Onset of donor warm ischemia time in DCD liver transplantation: hypotension or hypoxia?

Marit Kalisvaart
Jubi de Haan
Wojciech Polak
Diederik Gommers
Herold Metselaar
Jan IJzermans
Jeroen de Jonge

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SUMMARY

The aim of this study was to investigate the impact of hypoxia and hypotension during agonal phase of DWIT on hepatic IRI and complications in DCD liver transplantation. A retrospective single-centre study of 93 DCD liver transplants (Maastricht type III) was performed. DWIT was divided into two periods: agonal phase (from WoT until circulatory arrest) and asystolic phase (circulatory arrest until cold perfusion). During agonal phase, a drop to <80% in SpO2 was considered as the start of hypoxia (SpO2-Agonal) and a drop to <50 mmHg as the start of hypotension (SBP-Agonal). Peak postoperative AST level >3000 U/L was considered as severe hepatic IRI. SpO2 dropped within 2 minutes after WoT <80%, while the SBP dropped to <50 mmHg after 9 minutes, resulting in a longer SpO2-Agonal (13 minutes) than SBP-Agonal (6 minutes). In multiple logistic regression analysis, only duration of SpO2-Agonal was associated with severe hepatic IRI (p=0.006) and not SBP-Agonal (p=0.32). Also, recipients with long SpO2-Agonal (>13 minutes) had more complications with a higher CCI during hospital admission (43.0 vs 32.0; p=0.002) and 90-day graft loss (26% vs. 6%; p=0.01), compared to recipients with a short SpO2-Agonal (≤13 minutes). Furthermore, Cox proportional hazard modelling identified a long SpO2-Agonal as a risk factor for long-term graft loss (HR 3.30; 95% CI 1.15-9.48; p=0.03). In conclusion, the onset of hypoxia during agonal phase is related to severity of hepatic IRI and postoperative complications. Therefore, SpO2 <80% should be considered as the start of functional DWIT in DCD liver transplantation.

INTRODUCTION

DCD grafts are increasingly being used in liver transplantation and in some European countries more than one third of the deceased donor liver transplants are performed with DCD grafts (1–3). Nonetheless, the use of these marginal grafts is associated with various biliary and renal complications, resulting in inferior survival rates, compared to DBD grafts (4–11).

The inferior results are likely the consequence of the obligatory DWIT. This extra period of warm ischemia in DCD grafts is thought to aggravate hepatic IRI (12,13). In previous studies duration of DWIT has been linked to biliary complications and impaired graft survival (9–13). In general, DWIT consists of two periods (**Figure 3 – Chapter 2**): the agonal phase (from WoT until circulatory arrest) and asystolic phase (from circulatory arrest until cold perfusion). However, multiple definitions for DWIT are currently being used (14). The trajectory of agonal phase differs widely between donors and the exact moment of onset of hepatic tissue injury is still unknown. Furthermore, the lack of an uniform definition for the onset of DWIT compromises evaluation of its impact on recipient outcomes. Earlier studies, primarily focusing on the blood pressure during agonal phase, yielded varying results on its relation to postoperative complications (15–18). Abt et al showed that a slow decline in SBP during agonal phase is correlated with long term graft loss. Therefore, SBP is frequently used to mark the beginning of functional DWIT. However, from our clinical experience we have noticed that the peripheral oxygen saturation (SpO2) drops much quicker than SBP during the agonal phase. We therefore hypothesize that ischemic hepatic injury is more closely related to the onset of hypoxia, rather than hypotension during the agonal phase.

The aim of this study is first, to explore the relation between vital parameters during agonal phase and hepatic IRI and observe potential cut-off points for the start of functional DWIT. Secondly, we will explore the relation between the agonal phase and recipient outcome in DCD liver transplantation.

METHODS

This retrospective study was performed with approval of the Erasmus University Medical Centre Institutional Review Board (registration number: MEC-2014-670). All consecutive adult patients who underwent DCD liver transplantation in our centre

from July 2008 until March 2016 were included. All DCD grafts were retrieved from Maastricht type III donors. Exclusion criteria were retransplantation and liver transplantation for acute liver failure. None of the grafts were machine perfused. In the Netherlands, DCD donors older than 60 years of age or with a BMI >28 are regularly not considered for liver donation. Also, we allocate DCD livers preferably to recipients with HCC and/or a relatively low biological MELD-score of <20. The DRI was calculated to express graft quality (19). The amount of graft steatosis was assessed in biopsies taken after reperfusion. Data following duration and structure of different phases of DWIT were retrieved from the Eurotransplant database. An overview of DWIT is displayed in Figure 1A. The cut-off time points during agonal phase were considered at <80% for SpO2 (SpO2-Agonal) and <50 mmHg for SBP (SBP-Agonal). DCD grafts used in this study come from donors in the Netherlands only and are offered through the Eurotransplant waitlist, based on MELD status. In the Netherlands, WoT of the donor is on the ICU. After circulatory arrest, there is an obligatory 5 minutes waiting time, where after the donor is declared deceased and transported to the operation theatre (5-10 minutes) for organ procurement, using the super-rapid retrieval technique (20). The first flush (6-8 L) is performed with UW (University of Wisconsin) preservation fluid under a pressure of 200 mmHg via the aorta. The portal vein, hepatic artery and biliary tract are additionally flushed at the back-table with UW as well. Standard surgical technique included piggyback cavo-caval anastomosis with only incidental use of a portocaval shunt and the graft was routinely reperfused via portal vein. Duration of the surgical procedure, cold ischemia time, and recipient WIT, intraoperative blood loss with subsequent transfusion of red blood cell concentrates (RBC), and fresh frozen plasma (FFP) and end of surgery requirement of vasopressors were all recorded. The postreperfusion syndrome (PRS) was defined as a >30% drop in mean arterial pressure (MAP) in the 5 minutes after reperfusion, lasting at least 1 minute (21). The peak AST level in the first 72 postoperative hours was used as a marker for severity of hepatic IRI, a known marker for early allograft dysfunction (EAD) as well (22). The standard immunosuppression regime in our centre is based on the triple therapy strategy: prednisolone and MMF from day 0, basiliximab at day 0 and day 4 and introduction of tacrolimus on postoperative day 5. Duration of hospital and ICU admission, recipient and graft survival were all documented to assess postoperative morbidity and mortality. All major postoperative complications in the first year were documented. Major postoperative complications were defined as grade ≥ 3b by the Clavien-Dindo classification and the CCI was calculated at hospital discharge, 6 months and 12 months after transplantation (23,24).

Statistical analysis

Data were analysed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, NY, USA). The student's t test was used to compare normally distributed continuous variables. Nonparametric continuous variables were compared using the Mann-Whitney U test. To compare categorical variables the Chi-square test or Fisher's exact test were used. P-values <0.05 were considered statistically significant. Continuous variables were expressed as mean with standard deviation (SD) or median and inter-quartile range (IQR), where appropriate. A multiple logistic regression analysis was used to identify donor, DWIT and surgical risk factors associated with severe hepatic IRI (peak serum AST >3000 U/L). Two years graft and patient survival rates were estimated using Kaplan-Meier methods. A Cox proportional hazard model was used to identify donor, surgical and recipient factors associated with graft loss within the first two years after transplantation.

RESULTS

During the study period, 105 patients underwent DCD liver transplantation of whom 93 were included. Two cases were excluded because of re-transplantation and one recipient was transplanted for acute liver failure. In eight cases the duration of DWIT and donor's hemodynamic profile data was missing. In one case intraoperative data was not complete.

Baseline characteristics

Baseline characteristics are displayed in **Table 1**. Median recipient age was 57 years and 70% of the recipients were male. Viral hepatitis was the most common diagnosis of liver disease (27%), followed by alcohol-related cirrhosis (22%), and biliary cirrhosis (17%). Mean preoperative biological MELD score was 15. Median donor age was 47 years and 58% of the donors were male. The mean DRI was 2.6. Mean duration of total DWIT and asystolic phase were respectively 32 minutes and 16 minutes. Average duration of the total agonal phase was also 16 minutes. Of note, a variety in dispersion of the DWIT-phases was observed (**Table 1**): The SD and range in agonal phase were longer than in asystolic phase. SpO2 dropped already 2 minutes after WoT below 80%, while the SBP dropped below 50 mmHg after 9 minutes (**Figure 1**), resulting in a longer SpO2-Agonal (13 minutes) than SBP-Agonal (6 minutes). Also, the dispersion of SpO2-Agonal was greater than SBP-Agonal, with larger SD and range. The mean time between the drop in SpO2 and SBP was 7 minutes. The mean duration of cold ischemia time and RWIT was 6.6 hours and 29 minutes, respectively.

Table 1 - baseline donor, recipient and surgical characteristics in DCD liver transplantation

Donor	Total (n=93)	Donor warm ischemia (minutes)	Total (n=93)
Donor risk index	2.6 (0.46)	Total donor warm ischemia time	32 (32; 12-60)
Age (years)	47 (39-53)	Asystolic phase	16 (15; 7-28)
Male gender (%)	54 (58)	Agonal phase	
Body mass index	24.2 (22.0-26.0)	Total agonal phase	16 (15; 4-39)
serum AST (U/L)	46 (29-89)	SpO2-Agonal	13 (12; 2-38)
Graft steatosis		SBP-Agonal	6 (5; 1-17)
None	53 (57%)	Between SpO2 & SBP-Agonal	7 (6; 0-24)
1 - 10%	33 (36%)	**Transplant procedure**	
11 - 33%	4 (4%)	Operation time (hours)	7.9 (7.7; 6-12)
>33%	3 (3%)	Cold ischemia time (hours)	6.6 (6.4; 4-12)
Recipient		Recipient warm ischemia time (min)	29 (28; 17-61)
Age (years)	57 (49-63)	Blood loss (liters)	4.1 (3.2-6.2)
Male gender (%)	65 (70)	RBC transfusion (units)	3 (1-5)
Body mass index	26.5 (4.3)	FFP transfusion (units)	3 (0-6)
Etiology of liver disease (%)		Postreperfusion syndrome (%)	37 (40)
Biliary cirrhosis	16 (17)	Dose norepinephrine end of surgery (ug/kg/min)	0.30 (0.12-0.50)
Viral hepatitis	25 (27)	**Postoperative**	
Postalcoholic cirrhosis	20 (22)	EAD	56 (60%)
Other	32 (34)	Postoperative peak serum AST (U/L)	2287 (1305-4881)
Hepatocellular carcinoma (%)	31 (33)	Peak serum AST in categories	
LabMELD score	15 (7.8)	0 - 3000 U/L	53 (57)
Sodium (mmol/L)	138 (134-140)	>3000 U/L	40 (43)
Medical history (%)		AST = aspartate aminotransferase; EAD, early allograft dysfunction; FFP, fresh frozen plasma; MELD, model for end stage liver disease; RBC, red blood cells; SBP, systolic blood pressure. Continuous variables are displayed as mean (standard deviation) and median (IQR) where appropriate. Values of DWIT and intraoperative periods are displayed as follows: mean (median; range).	
Hypertension	19 (20)		
Coronary artery disease	2 (2)		
Diabetes mellitus	29 (31)		

Duration of ischemia periods and hepatic ischemia/reperfusion injury

More than half of the recipients developed EAD (60%) according to the Olthoff criteria (22), due to the relatively high peak AST levels (median 2287 U/L) in the first days after DCD liver transplantation. Therefore, this parameter was not considered suitable to quantify hepatic IRI. Instead, we used a cut off of 3000 U/L in peak AST in the first 72h after transplantation. The peak AST was >3000 U/L in 43% of the recipients. Univariable analysis of risk factors associated with severe hepatic IRI (**Table 2A**) showed that duration of total agonal phase was significantly associated to a peak AST >3000 U/L (OR 1.08; 95%CI 1.01-1.14; p=0.01), but the correlation for SpO2-Agonal was even stronger (OR 1.11; 95%CI 1.01-1.20; p=0.004). No relation was observed for SBP-Agonal and severe hepatic IRI (p=0.41). Multivariable analysis of the same

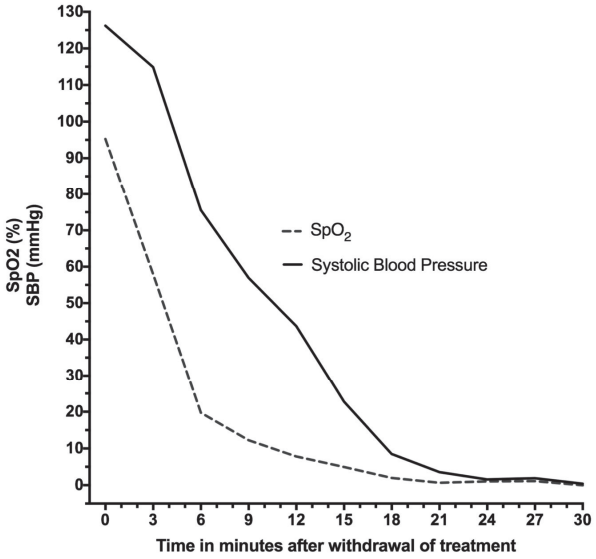

Figure 1 – The course of SpO2-Agonal and SBP-Agonal after withdrawal of treatment of the liver organ donors.

risk factors was used to correct for potential confounders in relation to severe hepatic IRI. Two multivariable analyses were performed, both with all factors of the univariable analysis and either with the SpO2-Agonal (**Table 2B**) or SBP-Agonal (**Table 2C**) period. In table 2B agonal phase was split into the time before and after the drop <80% in SpO2 and in table 2C agonal phase was equally split using the drop in SBP <50 mmHg. **Table 2B** shows that only the SpO2-Agonal is related to severe hepatic IRI (p=0.006) and not the period before the drop in SpO2 (p=0.26). On the contrary, Table 2C shows that the duration of the SBP-Agonal period was not an independent factor (p=0.32), while the period between the drop in SpO2 and the drop in SBP was significantly related to severe hepatic IRI (hypoxic agonal phase) (p=0.003).

Duration of SpO2-Agonal and clinically relevant outcome parameters

Early postoperative complications

Table 3 displays the recipient outcome for duration of SpO2-Agonal. After visualization of LOESS plots, the SpO2-Agonal was divided into two groups at the mean of 13 minutes. Recipients with a long SpO2-Agonal had significantly more postoperative complications and a longer length of stay, for both ICU and hospital admission. The CCI was measured at hospital discharge, six months and one year after transplantation and at each moment the CCI was significantly higher in recipient with a long

Table 2 - Univariable and multivariable analysis of risk factors associated with severe hepatic ischemia/reperfusion injury in DCD liver transplantation

2A. Univariable analysis				2B. Multivariable analysis SpO2-Agonal				2C. Multivariable analysis SBP-Agonal			
Donor	**OR**	**95% CI**	**P-value**	***Donor***	**OR**	**95% CI**	**P-value**	***Donor***	**OR**	**95% CI**	**P-value**
Age (years)	1.03	1.00 - 1.06	0.086	Age (years)	1.05	0.98 - 1.12	0.10	Age (years)	1.05	0.98 - 1.12	0.20
Female gender	0.67	0.29 - 1.53	0.34	Female gender	0.43	0.13 - 1.43	0.10	Female gender	0.51	0.15 - 1.74	0.29
Body mass index	1.06	0.95 - 1.18	0.30	Body mass index	0.87	0.72 - 1.06	0.19	Body mass index	0.88	0.73 - 1.06	0.19
Donor risk index	0.75	0.30 - 1.85	0.53	Donor risk index	0.51	0.08 - 3.42	0.37	Donor risk index	0.48	0.07 - 3.43	0.46
Graft steatosis				*Graft steatosis*				*Graft steatosis*			
No steatosis	1.00			No steatosis	1.00			No steatosis	1.00		
Steatosis 1-10%	2.54	1.04 - 6.22	0.04	Steatosis 1-10%	2.57	0.83 - 7.92	0.09	Steatosis 1-10%	2.43	0.78 - 7.63	0.13
Steatosis >10%	5.29	0.93 - 30.11	0.06	Steatosis >10%	9.17	1.32 - 63.94	0.02	Steatosis >10%	8.80	1.26 - 61.32	0.03
DWIT				***DWIT***				***DWIT***			
Total agonal phase	1.08	1.01 - 1.14	0.01	*Agonal phase*				*Agonal phase*			
SpO2-Agonal	1.11	1.04 - 1.20	0.004	Before SpO2-Agonal	0.88	0.70 - 1.10	0.26	Before SpO2-Agonal	0.89	0.71 - 1.11	0.30
SBP-Agonal	1.04	0.94 - 1.15	0.41	SpO2-Agonal	1.14	1.04 - 1.26	0.006	SBP-Agonal	1.07	0.94 - 1.23	0.32
.	.	.	.	.	.	.	.	Between SpO2 & SBP-Agonal	1.19	1.06 - 1.34	0.003
Asystolic phase	1.05	0.96 - 1.15	0.32	Asystolic phase	1.08	0.99 - 1.13	0.37	Asystolic phase	1.11	0.97 - 1.28	0.13
Intra-operative				***Intra-operative***				***Intra-operative***			
Cold ischemia time	0.97	0.69 - 1.35	0.84	Cold ischemia time	1.09	0.73 - 1.70	0.63	Cold ischemia time	1.09	0.71 - 1.69	0.69
RWIT	1.05	1.00 - 1.11	0.07	RWIT	1.06	0.99 - 1.13	0.07	RWIT	1.06	0.99 - 1.14	0.10
RBC transfusion	0.97	0.87 - 1.10	0.65	RBC transfusion	0.93	0.84 - 1.13	0.75	RBC transfusion	0.91	0.77 - 1.08	0.27

Figure 2A: Univariable analysis of factors associated with severe hepatic ischemia/reperfusion injury (peak postoperative AST >3000 U/L).
Figure 2B: Multivariable analysis with agonal phases splitted at the cut-off of saturation drop below 80%.
Figure 2C: Multivariable analysis with agonal phases splitted at the cut-off of systolic blood pressure below 50 mm Hg.
CI, confidence interval; DWIT, donor warm ischemia time; OR, odds ratio; RBC, red blood cells; RWIT, recipient warm ischemia time.

SpO2-Agonal. Also, recipients in the long SpO2-Agonal group experienced more 90-day graft loss (26% versus 6%; p=0.01). Three recipients had postoperative PNF of the graft requiring retransplantation and all of them had a long SpO2-Agonal. Surprisingly, no correlation was observed between duration of SpO2-Agonal and development of biliary complications in the first year after transplantation.

Long term graft loss

The median follow up was 2.2 years. The estimated 2-year graft and patient survival was 79% and 86%, respectively. **Figure 2** shows the 2-year graft survival for recipients with a short and long SpO2-Agonal. Recipients with a long SpO2-Agonal had the worst survival rate (70% versus 87%; p=0.03). No difference was observed in

Table 3 - Duration of SpO2-Agonal and recipient outcomes after DCD liver transplantation.

Length of SpO2-Agonal	<13 minutes (n=47)	≥13 minutes (n=46)	Total (n=93)	p-value
In-hospital				
≥ 1 Major postoperative complication*	10 (21%)	25 (54%)	35 (385)	0.001
Length of ICU admission (days)	2 (2-3)	3 (2-6)	2 (2-4)	0.02
Length of hospital admission (days)	16 (14-22)	22 (17-29)	18 (15-26)	0.009
Comprehensive Complication Index (median)				
In-hospital	32.0	43.0	38.1	0.002
Six months	46.0	58.4	48.2	0.006
One year	48.0	62.8	59.7	0.008
90-day graft loss	3 (6%)	12 (26%)	15 (16%)	0.01
Retransplantation - HAT	2 (4%)	3 (7%)	5 (5%)	
Retransplantation - PNF	0	3 (7%)	3 (3%)	
Retransplantation - ITBL	0	1 (2%)	1 (1%)	
Death	1 (2%)	5 (11%)	6 (6%)	
Biliary complications in first year				
Overall biliary complications**	17 (36%)	18 (39%)	35 (38%)	0.77
Anastomotic strictures***	12 (26%)	12 (26%)	24 (26%)	0.95
ITBL	6 (13%)	5 (11%)	11 (11%)	0.78
Retransplantation for ITBL	1 (2%)	2 (4%)	3 (3%)	0.62

*Major postoperative complication was defined as a Clavien Dindo classification ≥3B: reoperation or ICU re-admittance.

** Including all biliary complications requiring at least medical treatment (i.e. antibiotics / ursodeoxycholic acid).

*** Requiring at least endoscopic treatment.

HAT, hepatic artery thrombosis; ICU, intensive care unit; ITBL, ischemic-type biliary lesions; PNF, primary non function.

two-year patient survival for recipients with a short (92%) and long (80%) SBP-Agonal (p=0.12). The Cox proportional hazard model (**Table 4**) for graft loss in the first two years after transplantation showed that a long SpO2-Agonal leads to a three-fold increase in hazard ratio (HR) for graft loss (HR 3.30; 95% CI 1.15–9.48; p=0.03). Increasing recipient BMI was the other independent factor is this model (HR 1.13; 95% CI 1.02–1.26; p=0.02).

DISCUSSION

This study provides new insight in the impact of vital parameters during the agonal phase on hepatic IRI in DCD liver transplantation. Our findings confirm the hypothesis that ischemic hepatic injury is more closely related to the onset of hypoxia, rather than hypotension. Similarly, in regular medical practice we fear hypoxia for damaging a patient's organs, while a short period of severe hypotension is tolerated

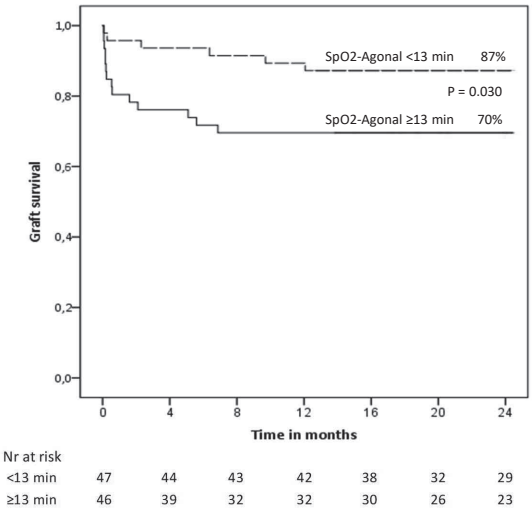

Figure 2 - Kaplan Meier curve for duration of SpO2-Agonal and graft survival after DCD liver transplantation.

Table 4. Cox proportional hazard model for graft loss after DCD liver transplantation.

Recipient	HR	95% CI	P-value
Age	1.01	0.96 - 1.07	0.63
Body mass index	1.13	1.02 - 1.26	0.02
LabMELD	0.99	0.92 - 1.08	0.97
Hepatocellular carcinoma	0.28	0.07 - 1.15	0.08
Donor			
Donor risk index	0.91	0.30 - 2.75	0.87
Graft steatosis			
None	1.00		
1-10%	1.84	0.70 - 4.79	0.21
>10%	4.11	0.77 - 22.01	0.09
Ischemia periods			
DWIT - SpO2-Agonal			
<13 minutes	1.00		
≥13 minutes	3.30	1.15 - 9.48	0.03
DWIT - asystolic phase	1.06	0.94 - 1.20	0.33
Cold ischemia time	1.02	0.95 - 1.08	0.64
Recipient warm ischemia time	1.23	0.89 - 1.71	0.20

CI, confidence interval; DWIT, donor warm ischemia time; HR, hazard ratio; MELD, model for end stage liver disease.

much better. Therefore, we hypothesized hypoxia would be more detrimental to DCD donor livers than hypotension during the agonal phase and our results indicate that the drop in SpO2 to <80% is much more relevant to assess the potential severity of hepatic IRI. Moreover, a prolonged SpO2-Agonal is associated with more postoperative complications and long-term graft failure.

Previous studies have used many different definitions for DWIT and consequently, the duration of an acceptable DWIT in these studies ranges from 10 to 35 minutes (15,17,25–27). The study by Abt et al showed that a quick drop in SBP after WoT was associated with an improved graft survival (15). Hong et al identified a period of MAP <60 mmHg before circulatory arrest longer than 20 minutes increased the risk for graft loss (17). Of note, the course of SpO2 was not considered in these studies. In a study of Firl et al the trajectory of agonal phase was divided into three categories by the course of SpO2 and MAP; a quick decline, a slow gradual decline, and a relatively long stable phase with initial good vital parameters with a quick decline just before circulatory arrest (16). The authors were not able to form strong conclusions donors with the worst prognosis, but in donors with a slow decline, only the course

of MAP was associated with graft loss. The course of SpO2 was not associated with graft loss, but is should be noted that pulse oximetry measurement of SpO2 is not reliable below a value of 80% (28–30). A more recent multicentre-study by Coffey et al investigated the relation between potential cut-offs of SpO2 and MAP and a composite endpoint consisting of early and late complications (31). Only the cut-off of SpO2 <60% was associated with postoperative complications, but these results were not confirmed in a multivariable analysis. Important pitfalls of this study are the low SpO2 cut-off of 60%, the uniform outcome that weakens potential effect on different complications and the lack of an analysis of the relation between DWIT and hepatic IRI.

In many countries, the cut-off for discarding a DCD liver is 30 minutes of functional DWIT (start of agonal phase at SBP<50 mm Hg until cold perfusion) and we also analysed these periods with this cut-offs for SBP. The cut-off for SpO2 was set at 80%, because the accuracy of pulse oximetry decreases significantly with arterial haemoglobin saturation levels below 75-80%. Using these cut-offs, the onset of hypoxia was on average almost directly after WoT and seven minutes earlier than hypotension, leading to a much longer SpO2-Agonal than SBP-Agonal period. In the multivariable analysis, only duration of SpO2-Agonal was associated with severe hepatic IRI. More importantly, the period between the drop in SpO2 and SBP (hypoxic agonal phase) was actually the period related to severe hepatic IRI in this multivariable model, suggesting that an agonal phase starting with a long hypoxic period is the driving force of the extra hepatic IRI in DCD grafts. These results are supported by studies investigating shock livers suggesting that hypoxia is an important cause for hepatic injury, even without hemodynamic shock (32). The increased hepatic IRI with the use of DCD grafts has previously been linked to impaired outcomes (13,33,34). We found that recipients of a graft with a SpO2-Agonal period longer than 13 minutes had more major complications, a higher CCI and more graft loss in the first 90 days. Moreover, the three recipients with PNF all had a SpO2-Agonal of ≥13 minutes. Considering the sum of SpO2-Agonal (13 min) and asystolic phase (16 min) is 29 min, we would advise to set the new cut-off for functional DWIT at 30 minutes.

The severity of hepatic IRI and complications has a multifactorial origin. Steatotic grafts are more vulnerable to IRI and graft steatosis was a significant factor in our multivariable analysis as well (36,37). We also observed a trend for increased RWIT, a known factor to increase hepatic IRI (38). Remarkably, the duration of the asystolic phase was not significant, this might be caused by the relative narrow dispersion of this phase, reflecting the legal five minutes no-touch period, transport of the

donor and cannulation time of the dedicated organ retrieval team, thus almost standardizing this period in all donors. Cold ischemia was not a factor, which could be explained by the relatively short cold ischemia time (mean <6.5h). The molecular pathways that could potentially explain our results regarding the importance of hypoxia have been previously studied before in renal transplantation by Damman et al (39). In this human study, transcriptomics of donor biopsies during retrieval and transplantation followed by functional pathway analysis showed that pathways related to prolonged and worsening deprivation of oxygen were associated with delayed graft function in DCD grafts. These pathways were already upregulated before organ retrieval and included metabolic pathways related to hypoxia and the complement-and-coagulation cascades. After reperfusion, these pathways were related to delayed kidney graft function. Furthermore, an experimental study in a rat liver transplant model with simulated prolonged WIT by Zhang et al confirmed that these complement-associated pathways are an important factor in the severity of hepatic IRI (40). To our knowledge, such analyses of prolonged warm ischemia, have not yet been performed in human liver transplantation. However, these studies confirm that hypoxia is an important factor in the warm phase of IRI. Interestingly, the duration of SpO2-Agonal was not related to development of biliary complications in our study. It is known that the biliary tree responds differently to ischemia than hepatocytes and previous experimental studies suggested that they are better resistant to hypoxia, but more susceptible to reoxygenation injury (41,42). Taner et al studied the impact of DWIT on ITBL and only observed a relation between the duration of asystolic phase and development of ITBL (10). This implicates that the biliary tree is less affected by hypoxia or hypotension during the agonal phase, compared to the full no-flow ischemia during the asystolic phase. Despite there was no relation between the duration of SpO2-Agonal and biliary complications, the 90-day outcomes and long-term survival rates were worse for grafts with a long SpO2-Agonal, due to an increased rate of HAT and PNF.

There are several limitations to this study. The retrospective design of this study means that recipient perioperative management varies, but our institution has a well-implemented liver transplant protocol and over 1000 liver transplants done. Donor data was retrieved from Eurotransplant, which has a well-organized database. However, in the first years of DCD organ retrieval, not all DWIT-data was available and we had to exclude eight cases. Peak serum AST levels are a surrogate marker for hepatic IRI, also depending on the weight of the donor liver. The only alternative option would be a histological assessment, but this would be subject to the interpretation by different pathologists. We specifically not use the classic Olthoff-criteria

for EAD, as we have observed that the median peak AST levels is above 2000 U/L in the majority of the DCD recipients. This study is reflecting the practice in the Netherlands, which can be different from other countries.

We believe our study provides strong evidence that hypoxia, rather than hypotension, marks the beginning of hepatic tissue injury in DCD grafts. We advise transplant professionals to consider functional DWIT as the period between SpO2 <80% and start of cold perfusion, irrespective of blood pressure. Duration of hypoxia during the agonal phase over 13 min could identify potential poor-quality grafts and thereby enable the transplant surgeons to engage in application of graft-improving methods such as machine perfusion.

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