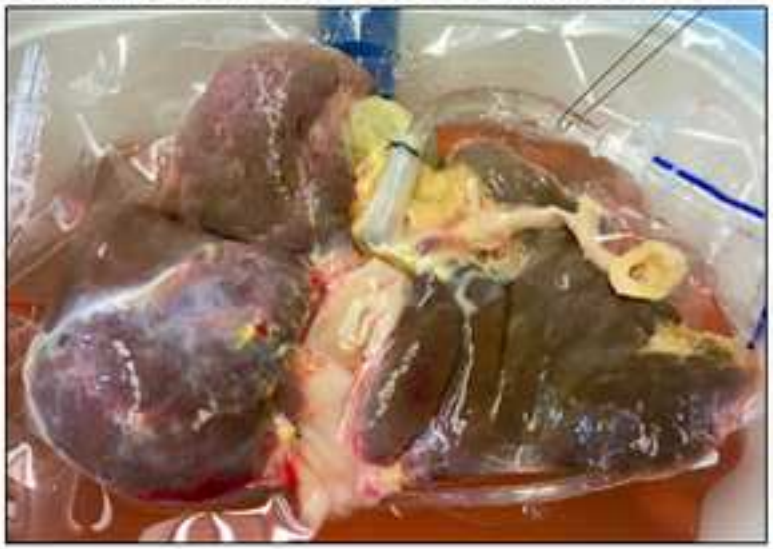
Table 2: Outcome parameters and complications

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 (PNF)	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 (1x conduit, 2x HAS, 1x pseu-doaneurysm, 2x 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 1y (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;

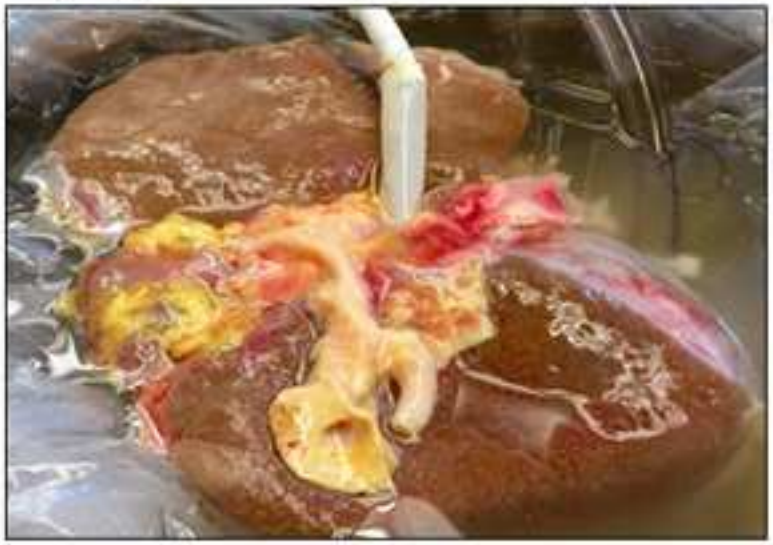
Figure 1: HOPE-treatment of human DCD liver grafts (4 examples)

A 86 years, 38 min fDWIT, UK-DCD-Risk-Score: 12



B 75 years, 43 min fDWIT, UK-DCD-Risk-Score: 8

C 74 years, 33 min fDWIT, UK-DCD-Risk-Score: 12



D 80 years, 32 min fDWIT, UK-DCD-Risk-Score: 11

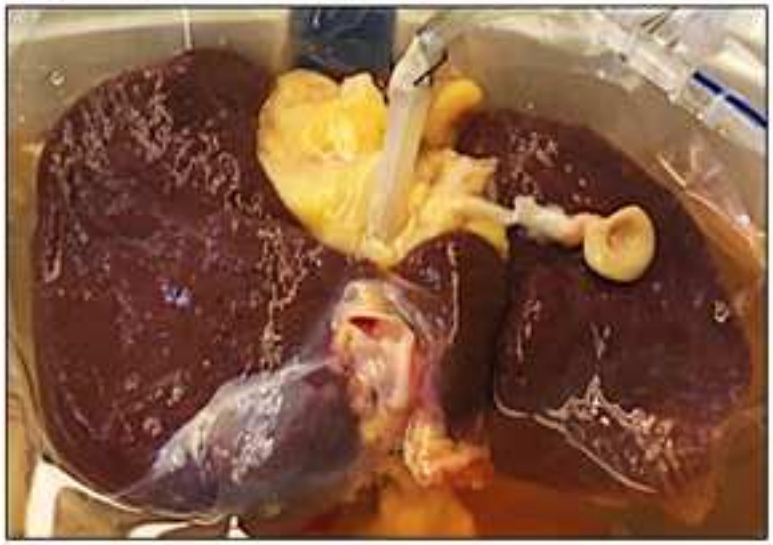


Figure 2

Figure 2: Liver function and Injury after Liver transplantation

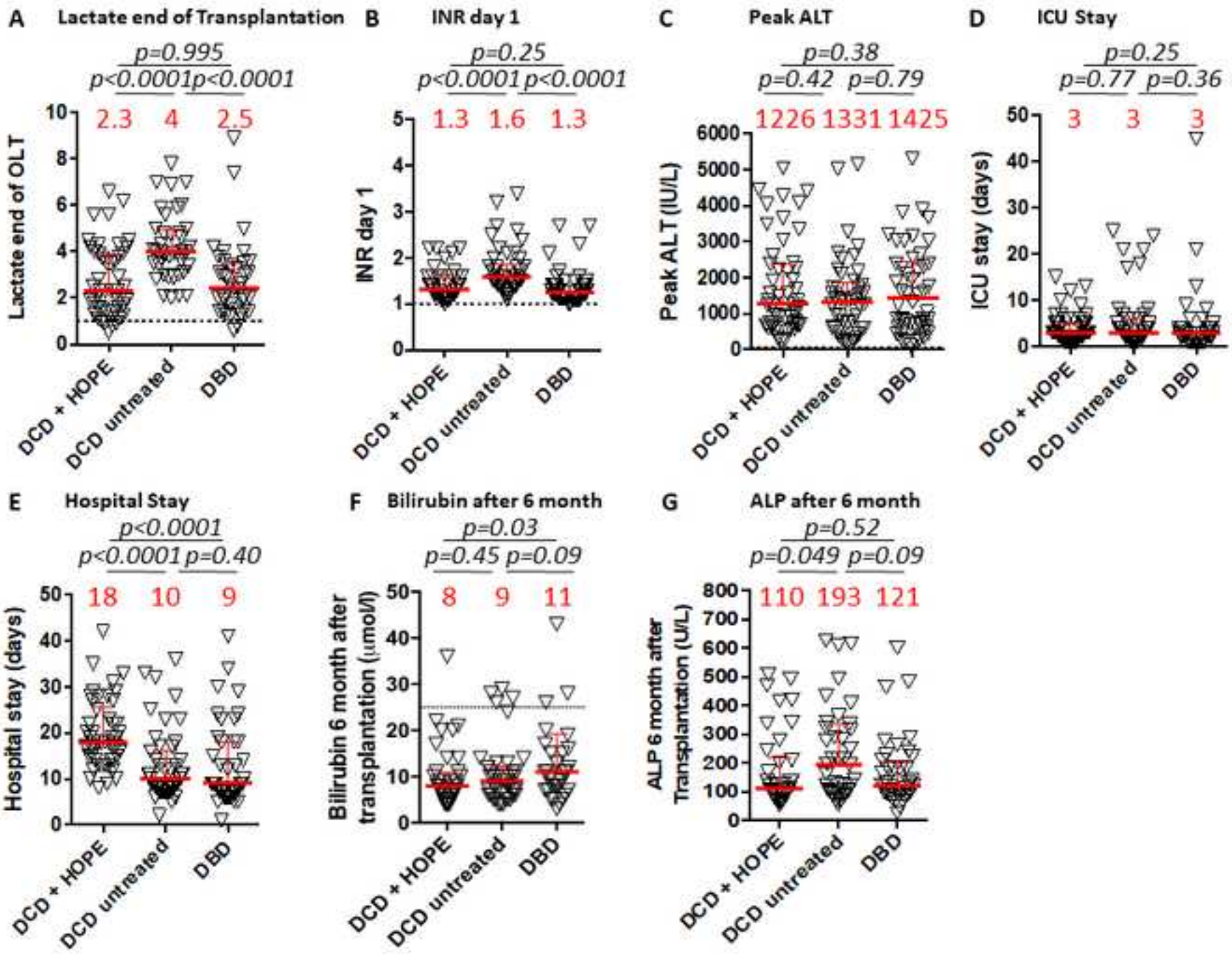
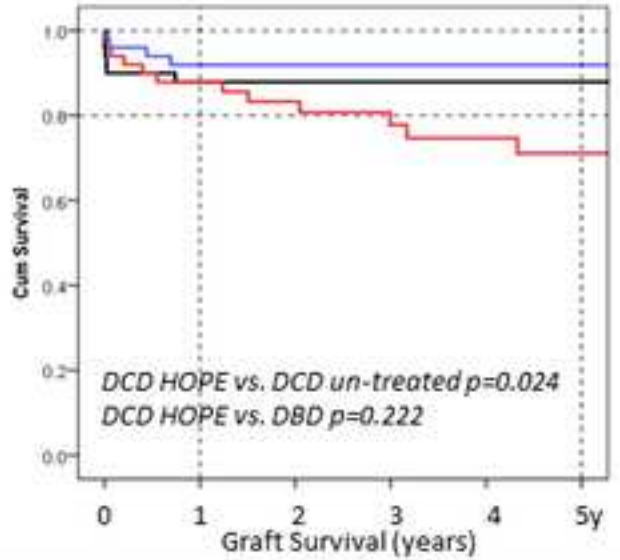


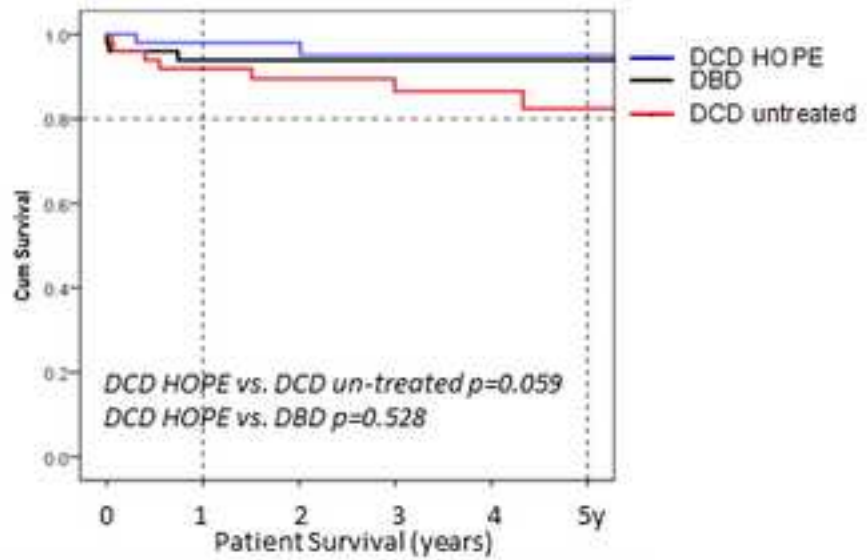
Figure 3: Five-year graft and patient survival after liver transplantation

A **5-year graft survival (censored for tumour related graft loss)**



No. at Risk	DCD untreated	50	41	33	26	22
No. at Risk	DCD HOPE	50	45	31	22	11
No. at Risk	DBD	50	41	36	23	16

B **5-year patient survival (censored for tumour related death)**

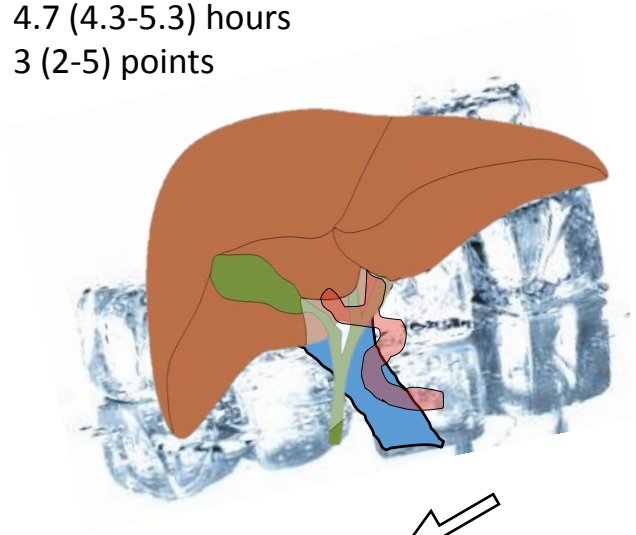


No. at Risk	DCD untreated	50	43	35	29	22
No. at Risk	DCD HOPE	50	49	33	23	12
No. at Risk	DBD	50	44	39	27	18

Five-year experience in human DCD liver transplantation treated by hypothermic oxygenated perfusion (HOPE) before implantation

Cold storage

53 (33-60) years
17 (15-19) minutes
4.7 (4.3-5.3) hours
3 (2-5) points

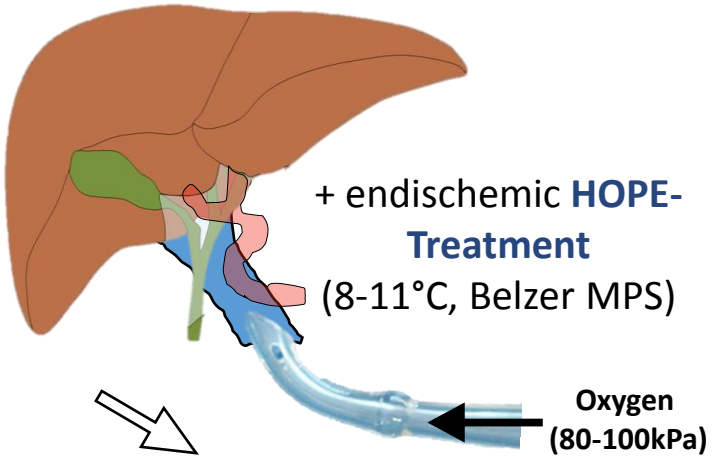


Risk Factors

Donor age
Functional donor warm ischemia
Cold storage
UK-DCD-Risk-Score

HOPE treatment after cold storage

57 (47-67) years
31 (27-36) minutes
4.4 (3.5-5.2) hours
9 (6-11) points



Early Liver Function after transplantation

Lactate end of transplant: 4 (3.2-5)
INR day one: 1.6 (1.4-1.9)

Lactate end of transplant: 2.3 (1.4-3.9)
INR day one: 1.3 (1.2-1.6)

Graft loss (non-tumour related)

32%

Primary non-function: 4%
Hepatic artery thrombosis: 12%
Ischemic cholangiopathy: 10%
Sepsis/chronic rejection: 6%

8%

Primary Non Function: 0%
Hepatic artery thrombosis: 4%
Ischemic cholangiopathy: 0%
Sepsis/chronic rejection: 4%

HOPE treatment protects from graft loss

Highlights:

-We report here, for the first time, on five-year graft survival in DCD livers, treated either by conventional cold storage, or by 1-2 hour hypothermic oxygenated perfusion (HOPE) after cold storage.

-Such end-ischemic HOPE treatment protected recipients from arterial and biliary complications with subsequent significant less graft loss.

-Despite significantly higher risk with a longer donor warm ischemia times, HOPE treatment achieved equivalent outcomes when compared to primary DBD liver transplants.

-HOPE after cold storage is a simple and effective method to treat high-risk DCD livers prior to implantation.