

Onset of Donor Warm Ischemia Time in Donation After Circulatory Death Liver Transplantation: Hypotension or Hypoxia?

Marit Kalisvaart,^{1*} Jubi E. de Haan,^{2*} Wojciech G. Polak,¹ Jan N. M. IJzermans,¹ Diederik Gommers,² Herold J. Metselaar,³ and Jeroen de Jonge¹

¹Department of Surgery, Division of Transplant Surgery, Erasmus University Medical Center, Rotterdam, the Netherlands;

²Department of Adult Intensive Care, Erasmus University Medical Center, Rotterdam, the Netherlands; and ³Department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, the Netherlands

The aim of this study was to investigate the impact of hypoxia and hypotension during the agonal phase of donor warm ischemia time (DWIT) on hepatic ischemia/reperfusion injury (IRI) and complications in donation after circulatory death (DCD) liver transplantation. A retrospective single-center study of 93 DCD liver transplants (Maastricht type III) was performed. DWIT was divided into 2 periods: the agonal phase (from withdrawal of treatment [WoT] until circulatory arrest) and the asystolic phase (circulatory arrest until cold perfusion). A drop to <80% in peripheral oxygenation (SpO₂) was considered as hypoxia in the agonal phase (SpO₂-agonal) and a drop to <50 mm Hg as hypotension in the agonal phase (SBP-agonal). Peak postoperative aspartate transaminase level >3000 U/L was considered as severe hepatic IRI. SpO₂ dropped within 2 minutes after WoT <80%, whereas the systolic blood pressure dropped to <50 mm Hg 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 Comprehensive Complication Index during hospital admission (43.0 versus 32.0; $P = 0.002$) and 90-day graft loss (26% versus 6%; $P = 0.01$), compared with recipients with a short SpO₂-agonal (≤ 13 minutes). Furthermore, Cox proportional hazard modeling identified a long SpO₂-agonal as a risk factor for longterm graft loss (hazard ratio, 3.30; 95% confidence interval, 1.15–9.48; $P = 0.03$). In conclusion, the onset of hypoxia during the agonal phase is related to the severity of hepatic IRI and postoperative complications. Therefore, SpO₂ <80% should be considered as the start of functional DWIT in DCD liver transplantation.

Liver Transplantation 24 1001–1010 2018 AASLD.

Received December 8, 2017; accepted May 26, 2018.

Donation after circulatory death (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 with donation after brain death grafts.^(4–11)

The inferior results are likely the consequence of the obligatory donor warm ischemia time (DWIT). This extra period of warm ischemia in DCD grafts is thought to aggravate hepatic ischemia/reperfusion injury (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 2 periods (Fig. 1A): the agonal phase (from withdrawal of treatment [WoT] until circulatory arrest) and the asystolic phase (from circulatory arrest until cold perfusion). However, multiple definitions for DWIT are currently being used.⁽¹⁴⁾ The trajectory of the agonal phase differs widely between donors, and the exact moment of onset of hepatic tissue injury is

Abbreviations: AST, aspartate transaminase; BMI, body mass index; CI, confidence interval; CCI, Comprehensive Complication Index; DCD, donation after circulatory death; DWIT, donor warm ischemia time; EAD, early allograft dysfunction; FFP, fresh frozen plasma; HAT, hepatic artery thrombosis; HR, hazard ratio; ICU, intensive care unit; IQR, interquartile range; IRI, ischemia/reperfusion injury; ITBL, ischemic-type biliary lesions; LOESS, locally weighted scatterplot smoother; MAP, mean arterial pressure; MELD, Model for End-Stage Liver Disease; OR, odds ratio; PNF, primary nonfunction; PRS, postreperfusion syndrome; RBC, red blood cell; RWIT, recipient warm ischemia time; SBP, systolic blood pressure; SBP-agonal, hypotension in the agonal phase; SD, standard deviation; SpO₂, peripheral oxygenation; SpO₂-agonal, hypoxia in the agonal phase; UW, University of Wisconsin; WoT, withdrawal of treatment.

still unknown. Furthermore, the lack of a uniform definition for the onset of DWIT compromises evaluation of its impact on recipient outcomes. Earlier studies, primarily focusing on the blood pressure during the agonal phase, yielded varying results on its relation to postoperative complications.⁽¹⁵⁻¹⁸⁾ Abt et al. showed that a slow decline in systolic blood pressure (SBP) during the agonal phase is correlated with longterm 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 oxygenation (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 the agonal phase and hepatic IRI and observe potential cutoff points for the start of functional DWIT. Second, we will explore the relation between the agonal phase and recipient outcome in DCD liver transplantation.

Patients and Methods

This retrospective study was performed with the approval of the Erasmus University Medical Center institutional review board (registration number: EMC-2014-670). All consecutive adult patients who underwent DCD liver transplantation in our center from July

2008 to 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 body mass index (BMI) > 28 kg/m² are regularly not considered for liver donation. Also, we allocate DCD livers preferably to recipients with hepatocellular carcinoma and/or a relatively low biological Model for End-Stage Liver Disease (MELD) score of <20. The donor risk index was calculated to express graft quality.⁽¹⁹⁾ The amount of graft steatosis was assessed in biopsies taken after reperfusion.

Data following length and structure of different phases of DWIT were retrieved from the Eurotransplant database. An overview of DWIT is displayed in Fig. 1A. The cutoff time points were considered at <80% for hypoxia in the agonal phase (SpO₂-agonal) and <50 mm Hg for hypotension in the agonal phase (SBP-agonal). DCD grafts used in this study come from donors in the Netherlands only and are offered through the Eurotransplant waiting list on the basis of MELD status. In the Netherlands, WoT of the donor is the responsibility of the intensive care unit (ICU). After circulatory arrest, there is an obligatory 5 minutes waiting time, after which 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 University of Wisconsin (UW) preservation fluid under a pressure of 200 mm Hg via the aorta. The portal vein, hepatic artery, and biliary tract are additionally flushed at the back table with UW as well. The standard surgical technique included piggyback cavocaval anastomosis with only incidental use of a portocaval shunt, and the graft was routinely reperfused via the portal vein. The length of the surgical procedure, cold ischemia time, recipient warm ischemia time (RWIT), intraoperative blood loss with subsequent transfusion of red blood cell (RBC) concentrates, 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 aspartate transaminase (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 regimen in our center is based on the triple therapy strategy: prednisolone and mycophenolate

Address reprint requests to Jeroen de Jonge, M.D., Ph.D., Department of Surgery, Division of Hepatopancreatobiliary and Transplant Surgery, Erasmus University Medical Center, Room H822-K, 's-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands. Telephone: +31643904182; FAX: +31107032396; E-mail: j.dejonge.1@erasmusmc.nl.

**These authors contributed equally to the work.*

© 2018 The Authors Liver Transplantation published by Wiley Periodicals, Inc. on behalf of American Association for the Study of Liver Diseases. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

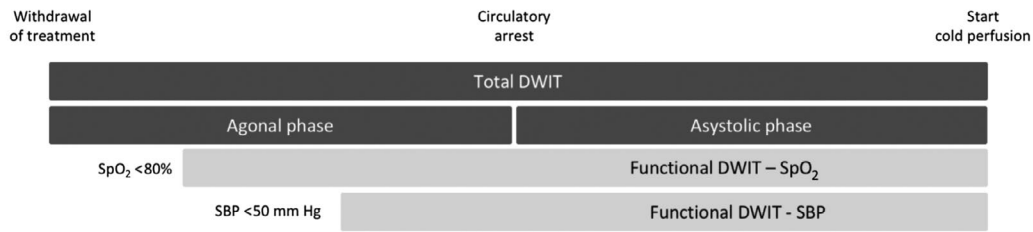
The copyright line for this article was changed on July 26, 2019 after original online publication.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/lt.25287

Potential conflict of interest: Nothing to report.

A. Phases of donor warm ischemia time



B. Decline of SpO₂ and SBP during agonal phase

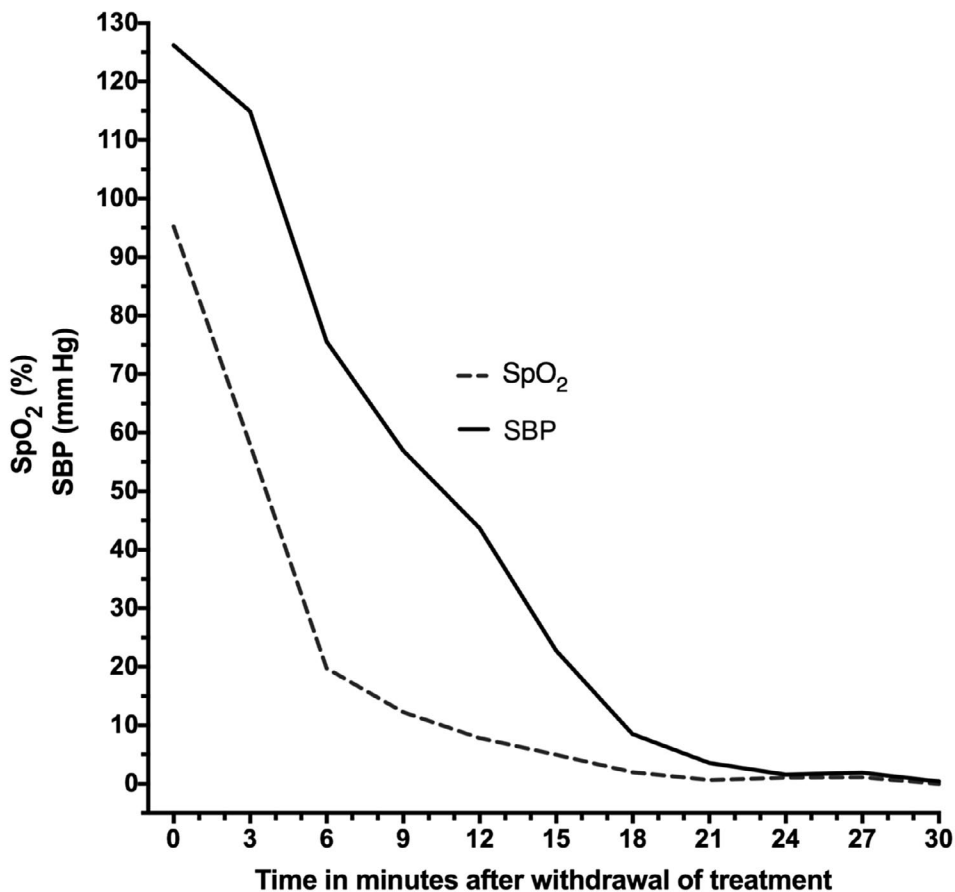


FIG. 1. DWIT in DCD liver transplantation. (A) The phases of donor warm ischemia; (B) the decline of SpO₂ and SBP during agonal phase.

mofetil from day 0, basiliximab at day 0 and day 4, and the introduction of tacrolimus on postoperative day 5. Length 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 $\geq 3b$ by the Clavien-Dindo classification, and the Comprehensive Complication Index (CCI) was

calculated at hospital discharge, 6 months, and 12 months after transplantation.^(23,24)

STATISTICAL ANALYSIS

Data were analyzed with IBM SPSS Statistics, version 24 (IBM, Armonk, NY). Student *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 was used. *P* values <0.05 were considered statistically significant. Continuous variables were expressed as mean with standard deviation (SD) or median and interquartile 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-year 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 2 years after transplantation.

Results

PATIENT SELECTION

During the study period, 105 patients underwent DCD liver transplantation of whom 93 were included. A total of 2 patients were excluded because of retransplantation, and 1 recipient was transplanted for acute liver failure. In 8 patients, the length of DWIT and donor's hemodynamic profile data were missing. In 1 patient, intraoperative data were 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 laboratory MELD score was 15. Median donor age was 47 years, and 58% of the donors were male. The mean donor risk index was 2.6. Mean length of total DWIT and asystolic phase were 32 and 16 minutes, respectively. Average length of the total agonal phase was also 16 minutes. Notably, a variety in dispersion of the DWIT phases was observed (Table 1). The SD and range in the agonal phase were longer than in the asystolic phase. SpO₂ dropped already 2 minutes after WoT below 80%, whereas the SBP dropped below 50 mm Hg after 9 minutes (Fig. 1B), resulting in a longer SpO₂-agonal (13 minutes) than SBP-agonal (6 minutes). Also, the dispersion of SpO₂-agonal was greater than the SBP-agonal, with larger SD and range. The mean time between the drop in SpO₂ and SBP was 7 minutes. The mean

TABLE 1. Baseline Donor, Recipient, and Surgical Characteristics in DCD Liver Transplantation

	Total (n = 93)
Donor	
Donor risk index	2.6 ± 0.5
Age, years	47 (39-53)
Sex, male	54 (58)
BMI, kg/m ²	24.2 (22.0-26.0)
Serum AST, U/L	46 (29-89)
Graft steatosis	
None	53 (57)
1%-10%	33 (36)
11%-33%	4 (4)
>33%	3 (3)
Total DWIT	32 (32; 12-60)
Asystolic phase	16 (15; 7-28)
Agonal phase	
Total agonal phase	16 (15; 4-39)
SpO ₂ -agonal	13 (12; 2-38)
SBP-agonal	6 (5; 1-17)
Between SpO ₂ and SBP-agonal	7 (6; 0-24)
Recipient	
Age, years	57 (49-63)
Sex, male	65 (70)
BMI, kg/m ²	26.5 ± 4.3
Etiology of liver disease	
Biliary cirrhosis	16 (17)
Viral hepatitis	25 (27)
Postalcoholic cirrhosis	20 (22)
Other	32 (34)
Hepatocellular carcinoma	31 (33)
Laboratory MELD score	15 (7.8)
Sodium, mmol/L	138 (134-140)
Medical history	
Hypertension	19 (20)
Coronary artery disease	2 (2)
Diabetes mellitus	29 (31)
Operation time, hours	7.9 (7.7; 6-12)
Cold ischemia time, hours	6.6 (6.4; 4-12)
RWIT, minutes	29 (28; 17-61)
Blood loss, liters	4.1 (3.2-6.2)
RBC transfusion, units	3 (1-5)
FFP transfusion, units	3 (0-6)
PRS	37 (40)
Dose norepinephrine end of surgery (ug/kg/minute)	0.30 (0.12-0.50)
EAD	56 (60)
Postoperative peak serum AST, U/L	2287 (1305-4881)
Peak serum AST in categories	
0-3000 U/L	53 (57)
>3000 U/L	40 (43)

NOTE: Continuous variables are displayed as mean ± SD, median (IQR), and n (%) where appropriate. Values of DWIT and intraoperative periods are displayed as follows: mean (median; range).

TABLE 2. Univariate and Multivariate Analysis of Risk Factors Associated With Severe Hepatic IRI in DCD Liver Transplantation

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

*Univariate analysis of factors associated with severe hepatic IRI (peak postoperative AST > 3000 U/L).

†Multivariate analysis with agonal phases split at the cutoff of saturation drop below 80%.

‡Multivariate analysis with agonal phases split at the cutoff of SBP below 50 mm Hg.

length of cold ischemia time and RWIT was 6.6 hours and 29 minutes, respectively.

LENGTH OF ISCHEMIA PERIODS AND HEPATIC IRI

More than half of the recipients developed EAD (60%) according to the Olthoff criteria⁽²²⁾ because of 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 cutoff of 3000 U/L in peak AST in the first 72 hours after transplantation. The peak AST was >3000 U/L in 43% of the recipients. Univariate analysis of risk factors associated with severe hepatic IRI (Table 2) showed that length of total agonal phase was significantly associated with a peak AST > 3000 U/L (odds ratio [OR], 1.08; 95% confidence interval [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 ($P = 0.41$). Multivariate analysis of the same risk factors was used to correct for potential confounders in relation to severe hepatic IRI. Two multivariate analyses were performed,

both with all factors of the univariate analysis and either with the SpO₂-agonal or SBP-agonal period (Table 2). In Table 2, the agonal phase for the multivariate analysis for SpO₂-agonal is split into the time before and after the drop <80% in SpO₂, and the agonal phase in the multivariate analysis for SBP agonal is also split using the drop in SBP <50 mm Hg. Multivariate analysis for SpO₂-agonal 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, the multivariate analysis for SBP-agonal shows that the length of the SBP-agonal period was not an independent factor ($P = 0.32$), whereas the period between the drop in SpO₂ and the drop in SBP was significantly related to severe hepatic IRI (hypoxic agonal phase; $P = 0.003$).

LENGTH OF SPO₂-AGONAL AND CLINICALLY RELEVANT OUTCOME PARAMETERS

Early Postoperative Complications

Table 3 displays the recipient outcome for the length of SpO₂-agonal. After visualization of locally weighted

TABLE 3. Duration of SpO₂-Agonal and Recipient Outcomes in the First Year After DCD Liver Transplantation

Length of SpO ₂ -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 (38)	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
CCI, median				
In-hospital	32.0	43.0	38.1	0.002
6 months	46.0	58.4	48.2	0.006
1 year	48.0	62.8	59.7	0.008
90-day graft loss	3 (6)	12 (26)	15 (16)	0.01
Retransplantation for HAT	2 (4)	3 (7)	5 (5)	
Retransplantation for PNF	0	3 (7)	3 (3)	
Retransplantation for ITBL	0	1 (2)	1 (1)	
Death	1 (2)	5 (11)	6 (6)	
Biliary complications in the 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

NOTE: Data are given as n (%) and median (IQR), unless otherwise noted.

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

†Including all biliary complications requiring at least medical treatment (ie, antibiotics/ursodeoxycholic acid).

‡Requiring at least endoscopic treatment.

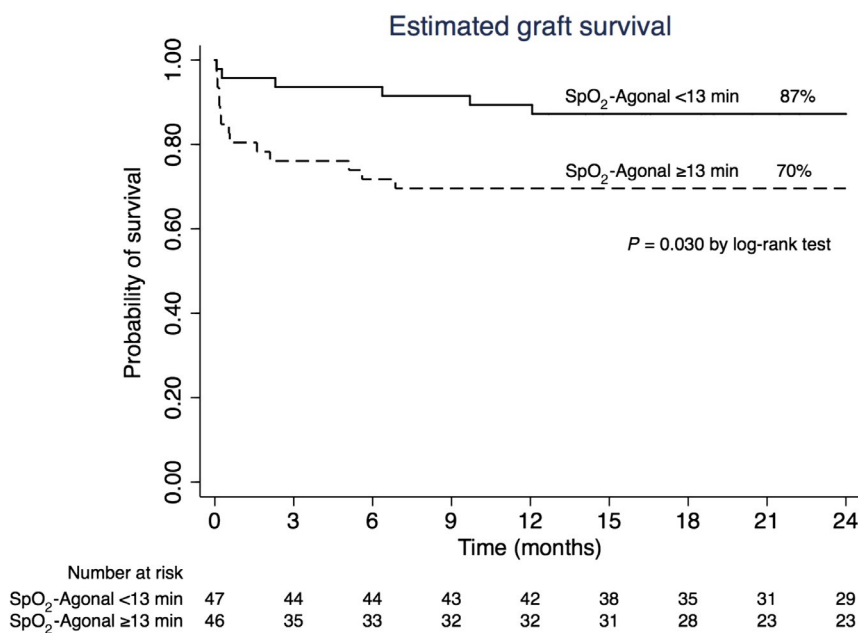


FIG. 2. Kaplan-Meier curve for length of SpO₂-agonal and graft survival after DCD liver transplantation.

TABLE 4. Cox Proportional Hazard Model for Graft Loss After DCD Liver Transplantation

	HR	95% CI	PValue
Recipient			
Age	1.01	0.96-1.07	0.63
BMI	1.13	1.02-1.26	0.02
Laboratory MELD	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 - SpO ₂ -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
RWIT	1.23	0.89-1.71	0.20

scatter-plot smoother (LOESS) plots, the SpO₂-agonal was divided into 2 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, 6 months, and 1 year after transplantation and at each moment the CCI was significantly higher in recipients with a long 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 primary nonfunction (PNF) of the graft requiring retransplantation, and all of them had a long SpO₂-agonal. Surprisingly, no correlation was observed between length of SpO₂-agonal and development of biliary complications in the first year after transplantation.

Longterm 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 2-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 2 years after transplantation showed that a long SpO₂-agonal leads to a 3-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 in 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, whereas a short period of severe hypotension is tolerated much better. Therefore, we hypothesized that 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 longterm graft failure.

Previous studies have used many different definitions for DWIT, and consequently, the length 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 mm Hg before circulatory arrest longer than 20 minutes increased the risk for graft loss.⁽¹⁷⁾ Notably, the course of SpO₂ was not considered in these studies. In a study by Firl et al., the trajectory of the agonal phase was divided into 3 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 about 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 it should be noted that pulse oximetry measurement of SpO₂ is not reliable below a value of 80%.⁽²⁸⁻³⁰⁾ A more recent multicenter study by Coffey et al. investigated the relation between potential cutoffs of SpO₂ and MAP and a composite endpoint consisting of early and late complications.⁽³¹⁾ Only the cutoff of SpO₂ <60% was associated with postoperative complications, but these results were not confirmed in a multivariate analysis. Important pitfalls of this study

are the low SpO₂ cutoff of 60%, the uniform outcome that weakens the potential effect on different complications, and the lack of an analysis of the relation between DWIT and hepatic IRI.

In many countries, the cutoff 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 analyzed these periods with this cutoff for SBP. The cutoff for SpO₂ was set at 80% because the accuracy of pulse oximetry decreases significantly with arterial hemoglobin saturation levels below 75%-80%. Using these cutoffs, the onset of hypoxia was on average almost directly after WoT and 7 minutes earlier than hypotension, leading to a much longer SpO₂-agonal than SBP-agonal period. In the multivariate analysis, only the duration of the 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 multivariate 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.^(13,33-35) We found that recipients of a graft with an 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 3 recipients with PNF all had an SpO₂-agonal of ≥13 minutes. Considering that the sum of the SpO₂-agonal (13 minutes) and asystolic phase (16 minutes) is 29 minutes, we would advise setting the new cutoff 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 multivariate analysis as well.^(36,37) We also observed a trend for increased RWIT, a known factor to increase hepatic IRI.⁽³⁸⁾ Remarkably, the length of the asystolic phase was not significant. This might be caused by the relatively narrow dispersion of this phase, reflecting the legal 5 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, <6.5 hours). 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 up-regulated 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 warm ischemia time 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 the 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 ischemic-type biliary lesions (ITBLs) and only observed a relation between the length of the asystolic phase and the development of ITBL.⁽¹⁰⁾ This implicates that the biliary tree is less affected by hypoxia or hypotension during the agonal phase, compared with the full no-flow ischemia during the asystolic phase. Even though there was no relation between the length of SpO₂-agonal and biliary complications, the 90-day outcomes and longterm survival rates were worse for grafts with a long SpO₂-agonal because of an increased rate of hepatic artery thrombosis (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 has done over 1000 liver transplants. Donor data were retrieved from Eurotransplant, which has a well-organized database. However, in the first years of DCD organ retrieval, not all DWIT data were available, and we had to exclude 8 patients. 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 did not use the classic Olthoff criteria for EAD because we have observed that median peak AST levels are above 2000 U/L in the majority of 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, regardless of blood pressure. The length of hypoxia during the agonal phase over 13 minutes could identify potential poor-quality grafts and thereby enable the transplant surgeons to apply graft-improving methods such as machine perfusion.

REFERENCES

- Merion RM, Pelletier SJ, Goodrich N, Englesbe MJ, Delmonico FL. Donation after cardiac death as a strategy to increase deceased donor liver availability. *Ann Surg* 2006;244:555-562.
- National Health Services Blood and Transplant. Organ Donation and Transplantation Activity Report 2015/16. <https://www.odt.nhs.uk/statistics-and-reports/annual-activity-report/>
- Eurotransplant International Foundation. Annual Report 2015. Branger P, Samuel U, eds. 2015; 49 p. https://www.eurotransplant.org/cms/index.php?page=annual_reports/
- Pine JK, Aldouri A, Young AL, Davies MH, Attia M, Toogood GJ, et al. Liver transplantation following donation after cardiac death: an analysis using matched pairs. *Liver Transpl* 2009;15:1072-1082.
- Jay CL, Lyuksemburg V, Ladner DP, Wang E, Caicedo JC, Holl JL, et al. Ischemic cholangiopathy after controlled donation after cardiac death liver transplantation: a meta-analysis. *Ann Surg* 2011;253:259-264.
- Foley DP, Fernandez LA, Levenson G, Anderson M, Mezrich J, Sollinger HW, D'Alessandro A. Biliary complications after liver transplantation from donation after cardiac death donors: an analysis of risk factors and long-term outcomes from a single center. *Ann Surg* 2011;253:817-825.
- DeOliveira ML, Jassem W, Valente R, Khorsandi SE, Santori G, Prachalias A, et al. Biliary complications after liver transplantation using grafts from donors after cardiac death: results from a matched control study in a single large volume center. *Ann Surg* 2011;254:716-722.
- Leithead JA, Taricciotti L, Gunson B, Holt A, Isaac J, Mirza DF, et al. Donation after cardiac death liver transplant recipients have an increased frequency of acute kidney injury. *Am J Transplant* 2012;12:965-975.
- Taner CB, Bulatao IG, Perry DK, Sibulesky L, Willingham DL, Kramer DJ, Nguyen JH. Asystole to cross-clamp period predicts development of biliary complications in liver transplantation using donation after cardiac death donors. *Transpl Int* 2012;25:838-846.
- Taner CB, Bulatao IG, Willingham DL, Perry DK, Sibulesky L, Pungpapong S, et al. Events in procurement as risk factors for

- ischemic cholangiopathy in liver transplantation using donation after cardiac death donors. *Liver Transpl* 2012;18:100-111.
- Kalisvaart M, de Haan JE, Polak WG, Metselaar HJ, Wijnhoven BPL, IJzermans JNM, de Jonge J. Comparison of postoperative outcomes between donation after circulatory death and donation after brain death liver transplantation using the comprehensive complication index. *Ann Surg* 2017;266:772-778.
 - Dubbeld J, Hoekstra H, Farid W, Ringers J, Porte RJ, Metselaar HJ, et al. Similar liver transplantation survival with selected cardiac death donors and brain death donors. *Br J Surg* 2010;97:744-753.
 - Xu J, Sayed BA, Casas-Ferreira AM, Srinivasan P, Heaton N, Rela M, et al. The impact of ischemia/reperfusion injury on liver allografts from deceased after cardiac death versus deceased after brain death donors. *PLoS One* 2016;11:e0148815.
 - Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ, et al. New classification of donation after circulatory death donors definitions and terminology. *Transpl Int* 2016;29:749-759.
 - Abt PL, Praestgaard J, West S, Hasz R. Donor hemodynamic profile presages graft survival in donation after cardiac death liver transplantation. *Liver Transpl* 2014;20:165-172.
 - Firl DJ, Hashimoto K, O'Rourke C, Diago-Uso T, Fujiki M, Aucejo FN, et al. Role of donor hemodynamic trajectory in determining graft survival in liver transplantation from donation after circulatory death donors. *Liver Transpl* 2016;22:1469-1481.
 - Hong JC, Yersiz H, Kositamongkol P, Xia VW, Kaldas FM, Petrowsky H, et al. Liver transplantation using organ donation after cardiac death: a clinical predictive index for graft failure-free survival. *Arch Surg* 2011;146:1017-1023.
 - Bradley JA, Pettigrew GJ, Watson CJ. Time to death after withdrawal of treatment in donation after circulatory death (DCD) donors. *Curr Opin Organ Transplant* 2013;18:133-139.
 - Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DeRoy MA, et al. Characteristics associated with liver graft failure: the concept of a donor risk index. *Am J Transplant* 2006;6:783-790.
 - Perera MT. The super-rapid technique in Maastricht category III donors: has it developed enough for marginal liver grafts from donors after cardiac death? *Curr Opin Organ Transplant* 2012;17:131-136.
 - Aggarwal S, Kang Y, Freeman JA, Fortunato FL Jr, Pinsky MR. Postreperfusion syndrome: hypotension after reperfusion of the transplanted liver. *J Crit Care* 1993;8:154-160.
 - Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010;16:943-949.
 - Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, et al. The Clavien-Dindo classification of surgical complications: five-year experience. *Ann Surg* 2009;250:187-196.
 - Slankamenac K, Graf R, Barkun J, Puhan MA, Clavien PA. The comprehensive complication index: a novel continuous scale to measure surgical morbidity. *Ann Surg* 2013;258:1-7.
 - Mathur AK, Heimbach J, Steffick DE, Sonnenday CJ, Goodrich NP, Merion RM. Donation after cardiac death liver transplantation: predictors of outcome. *Am J Transplant* 2010;10:2512-2519.
 - Blok JJ, Detry O, Putter H, Rogiers X, Porte RJ, van Hoek B, et al. for Eurotransplant Liver Intestine Advisory Committee. Longterm results of liver transplantation from donation after circulatory death. *Liver Transpl* 2016;22:1107-1114.
 - de Vera ME, Lopez-Solis R, Dvorchik I, Campos S, Morris W, Demetris AJ, et al. Liver transplantation using donation after cardiac death donors: long-term follow-up from a single center. *Am J Transplant* 2009;9:773-781.

- 28) Van de Louw A, Cracco C, Cerf C, Harf A, Duvaldestin P, Lemaire F, Brochard L. Accuracy of pulse oximetry in the intensive care unit. *Intensive Care Med* 2001;27:1606-1613.
- 29) Carter BG, Carlin JB, Tibballs J, Mead H, Hochmann M, Osborne A. Accuracy of two pulse oximeters at low arterial hemoglobin-oxygen saturation. *Crit Care Med* 1998;26:1128-1133.
- 30) Kohyama T, Moriyama K, Kanai R, Kotani M, Uzawa K, Satoh T, Yorozu T. Accuracy of pulse oximeters in detecting hypoxemia in patients with chronic thromboembolic pulmonary hypertension. *PLoS One* 2015;10:e0126979.
- 31) Coffey JC, Wanis KN, Monbaliu D, Gilbo N, Selzner M, Vachharajani N, et al. The influence of functional warm ischemia time on DCD liver transplant recipients' outcomes. *Clin Transplant* 2017;31.
- 32) Henrion J, Schapira M, Luwaert R, Colin L, Delannoy A, Heller FR. Hypoxic hepatitis: clinical hemodynamic study in 142 consecutive cases. *Medicine (Baltimore)* 2003;82:392-406.
- 33) Oniscu GC, Randle LV, Muiesan P, Butler AJ, Currie IS, Perera MT, et al. In situ normothermic regional perfusion for controlled donation after circulatory death—The United Kingdom experience. *Am J Transplant* 2014;14:2846-2854.
- 34) D'alessandro AM, Hoffmann RM, Knechtle SJ, Odorico JS, Becker YT, Musat A, et al. Liver transplantation from controlled non-heart-beating donors. *Surgery* 2000;128:579-588.
- 35) Selten J, Schlegel A, de Jonge J, Dutkowski P. Hypo- and normothermic perfusion of the liver: which way to go? *Best Pract Res Clin Gastroenterol* 2017;31:171-179.
- 36) Ali JM, Davies SE, Brais RJ, Randle LV, Klinck JR, Allison ME, et al. Analysis of ischemia/reperfusion injury in time-zero biopsies predicts liver allograft outcomes. *Liver Transpl* 2015;21:487-499.
- 37) Chu MJ, Hickey AJ, Phillips AR, Bartlett AS. The impact of hepatic steatosis on hepatic ischemia-reperfusion injury in experimental studies: a systematic review. *Biomed Res Int* 2013;2013:192029.
- 38) Ricca L, Lemoine A, Cauchy F, Hamelin J, Sebagh M, Esposti DD, et al. Ischemic postconditioning of the liver graft in adult liver transplantation. *Transplantation* 2015;99:1633-1643.
- 39) Damman J, Bloks VW, Daha MR, van der Most PJ, Sanjabi B, van der Vlies P, et al. Hypoxia and complement-and-coagulation pathways in the deceased organ donor as the major target for intervention to improve renal allograft outcome. *Transplantation* 2015;99:1293-1300.
- 40) Zhang J, Hu W, Xing W, You T, Xu J, Qin X, Peng Z. The protective role of CD59 and pathogenic role of complement in hepatic ischemia and reperfusion injury. *Am J Pathol* 2011;179:2876-2884.
- 41) Noack K, Bronk SF, Kato A, Gores GJ. The greater vulnerability of bile duct cells to reoxygenation injury than to anoxia. Implications for the pathogenesis of biliary strictures after liver transplantation. *Transplantation* 1993;56:495-500.
- 42) Seehofer D, Eurich D, Veltzke-Schlieker W, Neuhaus P. Biliary complications after liver transplantation: old problems and new challenges. *Am J Transplant* 2013;13:253-265.