Hemostatic system activation and reperfusion injury in liver machine preservation and transplantation of extended criteria donor livers

Karangwa, Shanice

DOI:
10.33612/diss.161905515


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CHAPTER 2

DCD liver transplantation is not associated with hyper-fibrinolysis and increased blood loss after graft reperfusion

Shanice A. Karangwa, Jelle Adelmeijer, Hans J. Burgerhof,
Ton Lisman, Vincent E. de Meijer, Ruben H. de Kleine,
Koen M.E.M. Reyntjens, Aad P van den Berg,
Robert J. Porte, Marieke T. de Boer

Transplantation 2021 (accepted)

ABSTRACT

Background: The specific effect of donation after circulatory death (DCD) liver grafts on fibrinolysis, blood loss, and transfusion requirements after graft reperfusion is not well known. The aim of this study was to determine whether transplantation of DCD livers is associated with an elevated risk of hyper-fibrinolysis, increased blood loss and higher transfusion requirements upon graft reperfusion, compared to livers donated after brain death (DBD).

Methods: A retrospective single-center analysis of all adult recipients of a primary liver transplantation between 2000 and 2019 was performed. Propensity score matching was used to balance baseline characteristics for DCD and DBD liver recipients. Intra- and post-operative hemostatic variables between DCD and DBD liver recipients were subsequently compared. Additionally, *in-vitro* plasma analyses were performed to compare the intraoperative fibrinolytic state upon reperfusion.

Results: No significant differences in median (interquartile range) post-reperfusion blood loss (1.2 L (0.5 – 2.2) vs. 1.3 L (0.6 – 2.2); p= 0.62), RBC transfusion (2 units (0 – 4) vs. 1.1 units (0 – 3), p= 0.21), or FFP transfusion requirements (0 units (0 - 2.2) vs. 0 units (0 – 0.9); p= 0.11) were seen in DCD compared to DBD recipients, respectively. Furthermore, plasma fibrinolytic potential was similar in both groups.

Conclusion: Transplantation of DCD liver grafts does not result in higher intraoperative blood loss or more transfusion requirements, compared to DBD liver transplantation. In accordance to this, no evidence for increased fibrinolysis upon reperfusion of DCD compared to DBD liver grafts was found.

INTRODUCTION

Despite over 21,000 orthotopic liver transplantations (OLT) performed worldwide in 2018 alone[1], the availability of donor livers for transplantation struggles to meet the ever-growing demand. This results in a high mortality rate on the waiting-list[2]. In an effort to expand the donor organ pool and thus lower waiting-list mortality, extended criteria donor (ECD) livers such as livers donated after circulatory death (DCD) are being increasingly utilized[3,4]. With improvements in donor management and graft preservation, surgical technique and anesthesiological advances, appropriate use of DCD livers, with minimal additional risk factors, results in satisfactory rates of graft and patient survival. However, DCD liver recipients still face an increased risk of postoperative morbidity, mainly as a result of ischemia-reperfusion (I/R) injury[5-7].

Restoration of blood flow and the re-establishment of oxygen supply following a period of ischemia to a donor liver often triggers a profound inflammatory response (reperfusion injury) during OLT[8,9]. One characteristic of I/R injury is the activation of fibrinolysis which is primarily mediated by endothelial-cell activation that is triggered by both direct and indirect I/R cytotoxic mechanisms. Hyper-fibrinolysis occurring after graft reperfusion could result in (severe) hemorrhage and the need for transfusion of substantial amounts of red blood cell (RBC), fresh frozen plasma (FFP) and platelet concentrate[10-15]. Previous studies have shown that high intraoperative blood loss and transfusions of human blood products is frequently associated with a higher incidence of surgical re-intervention[16]. Moreover, it has also been shown that transfusion of a total of ≥ 3 RBC units during liver transplantation is specifically associated with increased post-transplant morbidity[17].

The extent of IR injury in OLT is exacerbated by prolonged ischemia times that occur during donor demise, organ procurement and preservation prior to transplantation. Given the inevitable additional warm ischemia attributable to the agonal and cardiac arrest phases that occur, several studies have suggested that DCD liver transplantation is associated with

higher intra- and post- operative blood loss as well as, increased transfusion rates in comparison to DBD liver transplantation[18,19]. However, this specific effect of DCD livers on intra-operative hemostasis after reperfusion has yet to be investigated in depth. With 50% of all current donor livers available for transplantation derived from DCD donors in the Netherlands, the primary objective of this study was to investigate and assess the specific effect of DCD liver transplantation on intraoperative hemostatic dysfunction.

We hypothesize that as a result of the additional I/R injury DCD livers incur, DCD liver transplantation is indeed associated with exacerbated post-reperfusion hyper-fibrinolysis, leading to higher post-reperfusion blood loss and transfusion requirements compared to DBD liver transplantation.

METHODS

Study design and population

A retrospective analysis of an observational cohort study (www.trialregister.nl – Trial NL6334) of adult (age ≥18 years) patients who underwent a primary OLT between the 1st of January 2000 and the 20th of June 2019, was performed. Split/reduced liver graft transplantations (n= 22), combined organ transplantations (n = 27), domino transplantations (n = 2) and donor livers that underwent machine perfusion prior to transplantation (n= 37) were excluded. To allow for fair and valid comparison between the two groups, this cohort subsequently underwent propensity score matching analysis in order to minimize the differences between donor and recipient characteristics. 1:1 matching generated a final total cohort of 218 patients (n=109 patients per group) for further analysis.

This study was approved by the Medical Ethical Committee of our institute (METc 2014/77) and adhered to the Declaration of Helsinki and the Declaration of Istanbul[20].

Data collection

Donor and recipient characteristics, as well as intraoperative data during the distinct phases of OLT were obtained from a prospectively maintained computer database. When necessary, digital patient files were reviewed for missing information. Missing data per variable ranged between 0-8% in the total cohort, however, there were no missing data in the matched cohort. Variables determined to be relevant predictors of blood loss and transfusion requirements in the post-reperfusion phase were selected for our analyses based on clinical experience and after review of the literature.

Surgical technique and anesthetic management

Surgical techniques, anesthetic management and the blood transfusion policy in our center have been described previously[21,22]. The transfusion policy in our center is characterized by a restrictive use of blood products. RBC transfusions were administered to maintain a hematocrit level between 0.25 and 0.30 and administration of FFP and platelets was never solely dictated by laboratory values. These products were only given in the presence of excessive blood loss, which could not be controlled by standard surgical measures. Moreover, cell-saver blood is typically not used during OLT.

Outcome measures

The primary end-points for this study were post-reperfusion blood loss and RBC transfusion requirements. The secondary end-points were post-reperfusion FFP and platelet transfusions as well as incidence post-OLT hemorrhage occurring within the first 7 days after transplantation.

With respect to intra-operative blood loss and transfusion requirements, the following variables were assessed in the three phases (1. pre-anhepatic, 2. anhepatic (collectively noted as pre-reperfusion) and 3. post-reperfusion) of the transplantation procedure. Blood loss was measured through collection of all blood suctioned from the surgical field during the OLT procedure into measuring containers. All utilized surgical gauzes were wringed and the

blood was added to the above-mentioned containers. The total blood lost during each phase was subsequently recorded. Similarly, the number of units of allogeneic red blood cells (RBC; 1U approximately 250 mL), units of fresh frozen plasma (FFP; 1U approximately 300 mL) and units of thrombocyte/platelet concentrates (1U approximately 150 mL obtained from five donors) were recorded upon administration.

***In vitro* laboratory analysis**

To gain further insight into, and compare the fibrinolytic state of recipients of livers from DCD and DBD donors during the OLT procedure, blood samples routinely collected during 30 consecutive OLT procedures were analyzed.

Plasma samples were retrieved from 14 recipients of a DBD liver and 16 recipients of a DCD liver from which blood samples from all time-points were available. All these liver transplant recipients included in this sub-study analysis belonged to the propensity-score matched cohort.

Sample collection

Arterial blood was collected at 4 different time-points during OLT (i.e. 30 minutes after induction of anesthesia (baseline), 30 minutes after the start of the anhepatic phase, which we define as the moment the recipient native liver is taken out of the patient, 30 minutes after portal reperfusion and at the end of transplantation, after the abdomen was closed) in sodium citrated tubes. All samples were then centrifuged (2700 rpm for 10 min at 18℃) and plasma was collected, snap-frozen, and stored at -80 °C until analysis.

Assessment of fibrinolysis

Clot lysis time (CLT) was measured using a standard procedure in which lysis of a tissue factor–induced clot by exogenous tPA was studied by monitoring changes in turbidity during clot formation and subsequent lysis, as described in detail previously by our group[23]. Concentrations of tissue plasminogen activator (tPA) antigen, plasminogen activator

inhibitor-1 (PAI-1) antigen and plasmin-antiplasmin (PAP) complexes were measured using an IMUBIND® tPA ELISA kit, (Sekisui (USA) via Werfen, Breda, Netherlands), Quantikine Human Serpin E1/PAI-1 ELISA kit (Duoset DY1786 R&D systems, Abingdom, UK) and TECHNOZYM® PAP complex ELISA kit (Technoclone, Vienna, Austria), respectively. All ELISAs were performed according to the manufacturers' instructions. In addition, concentration of D-dimers in the perfusion fluid was measured using an automated latex enhanced immunoassay (D-dimer HS 500, ACL 300 TOP, Instrumentation Laboratory, Breda, The Netherlands).

Statistical analysis

Continuous normally distributed variables are presented as means and standard deviation whereas non-normally distributed variables are presented as medians and interquartile range. Categorical variables are presented as total numbers and percentages. Independent groups were compared using two-sample t-testing or the Mann-Whitney U test, depending on the distributions of the continuous variables. Categorical variables were compared using the Fisher's exact test or Pearson chi-square test. A p-value of <0.05 was considered to indicate statistical significance.

To account for the heterogeneity between the DBD and DCD liver recipient groups and to ensure to a valid comparison, propensity score matching was performed. A multivariate logistic regression model was performed and propensity scores were created. Patient groups were matched for donor age, donor BMI, donor sex, donor ICU admission duration, cause of donor death, grade of steatosis of liver graft, organ preservation fluid, recipient age and sex, recipient BMI, lab- MELD score, most recent serum creatinine and bilirubin, most recent recipient INR, indication for transplantation and status on the waiting list. Patients were matched 1:1 using a nearest-neighbor matching algorithm which attempted to match patients from either group based on the closest propensity score, with a difference of <10% of the standard deviation of the scores. Paired patients were then utilized for comparison analysis

on the degree of intraoperative blood loss and transfusion requirements between DBD and DCD liver transplantation. Unpaired patients were not added to this analysis. All statistical analyses were performed using SPSS version 25 for Windows (SPSS Inc., Chicago, Ill, USA). For PSM, Propensity Score Matching R (R Foundation for Statistical Computing, Vienna Austria, version 3.3.0) SPSS Python Essentials plug-in (IBM Corp., Armonk, NY, USA, version 25) and SPSS plug-in PS Matching in SPSS (Version 3.04) were additionally used.

RESULTS

Donor and recipient characteristics

From the total of 540 adult primary liver transplantations, 121 liver grafts (22%) were obtained from DCD donors (Maastricht category III). Baseline characteristics of this cohort are summarized in Table 1. DCD donors were significantly younger compared to DBD donors (mean ± SD: 45 ± 13 years vs. 50 ± 15 years, $p<0.001$). Recipients of DCD livers were slightly older, had lower lab-MELD scores and were transplanted with high urgency less frequently (Table 1).

After 1:1 propensity score matching (n= 109 per group), no differences were seen in the majority of donor and recipient baseline characteristics between the two groups (Table 2). The only two exceptions were; the type of organ preservation fluid used and serum creatinine. The difference in creatinine levels can be explained by the fact that DBD liver recipients tend to be sicker than recipients of DCD donor livers. Due to standard preservation protocol in the previous era, more DCD livers were mainly preserved with HTK in comparison to DBD livers (40% vs. 20%, $p<0.01$)(Table 2).

Table 1: Total Cohort baseline Donor - Recipient Demographics and Surgical Parameters

Variables	Total (n= 540)	DBD (n=419)	DCD (n=121)	P value
Donor characteristics				
Age (years) *(% missing)*	49 ± 15 *0.3%*	50 ± 15 *0.2%*	45 ± 13 *0.8%*	**<0.001**
BMI (kg/m^2) *(% missing)*	25 ± 3 *0.5%*	25 ± 4 *0.5%*	25 ± 3 *0.8%*	0.17
Sex Male Female *(% missing)*	 293 (54%) 247 (46%) *0%*	 220 (52%) 199 (48%) *0%*	 74 (61%) 47 (39%) *0%*	0.06
Duration of ICU admission (days) *(% missing)*	1 (1-3) *0.9%*	1 (1-3) *0.9%*	2 (1-4) *0.8%*	0.09
Cause of donor death Trauma Cerebrovascular accident Anoxia Other *(% missing)*	 126 (22%) 328 (62%) 19 (4%) 61 (11%) *1%*	 99 (24%) 268 (64%) 12 (3%) 38 (9%) *0.5%*	 28 (23%) 61 (51%) 7 (19%) 23 (6%) *1%*	**<0.01**
Macrovesicular steatosis Non Steatosis <30% 30-60% <60% *(% missing)*	 348 (64%) 128 (25%) 23 (4%) 1 (0.2%) 6.8%	 264 (63%) 101 (24%) 18 (4.8%) 1 (0.2%) 8%	 84 (70%) 27 (22%) 5 (4%) 0 (0%) 4%	0.93
Organ preservation fluid HTK UW IGL-1 *(% missing)*	 143 (27%) 376 (70%) 3 (0.5%) *2.5%*	 96 (23%) 305 (73%) 3 (0.7%) *3.3%*	 47 (39%) 71 (59%) 0 (0%) *2%*	**<0.01**
Recipient characteristics				
Sex Male Female *(% missing)*	 317 (59%) 223 (41%) *0%*	 242 (58%) 177 (42%) *0%*	 75 (62%) 46 (38%) *0%*	0.23
Age (years) (% missing)	50 ± 13 *0%*	49 ± 13 *0%*	53 ± 12 *0%*	**0.01**
BMI (kg/m^2) *(% missing)*	26 ± 5.0 *0.5%*	26 ± 4.5 *0.7%*	26 ± 5.0 *0%*	0.30
MELD score (lab-MELD) *(% missing)*	16 (10 – 23) *0.4%*	16 (11 – 24) *0.5%*	14 (8 – 20) *0%*	**<0.01**
Serum creatinin before OLT (µmol/L)[a] *(% missing)*	89 (72- 137) *0.9%*	90 (74-156) *1.0%*	88 (65- 121) *0.8%*	**0.02**
Serum total bilirubin before OLT (µmol/L)[b] *(% missing)*	52 (24-134) *0.2%*	54 (28-141) *0%*	41 (17-118) *0.8%*	**0.02**
INR before OLT *(% missing)*	1.4 (1.2- 1.8) 0.2%	1.4 (1.2-1.8) 0%	1.3 (1.2- 1.7) 0.8%	0.23
Indication for transplantation				**0.02**

Fulminant hepatic failure	47 (9%)	42 (10%)	5 (4%)	
Non-cholestasic				
Cholestatic	175 (32%)	142 (34%)	33 (28%)	
Metabolic	130 (24%)	101 (24%)	29 (24%)	
Malignant	70 (13%)	54 (13%)	16 (13.2%)	
Other	14 (2.6%)	8 (2%)	6 (5%)	
(% missing)	102 (19%)	70 (16.5%)	32 (26%)	
	0.4%	0.5%	0%	
Status on waiting list				**<0.01**
Elective	488 (90%)	371 (89%)	117 (97%	
High urgency	52 (10%)	48 (11%)	4 (3%)	
(% missing)	*0%*	*0%*	*0%*	
Surgical variables				
CIT of donor liver (hr:min)[c]	7:42 ± 1:54	7:46 ± 2:00	7:27 ± 1:25	**0.05**
(% missing)	*1.5%*	*1.4%*	*1.6%*	
WIT in recipient (min)[d]	43 ± 14	46 ± 14	44 ± 13	**0.02**
(% missing)	*1.7%*	*1.7%*	*1.6%*	

Normally distributed continuous variables are presented as mean ± standard deviation. Non-normally distributed continuous variables and categorical variables are presented as median (interquartile range) and frequency (valid percentage), respectively.

Abbreviations used: DBD: donation after brain death, DCD: donation after circulatory death, HTK: Histidine-tryptophan-ketoglutarate, UW: University of Wisconsin, MELD: model for end-stage liver disease, OLT: orthotopic liver transplantation CIT: cold ischemia time, WIT: warm ischemia time

[a)] Normal < 110 µmol/L, to convert the value for creatinine to mg/dL, divide by 88.4
[b)] Normal 0-17 µmol/L, to convert the value for bilirubin to mg/dL, divide by 17.1
[c)] Time from in situ flushing of the donor organ until the liver is removed from ice for implantation
[d)] Time from removal of liver from ice until reperfusion via portal vein, hepatic artery or both

Table 2: Propensity-Score Matched Cohort Donor - Recipient Demographics and Surgical Parameters

Variables	Total (n= 218)	DBD (n=109)	DCD (n=109)	P value
Donor characteristics				
Age (years) *(% missing)*	46 ± 14.6 *0%*	48 ± 15.6 *0%*	45 ± 13.5 *0%*	0.17
BMI (kg/m^2) *(% missing)*	25 ± 4 *0.5%*	25 ± 5 *0.5%*	25 ± 3 *0%*	0.56
Sex Male Female *(% missing)*	 124 (57%) 94 (43%) *0%*	 57 (52%) 52 (48%) *0%*	 67 (62%) 42 (38%) *0%*	0.11
Duration of ICU admission (days) *(% missing)*	1 (1-3) *0.0%*	1 (1-3) *0.0%*	2 (1-4) *0.0%*	0.32
Cause of donor death Trauma Cerebrovascular accident Anoxia Other *(% missing)*	 53 (24%) 122 (56%) 9 (4%) 33 (15%) *1%*	 26 (24%) 67 (61%) 3 (3%) 13 (12%) *0%*	 27 (25%) 55 (51%) 6 (5%) 20 (19%) *1%*	0.30
Macrovesicular steatosis Non Steatosis <30% 30-60% <60% *(% missing)*	 153 (70%) 56 (26%) 9 (4%) 0 (0%) 0%	 74 (68%) 31 (28%) 4 (4%) 0 (0%) 0%	 79 (73%) 25 (23%) 5 (4%) 0 (0%) 0%	0.68
Organ preservation fluid HTK UW IGL-1 *(% missing)*	 66 (30%) 152 (70%) 0 (0%) *0%*	 22 (20%) 87 (80%) 0 (0.6%) *0%*	 44 (40%) 65 (60%) 0 (0%) *0%*	**<0.01**
Recipient characteristics				
Sex Male Female *(% missing)*	 133 (61%) 85 (39%) *0%*	 67 (62%) 42 (38%) *0%*	 66 (60%) 43 (40%) *0%*	0.50
Age (years) (% missing)	54 (47 -60) *0%*	54 (47-58) *0%*	54 (46-61) *0%*	0.32
BMI (kg/m^2) *(% missing)*	26 ± 4.7 *0%*	26 ± 4.0 *0%*	26 ± 5.0 *0%*	0.64
MELD score (lab-MELD) *(% missing)*	16 ± 9 *0%*	17 ± 9 *0%*	15 ± 10 *0%*	0.16
Serum creatinine before OLT (µmol/L)[a] *(% missing)*	88 (72- 124) *0%*	90 (77-133) *0%*	88 (65- 116) *0%*	**0.03**
Serum total bilirubin before OLT (µmol/L)[b] *(% missing)*	42 (20-100) *0%*	45 (25-106) *0%*	40 (17-84) *0%*	0.17
INR before OLT	1.3 (1.2- 1.7)	1.3 (1.2-1.6)	1.3 (1.2- 1.7)	0.75

(% missing)	0%	0%	0%	
Indication for transplantation Fulminant hepatic failure Non-cholestasic Cholestatic Metabolic Malignant Other (% missing)	 13 (6%) 72 (33%) 47 (22%) 29 (13%) 9 (4%) 48 (22%) 0%	 9 (8%) 43 (39%) 22 (20%) 14 (13%) 2 (3%) 19 (17%) 0%	 4 (4%) 29 (26%) 25 (23%) 15 (14%) 6 (6%) 30 (27%) 0%	0.11
Status on waiting list Elective High urgency *(% missing)*	 203 (93%) 15 (7%) *0%*	 98 (90%) 11 (10%) *0%*	 105 (97%) 4 (3%) *0%*	0.06
Surgical variables				
CIT of donor liver (hr:min)[c] *(% missing)*	7:33 ± 1:52 *0%*	7:37 ± 2:13 *0%*	7:30 ± 1:26 *0%*	0.62
WIT in recipient (min)[d] *(% missing)*	44 ± 13 *0%*	45 ± 12 *0%*	43 ± 14 *0%*	0.18

Normally distributed continuous variables are presented as mean ± standard deviation. Non-normally distributed continuous variables and categorical variables are presented as median (interquartile range) and frequency (valid percentage), respectively.

Abbreviations used: DBD: donation after brain death, DCD: donation after circulatory death, HTK: Histidine-tryptophan-ketoglutarate, UW: University of Wisconsin, MELD: model for end-stage liver disease, OLT: orthotopic liver transplantation CIT: cold ischemia time, WIT: warm ischemia time

[a)] Normal < 110 µmol/L, to convert the value for creatinine to mg/dL, divide by 88.4
[b)] Normal 0-17 µmol/L, to convert the value for bilirubin to mg/dL, divide by 17.1
[c)] Time from in situ flushing of the donor organ until the liver is removed from ice for implantation
[d)] Time from removal of liver from ice until reperfusion via portal vein, hepatic artery or both

Post-reperfusion blood loss and transfusion requirements

In the matched cohort, no significant differences in median post reperfusion blood loss (DCD 1.2 (IQR 0.5 – 2.2) L vs. DBD 1.3 (IQR 0.6 – 2.2) L, p= 0.62) and median RBC transfusions (DCD 2 (IQR 0 – 4) U vs. DBD 1.1 (IQR 0 – 3) units, p= 0.21) were observed between the groups (Figures 1A and B). Similarly, there was no significant difference in post reperfusion FFP transfusion between the two groups and with the exception of a few cases, post-reperfusion platelet transfusions were generally not required for neither DBD nor DCD liver recipients (Table 3).

The incidence of (severe) post-operative hemorrhage within the first 7 days after transplantation in DCD liver recipients was similar to that of DBD liver recipients (Table 3).

Table 3: Propensity-Score Matched Cohort Blood Loss and Transfusion Requirements

Variables	Total (n= 218)	DBD (n=109)	DCD (n=109)	P value
Estimated blood loss pre-reperfusion (L) *(% missing)*	1.4 (0.8 -3.0) 8%	1.3 (0.6 -2.8) 8%	1.4 (0.8 -3.0) 8%	0.30
Estimated blood loss post-reperfusion (L) *(% missing)*	1.2 (0.5- 2.2) 6%	1.3 (0.6 – 2.2) 8%	1.2 (0.5 -2.2) 6%	0.62
Estimated total blood loss (L) *(% missing)*	2.9 (1.7 -5.5) 4%	2.5 (1.6 -5.0) 2%	3.1 (1.9 -5.7) 4%	0.34
RBC transfusion pre-reperfusion (U) *(% missing)*	1 (0 -4.0) 4%	0 (0 -4) 3%	1 (0 -4.4) 4%	0.45
RBC transfusion post-reperfusion (U) *(% missing)*	1.1 (0- 3.3) 3%	1.1 (0 -3) 3%	2 (0 -4) 3%	0.21
Total RBC transfusion (U) *(% missing)*	3 (0 -7) 2%	3 (0 -6) 1%	3.3 (0 -7.8) 2%	0.41
FFP transfusion phase pre-reperfusion (U) *(% missing)*	0 (0 – 1.5) 4%	0 (0 -1.5) 3%	0 (0 – 1.6) 6%	0.96
FFP transfusion post-reperfusion (U) *(% missing)*	0 (0 -1.5) 2%	0 (0 -0.9) 2%	0 (0 – 2.2) 4%	0.11
Total FFP transfusion (U) *(% missing)*	0 (0 -3.9) 3%	0 (0 -3) 2%	0 (0- 4) 3%	0.51
Platelet transfusion pre-reperfusion (U) *(% missing)*	0 (0- 0) 4%	0 (0- 0) 2%	0 (0- 0) 6%	0.91
Platelet transfusion post-reperfusion (U) *(% missing)*	0 (0- 0) 4%	0 (0- 0) 2%	0 (0- 0) 4%	0.10
Total platelet transfusion (U) *(% missing)*	0 (0- 0) 4%	0 (0- 0) 2%	0 (0- 0) 4%	0.39
Post-OLT bleeding complications				
7 day post-OLT haemorrhage None (Severe) bleeding requiring laparotomy (Severe) bleeding not requiring laparotomy *(% missing)*	 189 (86%) 17 (8%) 12 (6%) 0%	 97 (88%) 8 (8%) 4 (4%) 0%	 92 (85%) 9 (8%) 8 (7%) 0%	0.26

Continuous variables are presented as median (interquartile range) and categorical variables as frequency (valid percentage).

DBD: donation after brain death, DCD: donation after circulatory death, FFP: fresh frozen plasma, OLT: orthotopic liver transplantation

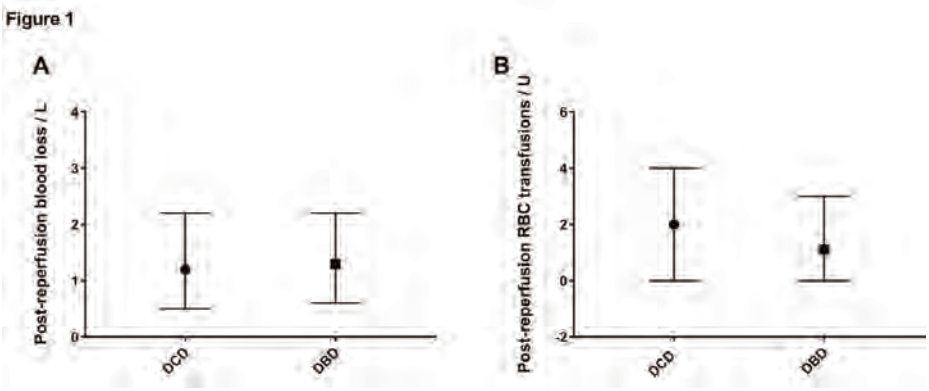

Figure 1: Scatter plots showing post-reperfusion blood loss and RBC transfusion in DCD liver recipients compared to DBD liver recipients
Panel A: Post-reperfusion blood loss (L); Panel B: Number of RBC units transfused post reperfusion. Graphs represent median values (error bars represent IQR)

In vitro laboratory assessment of fibrinolysis

In a subset of patients belonging to the matched cohort, we further investigated the intraoperative fibrinolytic profiles of patients undergoing OLT. Shortest CLTs were observed 30 minutes after reperfusion; median 49 (IQR 32 – 53) minutes and median 52 (IQR 35 - 95) minutes in recipients from DCD livers (n= 16) and DBD livers (n=14), respectively (p=0.13) (Figure 2A). Nonetheless, CLT at all points during transplantation did not significantly differ between the two groups. Post-reperfusion PAP complex, tPA and PAI-1 antigen levels were lower in DCD liver recipients, compared to DBD liver recipients. However, these differences were not statistically significant (Figures 2 B,C and D).

D-dimers levels increased following graft reperfusion in both groups; with significantly higher levels in DCD liver recipients. However, this difference was due to initially higher D-dimer levels observed at baseline. Baseline D-dimer levels in DCD liver recipients at the start of OLT were nearly three-fold higher than in the DBD liver recipients (median (IQR): 4399 (1477 – 13,248) vs. 1653 (691 – 2016) ng/ml; p= 0.03) (*Figure 2E*). To correct for these baseline differences such that the sole effect of reperfusion could be investigated, we calculated increases in D-dimer levels at 30 minutes post reperfusion and at the end of OLT

compared to baseline. Both groups exhibited slight increases in D-dimer levels post-reperfusion compared to pre-reperfusion levels and a negligible increase in levels at the end of OLT compared to pre-reperfusion, with the DBD liver recipients having slightly greater increments. These differences, however, were not statistically significant (p=0.26)(*Figure 2F*).

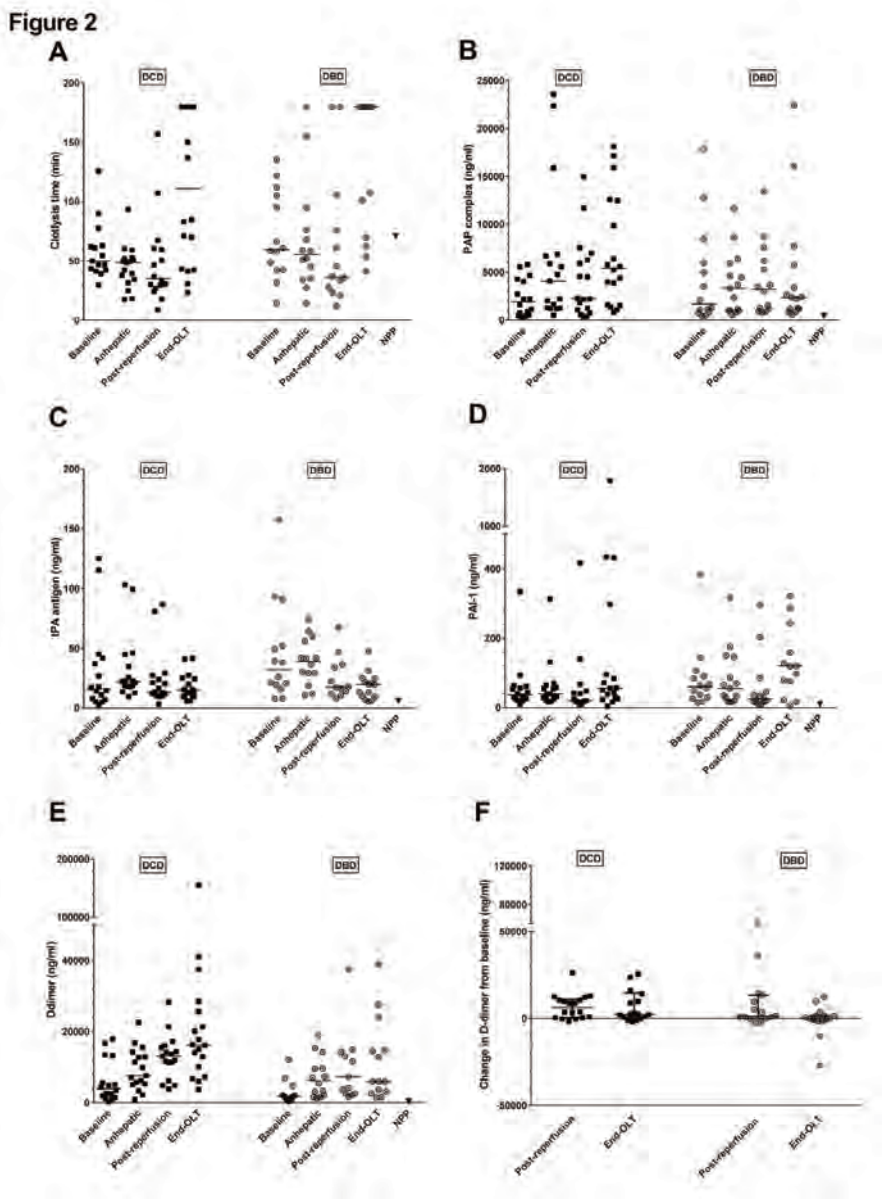

Figure 2: Comparison in the changes of levels of fibrinolysis markers in plasma between DCD and DBD liver recipients during transplantation.
Panel A: Clot lysis time (min); Panel B: Plasmin- anti-plasmin (PAP) complex (ng/ml); Panel C: Tissue plasminogen activator (tPA) antigen (ng/ml); Panel D: Plasminogen activator inhibitor-1 (PAI-1) antigen (ng/ml); Panel E: D-dimer (ng/ml); Panel F: Change in D-dimer level from baseline (ng/ml)
For all markers, samples of normal pooled plasma were used as a reference to normal physiological levels in vivo. NPP: Normal pooled plasma

DISCUSSION

Our findings demonstrate that DCD liver transplantation is not associated with greater post-reperfusion blood loss and transfusion requirements when compared to transplantation with DBD liver grafts. Moreover, recipients of DCD livers do not exhibit a hyper-fibrinolytic profile after reperfusion nor do they face an increased risk of (severe) post-operative hemorrhage.

A generally accepted consensus on the specific effect of DCD liver transplantation on blood loss, transfusion requirements and the incidence of the development of post-operative bleeding complications remains to be reached. In reference to recently published literature, our results dispute the findings and conclusions of a study by the London group in which DCD livers were more likely to develop aggressive fibrinolysis upon reperfusion evidenced by significantly higher fibrinolytic markers on TEG upon reperfusion. This accordingly resulted in higher blood loss and increased transfusion rates as compared to DBD livers[24]. Similarly, a recent study performed in North America report profound hyper-fibrinolysis, higher post-reperfusion blood loss, higher transfusion requirements and a higher incidence of post-reperfusion hemodynamic instability in DCD liver transplantation as compared to DBD liver transplantation[25]. Contrastingly, a single-center retrospective study by the group in Rotterdam describes similar transfusion requirements and a comparable incidence of the development of post-operative vascular complications in DCD and DBD liver recipients[26]. The contradictory findings of these studies together, highlight the difficulty in verifying the specific effect of DCD transplantation on intra-, and post-operative hemostasis in liver transplantation. We believe that these differences may potentially be attributable to factors such as the variation in selection criteria of donor organs amongst different transplant centers and the variation in cold and warm ischemia times of the grafts prior to implantation. Moreover, the administration of ante mortem heparin administration in the donor, the difference in preservation fluids used during cold storage of the donor liver or the administration of tissue plasminogen activator and/or other fibrinolytic agents into the liver allograft during implantation may influence intraoperative hemostasis.

In order to gain more insight from our results, we went further to investigate whether our clinical findings matched what occurred at biochemical level in plasma collected during the transplant procedures. We were able to conclude that DCD liver recipients do not exhibit significantly increased (hyper-) fibrinolytic profiles after reperfusion in comparison to DBD livers. This was evidenced by similar clot lysis times, absence of a significant release of D-dimer, PAP complexes and plasma tPA antigen levels upon reperfusion in DCD liver recipients as compared to DBD liver recipients.

This study is, to our knowledge, the first to primarily assess and compare bleeding risk and development of post-operative hemorrhage whilst simultaneously incorporating analysis of hyper-fibrinolysis at biochemical level in DBD and DCD liver recipients. Our findings convincingly demonstrate that DCD liver transplantation is not a particular risk factor for post-reperfusion hyper-fibrinolysis and consequently provides no increased risk of high intraoperative blood loss or greater transfusion requirements, compared to DBD liver transplantation. These findings may be due to the on-going universal practice to ensure the minimization of procurement and implantation times in order to limit ischemia and thus reduce the risk of I/R injury. Moreover, careful selection of suitable (ECD) donor organs (i.e. limitation of the use of heavily steatotic livers or livers from uncontrolled DCD donors with long warm ischemic periods), as well as, the careful selection of recipients that are capable of tolerating the particular physiological insult of end-ischemic reperfusion of a DCD organ, is key. In order to ensure favorable outcomes after DCD liver transplantation at our center, DCD liver transplantations are typically performed in relatively younger patients undergoing a primary OLT.

We acknowledge that this study bears some limitations. The retrospective nature of the study suggests that definite conclusions from our results cannot be drawn with absolute certainty. Additionally, given the lack of collected data on post-reperfusion syndrome, we were not able to investigate this phenomenon and compare the two groups. However, this study was still capable of achieving its aim of which intraoperative blood loss and transfusion requirements were the principle focus. Despite a relatively large cohort, these results are

based on data collected at a single center. Therefore, the sample size and heterogeneity of the study population is limited.

Future studies prospectively assessing intraoperative hemostatic data collected from multiple centers, perhaps also involving data from numerous countries, are necessary to investigate this further to ensure that a reliable, widely extrapolated consensus can be reached. Nevertheless, this study shows that the use of (controlled) DCD livers poses no increased risk of post-reperfusion bleeding, increased transfusion requirements and development of severe post-operative hemorrhage in primary OLT. These findings are encouraging as they emphasize the safety of the utilization of DCD livers which are beneficial in boosting the pool of donor organs to help tackle the high demand.

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