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The impact of combined warm ischemia time on development of acute kidney injury in DCD liver transplantation: Stay within the golden hour

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SUMMARY

AKI is a common complication after liver transplantation and more frequently observed when high-risk grafts, such as DCD grafts are used. Our aim was to investigate the impact of the ischemia periods on development of AKI in DCD liver transplantation. We performed a two-center retrospective study with 368 DCD graft-recipients. DWIT was divided into agonal phase (withdrawal of life-support – cardiac arrest) and asystolic phase (cardiac arrest – start cold perfusion). We introduced a new period of warm ischemia: the combined warm ischemia time (combined WIT), that was defined as the sum of DWIT and recipient WIT. AKI was observed in 65% of the recipients and severe AKI in 41% (KDIGO stage 2/3). The length of combined WIT increased significantly with AKI severity: 61 minutes in recipients without AKI up to 69 minutes in recipients with the most severe form of AKI ($p<0.001$). On multivariable analysis, increasing duration of the combined WIT was associated with an increased risk of developing severe AKI (OR 1.032 per every extra minute; 95% CI 1.014-1.051; $p<0.001$). No relation was observed between length of cold ischemia time and severe AKI. In conclusion, combined WIT is a newly defined period of warm ischemia in DCD liver transplantation. Length of combined WIT is associated with severity of postoperative AKI and should ideally not exceed 60 minutes.

INTRODUCTION

DCD grafts are increasingly used to overcome the donor shortage in liver transplantation (1,2). However, the use of these marginal grafts is associated with more PNF, EAD and ischemic cholangiopathy with subsequent impaired graft survival rates (3–10). These complications are the result of the additional DWIT, leading to an increase in hepatic IRI (11). The kidney is an organ known to suffer from hepatic IRI, and acute kidney injury (AKI) is also more frequently observed when DCD grafts are used (12–15). Overall, AKI affects up to 75% of the liver graft recipients and is related to CKD and impaired survival rates (13,15–20).

The additional DWIT of DCD livers can be divided into two periods: the agonal phase (withdrawal of life support - circulatory arrest) and the asystolic phase (circulatory arrest – start cold perfusion). Various factors impact on the length of DWIT: the course of vital parameters and length of agonal phase differs widely between donors and length of asystolic phase depends on institutional and national protocols (e.g. location of withdrawal of life support and the 'no touch' time after circulatory arrest). Both length of agonal and asystolic phase have been linked to postoperative development of ischemic cholangiopathy and graft survival rates (21–24). **Figure 1** shows an overview of the combined WIT in DCD liver transplantation: a new defined period that is the sum of agonal phase and asystolic phase during organ procurement and recipient warm ischemia time before reperfusion (recipient WIT). These periods have their own biological profile, where the blood and oxygen supply gradually decreases within agonal phase, followed by warm ischemia without any flow and oxygen distribution during asystolic phase. After cold storage, livers are exposed to another type of warm ischemia in the recipient, where a gradual rewarming occurs during reconstruction of the vascular anastomoses. Little is known about the impact of such different types of WIT on hepatic IRI and development of AKI. Furthermore,

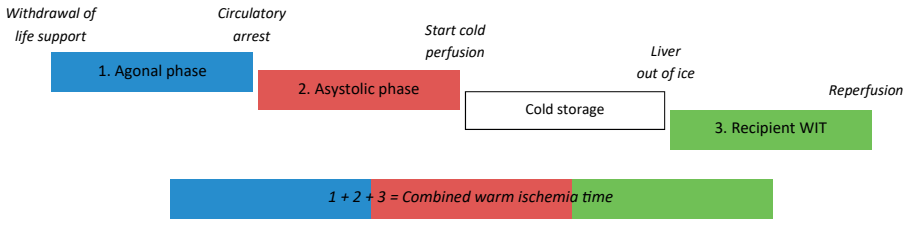

Figure 1 - Definition of combined warm ischemia time in DCD liver transplantation

the length of the separate phases varies in every transplant and they have not been assessed together before.

The negative impact of AKI on long term outcomes necessitates further research on this subject. Our aim was therefore to investigate the impact of such different periods of warm ischemia on development of postoperative AKI in DCD liver transplantation.

METHODS

This retrospective study was performed in two liver transplant centers: the Queen Elizabeth Hospital, NHS Foundation Trust, Birmingham, UK and the Erasmus MC University Medical Center, Rotterdam, the Netherlands. The study was approved by both institutional review boards (Birmingham, CARMS-13009; Rotterdam, MEC-2014-670). All consecutive adult patients, who underwent orthotopic DCD liver transplantation in both centers between July 2008 and July 2016 were included. Exclusion criteria were transplantation for acute liver failure, re-transplantation, AKI in the week prior to transplantation (but not pre-existent renal impairment) and machine perfusion of the graft.

The collected donor characteristics are displayed in **Table 1**, including the DRI (25). The most recent European classification for definitions and terminology in DCD donation was used to assess the different phases of DWIT (26). In the UK, all donor data are collected via the UK Transplant Registry. Rotterdam is part of the Eurotransplant allocation system. The recipient characteristics collected at admission are shown in

Table 1: Clinical characteristics of DCD liver transplantation recipients

Donor characteristics	Birmingham (n = 279)		Rotterdam (n = 89)		Total (n=368)		P-value
Age (years)	53	(39-64)	47	(40-53)	51	(39-61)	<0.001
Male gender (%)	192	(69)	64	(72)	256	(70)	0.581
Body mass index	24.8	(22.8-28.0)	24.2	(22.0-25.6)	24.7	(22.5-27.4)	0.022
Donor risk index	2.7	(2.2-3.1)	2.6	(2.2-2.9)	2.7	(2.2-3.1)	0.164
Donor γGT (U/L) (n=264)	40	(21-80)	37	(21-75)	39	(21-80)	0.895
Donor cause of death (%)							0.004
Hypoxia	72	(26)	14	(16)	86	(23)	
Trauma	34	(12)	18	(20)	52	(14)	
CVA	142	(51)	55	(62)	197	(54)	
Other	31	(31)	2	(2)	33	(9)	

Table 1: Clinical characteristics of DCD liver transplantation recipients (continued)

	Birmingham (n = 279)		Rotterdam (n = 89)		Total (n=368)		P-value
Donor warm ischemia time (min)							
Total DWIT	28.4	(8.7)	31.3	(8.4)	29.1	(8.7)	0.005
Agonal phase	15.8	(8.3)	15.3	(7.2)	15.7	(8.1)	0.632
Asystolic phase	12.6	(3.7)	16.0	(4.3)	13.4	(4.1)	<0.001
Recipient							
Age (years)	58	(51-65)	57	(50-63)	58	(51-64)	0.089
Male gender (%)	192	(69)	64	(72)	256	(70)	0.581
Body mass index	26.6	(24.0-29.9)	25.8	(23.5-29.5)	26.5	(23.9-29.8)	0.369
Etiology of liver disease (%)							0.010
Viral hepatitis	88	(32)	26	(29)	114	(31)	
Cholestatic disease	69	(25)	16	(18)	85	(23)	
Postalcoholic cirrhosis	81	(29)	19	(19)	100	(27)	
NASH	17	(6)	13	(15)	30	(8)	
Other	24	(9)	15	(17)	39	(11)	
Hepatocellular carcinoma (%)	109	(39)	31	(35)	140	(38)	0.473
LabMELD score							<0.001
< 15	170	(61)	44	(49)	214	(58)	
15 - 20	77	(28)	18	(20)	95	(26)	
> 20	32	(12)	27	(30)	59	(16)	
eGFR (ml/min/1.73m2)							0.057
> 90	142	(51)	35	(39)	177	(48)	
≤ 90	137	(49)	54	(61)	191	(52)	
Ascites (%)							0.056
Diuretic controlled	82	(29)	35	(39)	117	(32)	
Refractory ascites	53	(19)	21	(24)	74	(20)	
Medical history (%)							
Hypertension	44	(16)	18	(20)	62	(17)	0.328
Diabetes mellitus	50	(18)	28	(32)	79	(21)	0.007
Surgery							
Cold ischemia time (hours)	7	(6.1-8.1)	6.4	(5.8-7.2)	6.9	(5.9-7.9)	0.001
Recipient warm ischemia time (min)	37	(31-43)	27	(23-33)	35	(28-42)	<0.001
RBC transfusion (units)	2	(0-4)	2	(1-5)	2	(0-4)	0.020
FFP transfusion (units)	6	(2-11)	3	(0-7)	6	(2-10)	<0.001
Platelet transfusion (units)	2	(0-10)	5	(0-10)	2	(0-10)	0.891
Postoperative							
Peak ALT (U/L) (n=274)	1775	(1029-2630)	1603	(817-2834)	1718	(981-2656)	0.595
Tacrolimus peak trough level (ug/L)	9.2	(6.4-12.4)	<1.5	(<1.5-3.7)	7.5	(4.0-11.5)	<0.001

ALT, alanine transaminase; DWIT, donor warm ischemia time; eGFR, estimated glomerular filtration rate; FFP, fresh frozen plasma; ɣGT, gamma-glutamyltransferase; MELD, model for end-stage liver disease; NASH, non alcoholic steatohepatitis; Continuous variables are displayed as mean (standard deviation) and median (interquartile range) where appropriate.

Table 1. The eGFR was calculated using the MDRD-4 equation and the severity of liver disease was calculated using the preoperative MELD score (27–29). Duration of cold ischemia time and RWIT and the transfusion requirements were recorded.

In the UK, the donor WoT takes place either in ICU or in the anesthetic room next to theatres, while the withdrawal is always in ICU in the Netherlands. Both countries keep a maximum length of agonal phase at 60 minutes to proceed with organ retrieval and the 'no-touch' period after circulatory arrest is five minutes in both countries (30,31). In both countries, heparin is not administered to the donor prior to WoT. During the organ retrieval process, in Birmingham the preferred preservation fluid for procurement is heparinized UW solution (5-6 L) under 200 mm HG pressure via the aorta and 1 L under gravity via the portal vein. In Rotterdam, heparinized UW is used as well, 6-8 L via the aorta only. Additional back-table flushed is performed in both centers and the biliary tract is flushed in both centers as well. The standard surgical technique in both centers includes piggyback caval anastomosis (classical or side-to-side cavocavostomy) without the use of veno-venous bypass and only a portocaval shunt in selected cases.

The peak serum alanine aminotransferase (ALT) level in the first 48 hours was used as a surrogate marker for hepatic IRI. Postoperative creatinine levels were collected in the first week after liver transplantation. AKI was defined according to KDIGO criteria (32): an increase in serum creatinine by ≥26.5 umol/L within 48 hours or an increase in creatinine to ≥ 1.5 times baseline within the first 7 postoperative days. AKI was classified into 3 stages: stage 1, increase ≥26.5 umol/L or increase of 1.5-1.9 fold from baseline; stage 2, increase of 2-2.9 fold; stage 3, increase >3-fold or increase in serum creatinine to ≥354 umol/L or initiation of RRT. In both centers, patients received triple-therapy immunosuppression after transplant. In Birmingham, the regimen consisted of tacrolimus, azathioprine or MMF, and prednisolone, which were all introduced at day 0. Prednisolone therapy was generally discontinued after three months. In Rotterdam, the immunosuppression regimen was modified during the study period. From 2012, induction of tacrolimus was delayed until day 5, MMF was added from day 0 and basiliximab was given on day 0 and 4. The attending physicians adjusted the immunosuppression in case of impaired renal function or infection. Target peak trough levels for tacrolimus were 8-10 ug/L during the first month in both centers. To detect tacrolimus nephrotoxicity, the peak trough levels in the first week were recorded.

Statistical analysis

Data were analyzed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, New York, USA). Continuous variables were tested for normality with the Shapiro-Wilk test. The student's t test and the one-way analysis of variance were 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 the 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 IQR, where appropriate. Categorical variables were expressed in quantities and percentages. To relate donor-, recipient-, and peri-operative factors to the development of severe AKI after DCD liver transplantation, a multiple logistic regression analysis was performed with all clinical relevant factors. Long-term survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests.

RESULTS

During the study period 422 patients received a DCD liver graft; 317 in Birmingham and 105 in Rotterdam. The following 26 patients were excluded: one for acute liver failure, 1 re-transplantation, 5 patients with AKI in the week prior to transplantation, and 18 patients because of end-ischemic machine perfusion. One patient was excluded because of intra-operative death. Another 27 cases were excluded because of incomplete data (DWIT and intra-operative data). This resulted in an inclusion of 368 patients; 279 in Birmingham and 89 in Rotterdam

Recipient, donor, and perioperative characteristics in both centers

The baseline characteristics for both centers are displayed in **Table 1**. Median donor age was 53 years in Birmingham, compared to 47 years in Rotterdam ($p<0.001$). Donor BMI was also higher in Birmingham (median: 24.8 versus 24.2; $p=0.022$). The mean total DWIT was longer in Rotterdam (31 versus 28 min; $p=0.005$), because of a longer asystolic phase in this center (16 versus 13 min; $p<0.001$). The mean agonal phase length was 16 minutes in the entire cohort and comparable between centers. In Birmingham, livers were exposed to a longer period of cold ischemia (median 7.0 versus 6.4 hours; $p=0.001$) and RWIT was also longer in Birmingham (37 versus 27 min; $p<0.001$). Median recipient age (58 years) and BMI ($26.5 m^2/kg$) were comparable in both centers. The labMELD score was higher in Rotterdam, were 30% of the recipients had a score of 20 or higher, compared to 12% in Birmingham

(p<0.001). The preoperative kidney function (eGFR) was below 90 ml/min/1.73m^2 in 61% of the recipients in Rotterdam and 49% in Birmingham (p=0.057). Due to the delayed introduction of tacrolimus in Rotterdam, the median peak tacrolimus trough level in the first week was lower in this center (<1.5 versus 9.2 ug/L; p<0.001).

Development of acute kidney injury

The incidence of postoperative AKI is displayed in **Table 2**. A total of 239 (65%) recipients developed any form of AKI; 67% in Birmingham and 57% in Rotterdam (p=0.083). Of these 239 recipients, 37%, 18%, and 45% respectively developed AKI stage 1,2, and 3. More recipients in Birmingham required postoperative RRT (30% versus 6%; p<0.001). Correspondingly, the peak creatinine levels in the first week after transplant were higher in Birmingham (140 versus 119 umol/L; p=0.026). In the recipients who received RRT (n=89), the serum creatinine at the start of RRT was lower in Birmingham, (276 vs. 429 umol/L), suggesting a lower threshold to use RRT in this center. Recipients from the entire cohort were divided into three groups: no AKI, mild AKI (KDIGO stage 1), and severe AKI (KDIGO stage 2/3). Donor, recipient and surgical characteristics for the three AKI groups are displayed in **Table 3**. No significant differences were observed in the evaluated donor characteristics in relation to postoperative development of AKI. BMI was the only recipient factor increasing with severity of AKI (p=0.006). Transfusion requirements during surgery all increased with the extent of postoperative renal injury. The peak tacrolimus trough level was comparable for all AKI groups. The incidence of the postreperfusion syndrome was 33% and no relation was observed between the incidence of this phenomenon and the severity of AKI (p=0.987).

Table 2: Incidence and severity of acute kidney injury after DCD liver transplantation

	Birmingham		Rotterdam		Total		P-value
Total recipients	279	(100%)	89	(100%)	368	(100%)	.
Peak serum creatinine (umol/L)	140	(98-235)	119	(89-184)	133	(95-218)	0.026
*AKI total**	188	(67%)	51	(57%)	239	(65%)	0.083
Stage 1	56	*(30%)*	32	*(63%)*	88	*(37%)*	.
Stage 2	32	*(17%)*	12	*(24%)*	44	*(18%)*	.
Stage 3	100	*(53%)*	7	*(14%)*	107	*(45%)*	.
Severe AKI (KDIGO stage 2/3)	132	(47%)	19	(21%)	151	(41%)	<0.001
Renal replacement therapy (RRT)	84	(30%)	5	(6%)	89	(24%)	<0.001
Serum creatinine at start RRT (umol/L)	276	(201-423)	429	(216-470)	279	(205-429)	0.345

AKI, acute kidney injury; KDIGO, Kidney Disease: Improving Global Outcomes.
*All the percentages in *Italic* are the percentage of all recipients that developed AKI.

Table 3: Donor, recipient characteristics and surgical parameters and mild and severe acute kidney injury in DCD liver transplantation

Donor	No AKI (n=129)		Mild AKI (n=88)		Severe AKI (n=151)		P-value
Age	50	(39-64)	52	(37-59)	51	(42-61)	0.710
Male gender (%)	87	(67)	66	(75)	103	(68)	0.442
Body mass index	24.5	(22.5-26.7)	24.5	(22.5-26.2)	25.1	(22.8-28.4)	0.093
Donor risk index	2.7	(2.2-3.1)	2.7	(2.2-3.02)	2.7	(2.3-3.0)	0.852
Donor ɣGT (U/L) (n=264)	38	(19-66)	36	(20-94)	41	(23-86)	0.228
Recipient							
Age (years)	57	(51-64)	58	(51-65)	59	(52-64)	0.832
Male gender (%)	87	(67)	66	(75)	103	(68)	0.442
Body mass index	25.4	(22.9-29.4)	25.9	(23.8-29.5)	27.3	(24.6-30.8)	0.006
Etiology of liver disease (%)							0.715
Viral hepatitis	37	(29)	23	(26)	54	(36)	
Biliary cirrhosis	32	(25)	24	(27)	29	(19)	
Postalcoholic cirrhosis	33	(26)	25	(28)	42	(28)	
NASH	10	(8)	8	(9)	12	(8)	
Other	17	(13)	8	(9)	14	(9)	
Hepatocellular carcinoma (%)	47	(36)	29	(33)	64	(42)	0.314
LabMELD score							0.160
< 15	74	(57)	44	(50)	96	(64)	
15 - 20	33	(26)	24	(27)	38	(25)	
> 20	22	(17)	20	(23)	17	(11)	
eGFR (ml/min/1.73m2)							0.139
> 90	69	(54)	35	(40)	73	(48)	
≤ 90	60	(47)	53	(60)	78	(52)	
Sodium (mmol/L)	138	(135-141)	138	(134-140)	138	(136-141)	0.082
Ascites (%)							0.341
Diuretic controlled	35	(27)	28	(32)	54	(36)	
Refractory ascites	27	(21)	22	(25)	25	(17)	
Medical history (%)							
Hypertension	19	(15)	12	(14)	31	(21)	0.283
Diabetes mellitus	21	(16)	21	(24)	36	(24)	0.238
Peri-operative							
RBC transfusion (units)	1	(0-3)	2	(1-5)	3	(1-6)	<0.001
FFP transfusion (units)	4	(0-6)	6	(1-10)	8	(4-12)	<0.001
Platelet transfusion (units)	0	(0-5)	1	(0-5)	4	(0-10)	0.003
Postreperfusion syndrome (n=117)	16	(34)	12	(33)	11	(32)	0.987
Tacrolimus peak trough level (ug/L)	7.5	(4.0-11.5)	6.3	(0.8-11.7)	4.8	(7.7-11.5)	0.293

AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; FFP, fresh frozen plasma; ɣGT, gamma-glutamyltransferase; MELD, model end-stage liver disease; NASH, non alcoholic steato hepatitis; RBC, red blood cells. Continuous variables are displayed as mean (standard deviation) and median (IQR) where appropriate.

Warm ischemia time and acute kidney injury

Table 4 shows the length of ischemia periods for all AKI stages. Interestingly, length of asystolic phase was not found to be significantly associated with severity of AKI in either center. The duration of agonal phase was associated with renal injury in Rotterdam (p=0.042), but not in Birmingham (p=0.935). In contrast, in Birmingham severity of AKI increased with length of RWIT (p=0.007), while no significant association was observed in Rotterdam (p=0.136). To explore the impact of the total amount of warm ischemia (combined WIT), duration of the donor agonal and asystolic phase were added to the secondary warm ischemia of the graft in the recipient (RWIT). **Figure 2** demonstrates the relation of combined WIT to the different AKI stages in both centers and the total cohort. Duration of combined WIT was longer in recipients with an increased severity of AKI in both centers. In the total cohort, the combined WIT was 61 minutes in recipients without AKI and increased up to 69 minutes in recipients with the most severe form of AKI (stage 3, p<0.001). Cold ischemia time was not found to be associated with the development of AKI in both centers. A further analysis confirmed that severe AKI was not more frequently observed in recipients with a prolonged cold ischemia time (incidence severe AKI: <6 hours 40%; 6-8 hours 40%; 8-10 hours 45%; >10 hours 45%; p=0.901).

Table 4: Length of ischemia periods and severity of acute kidney injury after DCD liver transplantation.

Birmingham	No AKI (n=91)		AKI stage 1 (n=56)		AKI stage 2 (n=32)		AKI stage 3 (n=100)		P-value
Donor warm ischemia time (min)									
Agonal phase	15	(±7.4)	16	(±8.8)	16	(±8.3)	16	(±9.0)	0.915
Asystolic phase	13	(±3.2)	12	(±3.9)	12	(±2.2)	13	(±4.3)	0.207
Total	28	(±7.5)	28	(±9.6)	28	(±9.0)	29	(±9.2)	0.726
Cold ischemia time (hours)	7.1	(±1.6)	7.1	(±1.5)	6.9	(±1.7)	7.1	(±1.5)	0.911
Recipient warm ischemia time (min)	35	(±8.5)	38	(±8.6)	40	(±13.2)	40	(±11.8)	0.007
Rotterdam	**No AKI (n=38)**		**AKI stage 1 (n=32)**		**AKI stage 2 (n=12)**		**AKI stage 3 (n=7)**		**P-value**
Donor warm ischemia time (min)									
Agonal phase	14	(±6.0)	15	(±5.9)	17	(±10.7)	22	(±8.7)	0.042
Asystolic phase	17	(±4.0)	15	(±4.9)	15	(±3.8)	16	(±3.9)	0.548
Total	31	(±6.8)	30	(±7.6)	32	(±12.3)	38	(±10.4)	0.128
Cold ischemia time (hours)	6.3	(±1.1)	6.7	(±1.2)	6.7	(±1.2)	6.7	(±1.0)	0.431
Recipient warm ischemia time (min)	28	(±5.9)	29	(±8.3)	34	(±9.6)	28	(±8.8)	0.136

AKI, acute kidney injury. Values are demonstrated as mean and standard deviation.

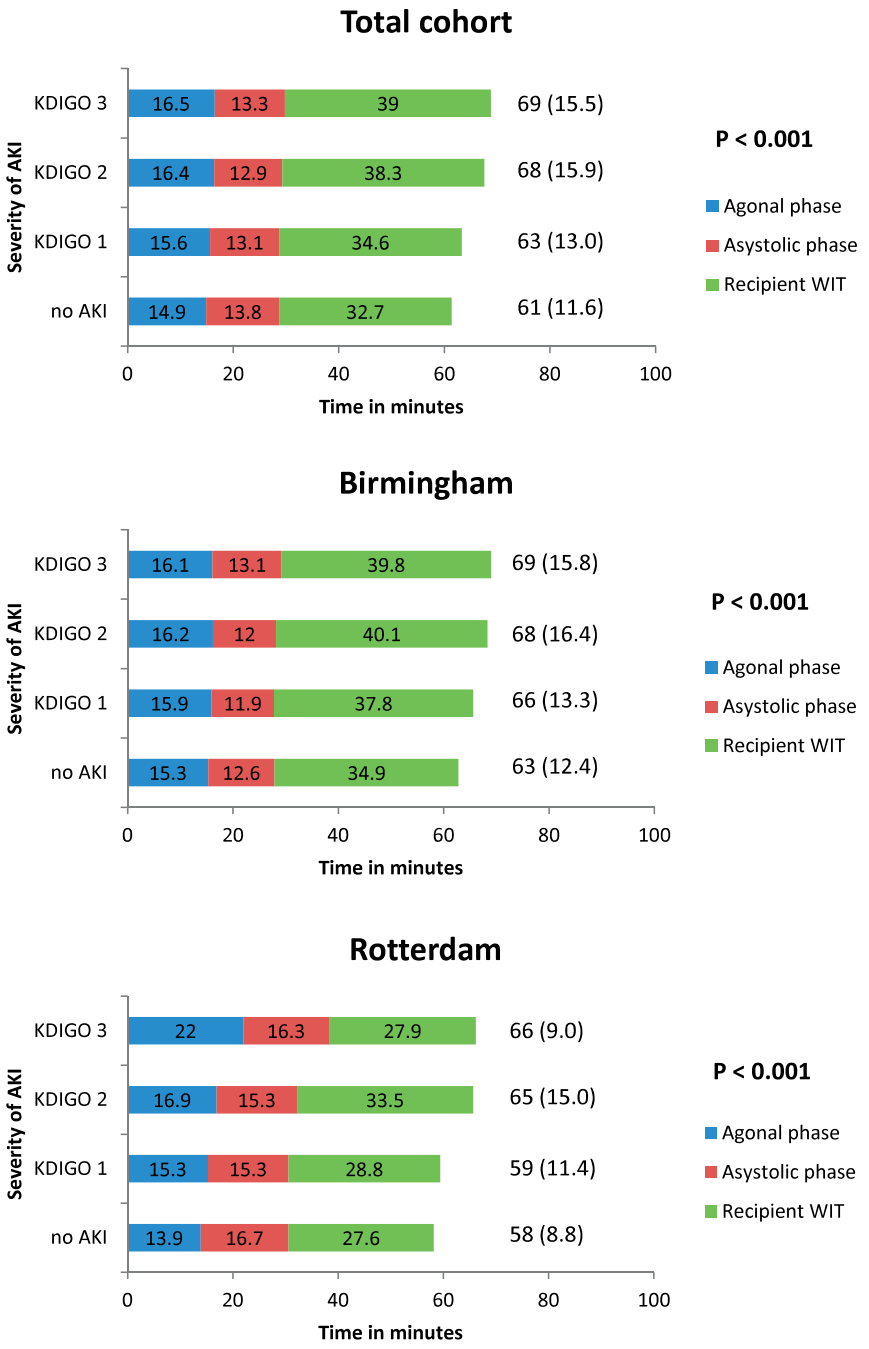

Figure 2 - Mean length of total warm ischemia time and severity of acute kidney injury after DCD liver transplantation

Multivariable analysis for development of severe acute kidney injury

Multiple binary logistic regression was used to identify donor, graft, recipient and surgical factors associated with development of severe AKI (**Table 5**). Independent predictors were an increased recipient BMI (OR 1.09 per kg/m^2; 95%CI 1.03-1.15; p=0.003), transfusion of RBC during liver transplant (OR 1.10 per unit; 95%CI 1.03-1.18; p=0.004) and the length of combined WIT (OR 1.03 per one extra minute; 95% CI 1.01-1.05; p<0.001). Duration of old ischemia time (CIT) was not found to be significantly associated with development of severe AKI (OR 0.98; 95%CI 0.84-1.15; p=0.822).

Table 5 - Univariable and multivariable analysis of factors associated with development of severe acute kidney after DCD liver transplantation

	Unvariable analysis				Multivariable analysis			
Donor	**OR**	**95% CI**		**P-value**	**OR**	**95% CI**		**P-value**
Age (years)	1.008	0.995	1.021	0.238	1.005	0.989	1.021	0.574
Female gender	0.967	0.633	1.477	0.875	0.943	0.570	1.559	0.819
Body mass index	1.069	1.014	1.128	0.014	1.050	0.986	1.118	0.127
Recipient								
Age (years)	1.000	0.999	1.001	0.947	0.999	0.998	1.001	0.420
Female gender	1.114	0.710	1.747	0.002	1.764	0.981	3.173	0.058
Body mass index	1.074	1.026	1.123	0.002	1.086	1.028	1.148	0.003
Diagnosis liver disease								
Viral hepatitis	1.000				1.000			
Biliary cirrhosis	0.575	0.322	1.028	0.062	0.808	0.386	1.692	0.808
Alcohol related cirrhosis	0.805	0.468	1.382	0.432	0.817	0.433	1.541	0.817
NASH	0.741	0.327	1.678	0.472	0.713	0.279	1.820	0.713
Other	0.622	0.294	1.318	0.215	0.867	0.372	2.021	0.867
Hepatocellular carcinoma	1.365	0.891	2.091	0.617	1.001	0.552	1.815	0.998
labMELD	0.957	0.920	0.995	0.029	0.955	0.907	1.006	0.081
eGFR preoperative (ml/min/1.73m2)	1.005	0.998	1.011	0.154	1.007	0.999	1.015	0.075
Graft								
Cold ischemia time (hours)	1.046	0.910	1.203	0.526	0.982	0.842	1.147	0.822
Combined WIT (minutes)	1.035	1.018	1.052	<0.001	1.032	1.014	1.051	<0.001
RBC transfusion (units)	1.080	1.020	1.143	0.008	1.101	1.031	1.175	0.004
Postoperative								
Tacrolimus peak level week 1 (ug/L)	1.016	0.981	1.052	0.006	1.016	0.975	1.058	0.447

CI, confidence interval; eGFR, estimated glomerular filtration rate; MELD, model for end stage liver disease; NASH, non alcoholic steatohepatitis; OR, odds ratio; RBC, red blood cells; WIT, warm ischemia time.

Hepatic IRI, graft-related outcomes and acute kidney injury

The median peak serum ALT level in the first 48 hours, a surrogate marker for hepatic IRI, was comparable in both centers (1775 U/L in Birmingham vs. 1603 U/L in Rotterdam; p=0.595). **Figure 3** shows the relation between peak serum ALT levels and severity of AKI. In both centers, peak ALT levels were associated with severity of AKI. Short term outcomes after transplant in relation to the development of postoperative AKI are shown in **Table 6.** EAD was more frequently observed in recipients with AKI in both centers In addition, graft loss in the first six months after transplantation was 6% in recipients without AKI and increased up to 10% and 20% in recipients with mild and severe AKI, respectively (p=0.002). **Figure 4** displays the KM-curves for the 3-year graft survival (A) and recipient survival (B) for the entire cohort. The overall

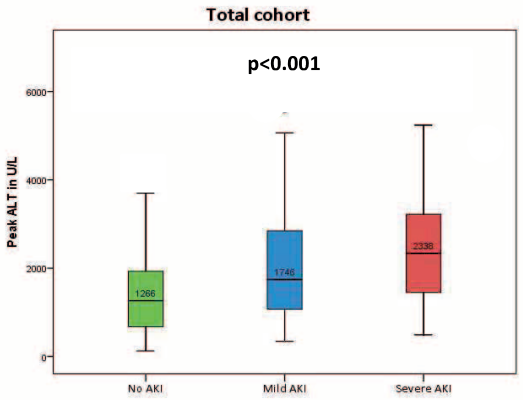

Figure 3 - hepatic IRI and acute kidney injury after DCD liver transplantation

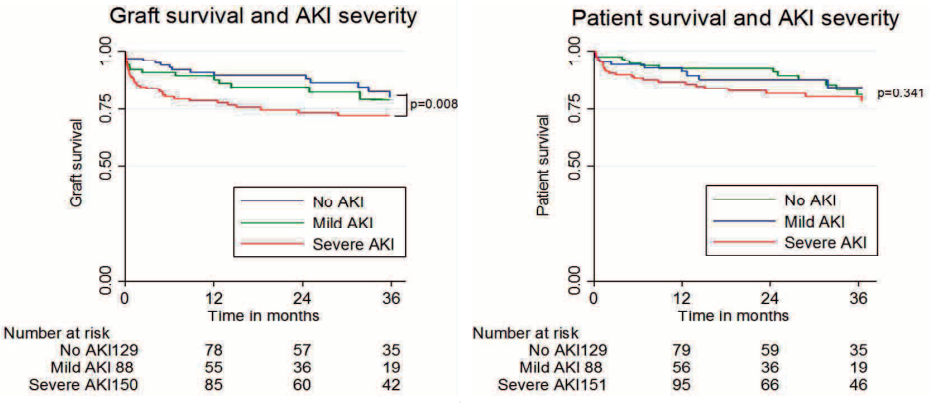

Figure 4 - Acute kidney injury and estimated 3-year graft (left) and recipient (right) survival and severity of acute kidney injury after DCD liver transplantation

Table 6: severity of acute kidney injury and in-hospital and 90 days outcome parameters after DCD liver transplantation

Total cohort	**No AKI (n=129)**		**Mild AKI (n=88)**		**Severe AKI (n=151)**		**P-value**
EAD (%)	42	(33)	34	(39)	88	(58)	<0.001
Length of stay ICU	2	(1-3)	3	(2-4)	5	(3-9)	<0.001
Length of stay hospital	9	(7-15)	12	(8-18)	16	(10-24)	<0.001
6 month graft loss	8	(6)	9	(10)	30	(20)	0.002
Death (%)	2	(2)	4	(5)	12	(8)	
Retransplantation - PNF (%)	2	(2)	2	(2)	7	(5)	
Retransplantation - HAT (%)	2	(2)	2	(2)	9	(6)	
Retransplantation - ITBL (%)	3	(1)	1	(1)	2	(1)	
Birmingham	**No AKI (n=91)**		**Mild AKI (n=56)**		**Severe AKI (n=132)**		
EAD (%)	23	(25)	16	(29)	72	(55)	<0.001
Length of stay ICU	2	(1-3)	3	(2-4)	5	(3-9)	<0.001
Length of stay hospital	8	(7-11)	9	(7-12)	16	(9-23)	<0.001
6 month graft loss	7	(8)	4	(7)	23	(17)	0.040
Death (%)	1	(1)	2	(4)	6	(5)	
Retransplantation - PNF (%)	1	(1)	1	(2)	7	(5)	
Retransplantation - HAT (%)	3	(2)	0		1	(1)	
Retransplantation - ITBL (%)	2	(3)	1	(2)	9	(7)	
Rotterdam	**No AKI (n=38)**		**Mild AKI (n=32)**		**Severe AKI (n=19)**		
EAD (%)	19	(50)	18	(56)	16	(84)	0.041
Length of stay ICU	2	(1-3)	3	(2-4)	6	(2-10)	0.001
Length of stay hospital	15	(14-23)	20	(17-29)	21	(16-29)	0.002
6 month graft loss (%)	1	(3)	5	(16)	7	(37)	0.003
Death (%)	0		3	(9)	3	(16)	
Retransplantation - PNF (%)	0		0		1	(5)	
Retransplantation - HAT (%)	1	(3)	1	(3)	2	(11)	
Retransplantation - ITBL (%)	0		1	(3)	1	(3)	

AKI, acute kidney injury; EAD, early allograft dysfunction; ICU, intensive care unit; LOS, length of stay. Continuous variables are displayed as mean (standard deviation) and median (interquartile range) where appropriate.

3-year estimated graft survival was 76% and decreased significantly with severity of AKI (p=0.019). Recipients without AKI and mild AKI had comparable survival rates (80% and 79%; p=0.491), while the 3-year graft survival decreased significantly for recipients with severe AKI, compared to recipients with no or mild AKI (70% versus 80%; p=0.008). The 3-year overall estimated recipient survival was 81%, but was not significantly associated with AKI stages (p=0.341).

DISCUSSION

AKI has been shown to be an important complication after transplantation of marginal liver grafts and with this study we provide new insight regarding its etiology in the context of DCD liver transplantation (12–14). First, we combined two main European cohorts of DCD liver transplantation and found a significant lower graft survival in recipients with the severe form of AKI. Second, we introduce the combined WIT as an important factor related to the development of AKI in recipients who receive a DCD liver graft, especially the severe form of AKI.

The general definition and consecutive impact of warm ischemia on outcome after DCD liver transplantation is differently interpreted amongst transplant professionals. Here we introduced combined WIT, the period that combines all warm ischemia of a DCD graft, and our results indicate that this period is associated with the development and severity of postoperative AKI. Postoperative AKI in surgery in general is known for its impact on length of stay, costs, and development of CKD (20,33,34). Additionally, severe AKI is associated with higher complication rates, longer ICU and hospital stay and consecutively inferior survival rates (35,36). Therefore, we chose to evaluate mild and severe AKI separately and the results confirm our hypothesis that the severity of AKI impacts recipient outcomes: severe AKI correlated with both early postoperative outcomes and long term graft survival.

This is the first study investigating post-transplant AKI in more than one center. In our analysis, almost two out of three recipients developed AKI. In general, more recipients in Birmingham required RRT, which is started generally at a lower peak creatinine threshold in this center. Despite the similar retrieval and surgical technique in both centers, a few donor and recipient parameters were different. For example, the higher donor age in Birmingham is explained by the maximum age limit of 60 years for DCD liver donors in the Netherlands, while transplant centers in the UK also accept older donors (30,37). The duration of total DWIT was significantly longer in Rotterdam due to a three-minute longer asystolic phase. This is probably the result of the different location of WoT the donor: in the Netherlands, the withdrawal mainly takes place on ICU, while in the UK some withdrawals are performed in the anesthetic room. The association between location of treatment withdrawal and impaired recipient outcome has been previously shown, because prolonged donor transport time from ICU to theatre may impact negatively (38). However, in our study this additional time is already included in the asystolic phase. The allocation system for liver transplantation in the Netherlands follows the MELD principle. In the UK, the

allocation of designated liver grafts is decided by the recipient institution. This creates a window of opportunity for the transplant surgeons to match DCD grafts with a higher risk to recipients with a relatively low labMELD score. In this context, the preoperative labMELD score was lower in Birmingham and these recipients had a better pre-transplant kidney function. As expected, the peak trough tacrolimus levels were higher in Birmingham, due to the earlier introduction of this immunosuppressant. Our multivariable analysis identified an increased recipient BMI and transfusion requirement during surgery as independent factors associated with development of severe AKI. Recipient BMI has previously been linked to post-transplant AKI and transfusion requirements represent the amount of blood loss and hypotensive periods during surgery, which are also factors known to damage the kidney (16,17,20,39–41). The last factor identified by our model was the length of combined WIT. Warm ischemia is not only well known to significantly aggravate hepatic IRI, previous studies also showed a link to the development of AKI after liver transplantation (13,15,42). In our cohort, we used the peak serum ALT levels as a surrogate marker for hepatic IRI and these levels correlated well with the extent of AKI in both centers.

Hepatic IRI during DCD liver transplantation is the result of four consecutive periods of warm and cold ischemia. The exact impact of each period on development of AKI remains unclear. Interestingly, the duration of the asystolic phase and cold ischemia time were not found to be significantly associated with AKI. Longer asystolic phase has previously been related to impaired graft survival, but only when it exceeds 25 or 30 minutes (8,43). This could explain why our relatively short asystolic phase (mean of 13 minutes) was not associated with hepatic IRI or AKI. Also, in contrast to the agonal phase, the range of length of asystolic phase is relatively small, as the retrieval procedure is a standardized surgical procedure in these two centers with experience in DCD retrievals. Previous studies have not identified cold ischemia as an influencing factor for AKI or graft survival in DCD liver transplantation and, with a mean of only 7 hours, the cold ischemia duration was relatively limited in our cohort (13,44). Due to the lack of transplant cases with a prolonged cold ischemia in our study, larger cohort studies with a wider range of cold ischemia would be of interest to further support this result. RWIT has been related to AKI before, but this is the first analysis of the relation between DWIT and postoperative AKI (40,45). Several studies have shown a relation between DWIT and postoperative outcomes: a prolonged course of agonal phase with a slow decrease of vital parameters has been related to impaired graft survival, while a longer asystolic phase is associated with ischemic cholangiopathy and impaired graft survival (8,21,23,24,43). In our cohort, the structure of combined WIT and severity of AKI varied between the two centers. Length of agonal phase was

significantly associated with postoperative AKI in Rotterdam, but not in Birmingham. On the contrary, length of RWIT was longer in Birmingham and increased significantly with severity of AKI, whilst there was no significant relation between RWIT and AKI in Rotterdam. In donors with prolonged WIT, the transplant surgeon could limit the combined WIT by choosing a technically easier transplant candidate with quicker graft implantation, to further reduce the risk of severe AKI. In contrast, when DWIT is short, there is more time left for graft implantation. Both scenarios could be of interest in the context of technically challenging recipients or teaching scenarios. One advantage of the combined WIT is, that the overall WIT is included in one parameter, where our analysis showed that a prolonged combined WIT significantly predicts the development of more severe AKI in both centers.

This study has several limitations. The retrospective design of this study has its inherent shortcomings. A total of 27 cases were excluded because of missing data, 17 of them due to missing information on DWIT. This is a known problem in DCD liver transplantation, because in the early years of this study not all DWIT data were registered. The two-center study design has several advantages, but several potential confounders should be addressed: comparisons of the two cohorts in this study confirmed that there are differences in daily practice and protocols between transplant programs that influence postoperative development of AKI. For example, in Birmingham tacrolimus is introduced at day 0 after transplant, while in Rotterdam most of the recipients received tacrolimus from day 5. However, in the multivariable analysis, the peak tacrolimus trough level was not found to be significantly associated with development of severe AKI, probably because recipients who are suspected to experience renal injury receive a low-dose or late introduction of tacrolimus. Also, the treatment of recipients with severe AKI was more aggressive in Birmingham, as shown in the lower median serum creatinine level at start of RRT. This could result in a relatively larger proportion of recipients in the AKI stage 3 group in this center, that would not receive RRT in Rotterdam and be assigned to the AKI stage 2 group. Therefore, we bundled the recipients with AKI stage 2 and 3 into one group as severe AKI.

The potential amount and impact of ischemic graft injury varies during the different types of warm ischemia in DCD grafts. The injury, conveyed through a slow decrease of oxygen and graft cooling in the donor might be in contrast to the rewarming injury during graft implantation prior to reperfusion. Unfortunately, retrospective studies are not ideal for this purpose and in order to separately assess the impact of donor or recipient WIT with and without remaining blood in the liver, experimental transplant

studies might be of further benefit. However, this study is the first step into large multicenter cohort studies on the etiology of AKI after DCD liver transplantation. The results of this study help to earlier anticipate a high risk for later kidney injury and to implement kidney protective strategies to prevent additional injury during or after transplantation. For example, a long agonal phase could be used as a threshold to use novel preservation techniques, such as machine perfusion after or instead of cold storage, and to limit the duration of RWIT through a quick implantation of the DCD graft by an experienced surgeon (46,47). When combined WIT exceeds 60 minutes, preventive management can be undertaken to limit further AKI, such as adjustment of the immunosuppression regimen with delayed introduction of calcineurin inhibitors. Combined WIT is a new concept and its relation to other postoperative complications in DCD liver transplantation should be investigated as well.

In conclusion, this study provides new insight in the impact of warm ischemia during DCD liver transplantation and the development of post-transplant AKI. We introduced combined WIT as a new variable to explore the impact of the total burden of warm ischemia. Although the structure of warm ischemia varies, the duration of the combined WIT is important in the development of severe postoperative AKI and should ideally not exceed 60 minutes.

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