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

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CHAPTER 6

The effect of arterialization time on outcomes in DCD liver transplantation

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ABSTRACT

Background and aim: In comparison to liver transplantations (LT) with grafts donated after brain death (DBD), LT with grafts obtained after circulatory death (DCD) face a higher risk of developing biliary complications. Since the biliary tree relies solely on arterial blood supply, it is questioned whether an initial arterial reperfusion (IAR) approach could lead to less biliary complications. In the vast majority of LT however, an initial portal vein reperfusion (IPR) approach is used. Therefore, the aim of this study was to assess the influence of the additional time between portal and arterial reperfusion on outcomes after DCD-LT.

Patients and methods: All controlled DCD-LT with IPR performed in the Netherlands between 2001 and 1st of June 2018, were included. The primary endpoint was the development of non-anastomotic strictures (NAS) after DCD-LT. Secondary endpoints were graft failure and patient death. Cox Proportional-Hazard regression analyses were used to assess the influence of arterialization time on all endpoints.

Results: A total of 289 DCD-LT were included. Median arterialization time was 33 minutes (interquartile range 25 - 48). A prolonged arterialization time was not a significant risk factor for the development of NAS, patient death or graft failure. Donor age and cold ischemia time were both significant risk factors for the development of NAS.

Conclusions: The time between portal and arterial reperfusion in DCD-LT is not a significant risk factor for developing NAS, patient death and graft failure.

INTRODUCTION

As a result of the ongoing critical organ shortage, the use of grafts from donation after circulatory death (DCD) donors has substantially increased in Europe. In 2019, the proportion of DCD liver transplantations (LT) among all deceased donor LT in the Eurotransplant (ET) region exceeded 20% for the first time[1]. DCD donation is not yet widely practiced and in fact, Austria, Belgium and the Netherlands are currently the only countries within the Eurotransplant region that permit DCD donation.

LT with DCD grafts (DCD-LT) are known to have inferior post-transplant graft survival rates compared to liver transplantations performed with grafts donated after brain death (DBD) [2,3]. This is mainly attributed to the fact that DCD livers are more prone to developing post-transplant cholangiopathy[4-6]. Non-anastomotic strictures (NAS), also known as ischemic cholangiopathy (IC) or ischemic type biliary lesions (ITBL) are the most severe biliary complications and frequently result in re-transplantation[7,8].

An important underlying mechanism in the development of NAS is ischemia-reperfusion (IR) injury. During ischemia, adenosine triphosphate (ATP) in cholangiocytes is depleted which eventually leads to cell swelling and lysis. Moreover, ischemia itself can lead to irreversible damage of the peribiliary glands, impairing their capacity to regenerate the biliary epithelium[9]. During subsequent reperfusion, formation of reactive oxygen species (ROS) leads to the activation of an inflammatory cascade[10]. This then results in apoptosis and necrosis of cholangiocytes and subsequent loss of the biliary epithelium. Previous studies have shown that cholangiocytes are more susceptible to IR injury than hepatocytes[11].

Since the biliary tree relies solely on arterial blood supply by both the hepatic artery and branches from the gastroduodenal artery[12], it is questioned whether minimizing the biliary ischemia time by performing initial artery reperfusion (IAR) i.e. reconstruction of hepatic artery followed by reconstruction of the portal vein, could result in a lower incidence of post-transplant cholangiopathy. This would be especially beneficial in the higher risk liver grafts such as DCD grafts. However, in majority of liver transplants across the Eurotransplant region, initial portal vein reperfusion (IPR) is performed[13-15]. This is mainly because portal

vein anastomosis is normally less complex and less time-consuming thereby minimizing the anhepatic phase in the recipient. Moreover, portal blood flow alone is sufficient for the liver to adequately resume metabolic and synthetic activity[16].

Based on two recent published meta-analyses, there seems to be no difference in the occurrence of NAS between grafts revascularized through IPR or IAR approaches[17,18] However, all of the included studies involved livers derived from DBD donors, making it difficult to extrapolate these results to DCD transplantation.

In absence of validated clinical evidence regarding the most protective reperfusion approach, we aimed to determine the effect of the additional period of arterial ischemia between reperfusion of the portal vein and hepatic artery on the development of NAS as well as on graft and patient survival. We hypothesize that a prolonged arterialization time results in a higher incidence of NAS in DCD liver transplantation.

METHODS

In this national, retrospective cohort study, all adults who underwent primary liver transplantation with a DCD graft in the Netherlands between the start of the first DCD program in 2001 and the first of June 2018 were included. Multi-organ transplantation, re-transplantation, transplantation with split livers and grafts that underwent machine perfusion were excluded from this study. Furthermore, cases with an IAR approach or IPR approach with missing information on the time between portal and arterial reperfusion were excluded. This study was approved by the institutional review board of the Erasmus MC University Medical Center Rotterdam (MEC-2019-0434).

Data collection and definitions

The data for this study were retrieved from the local, prospectively maintained databases from the three liver transplant centers in the Netherlands. In the case of missing data, individual medical records or the Eurotransplant online application DonorData were consulted. Arterialization time was defined as the additional time between the reperfusion of

the portal vein and the removal of the cross clamp of the hepatic artery. The definition of donor warm ischemia time was the time between the circulatory arrest in the donor and the start of the cold perfusion of the donor liver graft. Cold ischemia time was defined as the time between the start of cold perfusion in the donor and the liver being removed from ice during implantation in the recipient. The definition of recipient warm ischemia time (rWIT) used in this study is the interval between removal of the liver from ice and portal reperfusion.

Primary and secondary outcomes

The primary endpoint of this study was the occurrence of non-anastomotic strictures (NAS) after DCD-LT. NAS was defined as donor bile duct strictures located anywhere within the biliary tree, with exception of at the anastomosis, and in absence of hepatic artery thrombosis. Furthermore, patients had to have clinical symptoms of cholestasis with subsequent imaging demonstrating the stricture. Secondary endpoints were graft failure and patient survival, which were defined as re-transplantation and patient death, respectively.

Statistical analysis

Continuous and categorical variables are presented as median (interquartile range) and frequency (valid percentage), respectively. To evaluate the influence of arterialization time on the development of NAS, as well as on graft and patient survival, univariate and multivariate Cox Proportional-Hazards regression models were used. In all multivariate, backward stepwise models, arterialization time was included, regardless of the p-value in univariate analysis. Co-variables were included in the multivariate, backward stepwise Cox model if univariate Cox regression yielded a p-value < 0.20. The threshold of 0.20 was chosen to decrease the risk of overfitting of the model. The reported hazard ratios (HR) for arterialization time refer to an increase of ten minutes in arterialization time. For the cold ischemia time and recipient warm ischemia time, the HR represents an increase of one hour and ten minutes, respectively. All statistical analyses were performed in SPPS, version 25

(SPSS Inc. Chicago, IL, USA). A p-value below 0·05 was considered as statistically significant.

RESULTS

A total of 289 DCD liver transplants were included in this study. The median follow up period of the complete cohort was 4.3 years (IQR 2.5 - 7.9). Baseline characteristics are presented in Table 1. Fifty-five percent of the donors were male and the median age of all donors was 47 years (IQR 37 - 53). The cause of death for the majority of the donors was a cerebrovascular accident (CVA), followed by trauma (42.6% and 25.6%, respectively). The median dWIT was 16 minutes (IQR 13 – 18).

The majority of the DCD-LT recipients were male (68.2%). Hepatocellular carcinoma was the most common primary indication for transplantation in this cohort (34.6%). The median laboratory MELD score at time of transplantation was 15 (IQR 10 – 20). The median CIT and rWIT were 420 minutes (IQR 362 - 479) and 33 minutes (IQR 26 - 40), respectively. Median arterialization time was 33 minutes (IQR 25 - 48).

Graft survival rates at one- three- and five-year follow up were 76%, 63% and 58%, respectively. A total of 60 patients required a re-transplantation during the follow up period and post-transplant cholangiopathy was the most common indication for a re-transplantation among these recipients. During follow-up, 131 recipients (45.3%) developed at least one biliary complication (i.e. anastomotic stricture, bile leakage and/or non-anasatomotic strictures). Seventy five patients (26.0% of the total cohort n= 289) were diagnosed with NAS, of which the majority (58 out of 75, 77.3%) was diagnosed during the first year post-transplant. Actuarial one-, three- and five-year patient survival rates in the total cohort were 89%, 79% and 74%.

Table 1: Baseline donor, recipient, transplantation and post-operative characteristics

Characteristic	Result (n=289)
Donor and procurement	
Age (years)	47.0 (37.0-53.0)
Gender	
Male	159 [55.0%]
Female	130 [45.0%]
Body mass index (kg/m^2)	24.0 (22.0-26.0)
Cause of death	
CVA	123 [42.6%]
Trauma	74 [25.6%]
Anoxia	67 [23.2%]
Other	25 [8.7%]
Last AST	44.0 (28.5-83.5)
Last ALT (U/L)	32.0 (20.0-62.0)
Last γGT (U/L)	31.5 (19.0-66.0)
Donor warm ischemia time (min)*	16.0 (13.0-18.0)
Recipient	
Age (years)	56.0 (46.0-62.0)
Gender	
Male	197 [68.2%]
Female	92 [31.8%]
Body mass index (kg/m^2)	25.9 (23.7-28.9)
Indication for LT	
HCC	100 [34.6%]
Cholestatic liver diseases (PBC/PSC)	55 [19.0%]
Alcoholic liver disease	34 [11.8%]
Viral hepatitis related cirrhosis	20 [6.9%]
NASH	20 [6.9%]
Cryptogenic liver cirrhosis	17 [5.9%]
Other	43 [14.9%]
Laboratory MELD score	15.0 (10.0-20.0)
Transplantation	
Cold ischemia time (min)†	420 (362-479)
Recipient warm ischemia time (min)‡	33.0 (26.0-40.0)
Arterialization time (min)§	33.0 (25.0-48.0)
Type of biliary anastomosis	
Duct to duct	253 [89.7%]
Hepaticojejunostomy	29 [10.3%]
Blood loss (mL)	3500 (2100-5500)
Post-operative	
Bilirubin day 7 (µmol/L)	35.0 (18.0-88.0)
AST peak (u/L)	2204 (1158-4263)
ALT peak (u/L)	1645 (808-2904)
Intensive care unit stay (days)	2.0 (1.0-5.0)
Total hospital stay (days)	19.0 (14.0-29.5)

Values are presented as median (interquartile range) or number [%].

**The time between circulatory arrest and cold flush in the donor. †The time between the start of the cold perfusion in the donor and the removal of the liver from ice prior to implantation. ‡The time*

between removal of the liver from ice until portal reperfusion. §The time between portal and arterial reperfusion. ALT, alanine aminotransferase; , AST; aspartate aminotransferase, CVA , cerebrovascular accident; γGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Table 2 depicts the multivariable Cox Proportional-Hazards regression model for the development of NAS. Arterialization time was not a significant risk factor for the development of NAS after transplantation. However, donor age (HR 1.029) and cold ischemia time (HR 1.167) significantly increase the risk for the development of NAS. Furthermore, higher levels of alanine transaminase in the donor seemed to provide a protective effect on the development of NAS (HR 0.991, 95% CI 0.984 – 0.999, p-value 0.023) (Table 2).

In regard to graft failure, arterialization time yielded no significance in both univariate and multivariate models (Table 3). In the multivariate model, donor age was a significant risk factor for graft failure (HR 1.022, 95% CI 1.007 – 1.037, p-value 0.003). Similarly, in both univariate and multivariate regression models, arterialization time was not a statistically significant risk factor for patient death (Table 4). Recipient age was the only significant risk factor for patient death (Hazard ratio 1.029, 95% confidence interval 1.007 – 1.051, p-value = 0.009).

Table 2: Univariate and multivariate Cox Proportional-Hazards regression model for the development of non-anastomotic strictures

	Univariate			Multivariate		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Donor and procurement						
Age (years)	1.034	1.013 – 1.056	0.001	1.029	1.008 – 1.051	***0.007***
Gender						
Male	REF					
Female	0.824	0.519 – 1.308	0.451			
Body mass index (kg/m^2)	0.981	0.918 – 1.048	0.575			
CVA as cause of death						
No	REF					
Yes	1.412	0.898 – 2.222	0.135			
Last AST	0.993	0.987 – 0.999	0.021	Removed from model		0.995
Last ALT (U/L)	0.990	0.983 – 0.998	0.010	0.991	0.984 – 0.999	***0.023***
Last γGT (U/L)	1.001	0.999 – 1.003	0.529			
Donor warm ischemia time (min)*	1.034	0.986 – 1.084	0.164	Removed from model		0.203
Recipient						
Age (years)	0.992	0.973 – 1.011	0.412			
Gender						
Male	REF					
Female	0.947	0.576 – 1.557	0.829			
Body mass index (kg/m^2)	1.015	0.966 – 1.068	0.548			
HCC as indication for transplantation						
No						
Yes	0.759	0.461 – 1.248	0.277			
Laboratory MELD score	0.995	0.968 – 1.023	0.730			
Transplantation						
Cold ischemia time (hours)‡	1.147	1.003 – 1.311	0.044	1.167	1.020 – 1.335	***0.025***
Recipient warm ischemia time (min)§	1.090	0.908 – 1.309	0.354			
Arterialization time (min)	1.095	0.995 – 1.206	0.063	Removed from model		0.174
Type of biliary anastomosis						
Duct to duct	REF					
Hepaticojejunostomy	1.067	0.512 – 2.227	0.862			

Multivariable model was conducted via backward stepwise approach.

**The time between circulatory arrest and cold flush in the donor, reported hazard ratio (HR) refers to an increase in donor warm ischemia time of one minute. †The time between the start of the cold perfusion in the donor and the removal of the liver from ice prior to implantation, reported HR refers to a one hour increase in cold ischemia time. ‡The time between removal of the liver from ice until portal reperfusion, reported HR refers to an increase in recipient warm ischemia time of ten minutes. §The time between portal and arterial reperfusion, reported HR refers to a ten minutes increase of the arterialization time. ALT, alanine aminotransferase; , AST; aspartate aminotransferase, CVA , Cerebrovascular accident; γGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease.*

Table 3: Univariate and multivariate Cox Proportional-Hazards regression model for graft failure

	Univariate			Multivariate		
	HR	95% CI	p-value	HR	95% CI	p-value
Donor and procurement						
Age (years)	1.021	1.006 – 1.036	0.006	1.022	1.007 – 1.037	***0.003***
Gender						
Male	REF					
Female	1.023	0.727 – 1.440	0.896			
Body mass index (kg/m^2)	0.995	0.945 – 1.046	0.836			
CVA as cause of death						
No	REF					
Yes	1.183	0.841 – 1.665	0.335			
Last AST	0.996	0.992 – 1.000	0.060	Removed from model		0.449
Last ALT (U/L)	0.998	0.995 – 1.002	0.345			
Last γGT (U/L)	0.998	0.996 – 1.001	0.161	0.998	0.995 – 1.001	0.125
Donor warm ischemia time (min)*	1.033	0.996 – 1.071	0.081	1.033	0.997 – 1.071	0.072
Recipient						
Age (years)	0.994	0.979 – 1.009	0.413			
Gender						
Male	REF					
Female	0.834	0.569 – 1.223	0.353			
Body mass index (kg/m^2)	1.020	0.981 – 1.061	0.312			
HCC as indication for transplantation						
No	REF					
Yes	1.091	0.759 – 1.570	0.637			
Laboratory MELD score	1.004	0.985 – 1.023	0.687			
Transplantation						
Cold ischemia time (hours)‡	1.088	0.978 – 1.210	0.120	Removed from model		0.102
Recipient warm ischemia time (min)§	1.039	0.897 – 1.204	0.609			
Arterialization time (min)	0.980	0.896 – 1.072	0.660	Removed from model		0.422
Type of biliary anastomosis						
Duct to duct	REF					
Hepaticojejunostomy	1.281	0.758 – 2.164	0.355			

Multivariable model was conducted via backward stepwise approach.

**The time between circulatory arrest and cold flush in the donor, reported hazard ratio (HR) refers to an increase in donor warm ischemia time of one minute.*
†The time between the start of the cold perfusion in the donor and the removal of the liver from ice prior to implantation, reported HR refers to a one hour

increase of cold ischemia time. ‡The time between removal of the liver from ice until portal reperfusion, reported HR refers to an increase in recipient warm ischemia time of ten minutes. §The time between portal and arterial reperfusion, reported HR refers to a ten minutes increase of the arterialization time. ALT, alanine aminotransferase; , AST; aspartate aminotransferase, CVA , Cerebrovascular accident; γGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease.

Table 4: Univariate and multivariate Cox Proportional-Hazards regression model for patient death

	Univariate			Multivariate		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Donor and procurement						
Age (years)	1.014	0.997 – 1.032	0.116	1.016	0.997 – 1.034	0.096
Gender						
Male	REF					
Female	1.026	0.672 – 1.563	0.905	Removed from model		0.128
Body mass index (kg/m^2)	1.018	0.958 – 1.083	0.560			
CVA as cause of death						
No	REF					
Yes	0.978	0.641 – 1.492	0.918			
Last AST	0.999	0.995 – 1.004	0.824			
Last ALT (U/L)	1.000	0.997 – 1.003	0.784			
Last γGT (U/L)	0.999	0.996 – 1.002	0.371			
Donor warm ischemia time (min)*						
Recipient						
Age (years)	1.026	1.005 – 1.047	0.014	1.029	1.007 – 1.051	***0.009***
Gender						
Male	REF					
Female	0.590	0.355 – 0.981	0.042			
Body mass index (kg/m^2)	1.039	0.994 – 1.086	0.091	Removed from model		0.301
HCC as indication for transplantation						
No	REF					
Yes	1.574	1.015 – 2.441	0.043	Removed from model		0.296
Laboratory MELD score	0.998	0.975 – 1.023	0.902			
Transplantation						
Cold ischemia time (hours)†	0.981	0.854 – 1.128	0.790			
Recipient warm ischemia time (minutes)‡	1.159	0.983 – 1.366	0.078	1.187	0.998 – 1.412	0.053
Arterialization time (minutes)§	0.983	0.887 – 1.090	0.745	Removed from model		0.121
Type of biliary anastomosis						

Duct to duct Hepaticojejunostomy	REF 0.609	0.280 – 1.324	0.210			

Multivariable model was conducted via backward stepwise approach.

**The time between circulatory arrest and cold flush in the donor, reported hazard ratio (HR) refers to an increase in donor warm ischemia time of one minute. †The time between the start of the cold perfusion in the donor and the removal of the liver from ice prior to implantation, reported HR refers to a one hour increase of cold ischemia time. ‡The time between removal of the liver from ice until portal reperfusion, reported HR refers to an increase in recipient warm ischemia time of ten minutes. §The time between portal and arterial reperfusion, reported HR refers to a ten minutes increase of the arterialization time. ALT, alanine aminotransferase; , AST; aspartate aminotransferase, CVA , Cerebrovascular accident; γGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease.*

DISCUSSION

Based on the results of this national, retrospective cohort study, we concluded that the additional time between portal and arterial reperfusion has no impact on the development of NAS or on the risk of graft failure and patient death in DCD-LT with an initial portal reperfusion approach. This implies that transplant surgeons need not worry that the development of NAS is due to prolonged arterialization times.

The finding that arterialization time has no impact on the development of NAS, is in line with results from studies conducted by Rammohan and colleagues in 2016 and Cag et al. in 2010[19,20]. Both these studies investigated the effect of arterialization time on the development of NAS in a general cohort of living- and deceased-donor liver transplantation procedures. In a multivariate analysis, Rammohan et al. found that arterialization time was not a significant risk factor for the development of biliary complications[19]. Similarly, Cag and colleagues also found no differences in arterialization time between LT recipients with and without post-transplant cholangiopathy[20]. Furthermore, both Cag et al. and Rammohan et al. showed that the cold ischemia time was a significant risk factor for the development of biliary complications and emphasized the importance of limiting the CIT to as short as possible. Given that CIT is indeed a risk factor for developing NAS in our cohort, we fully support this statement. Similar to the findings from the aforementioned studies, our results showed that donor age is associated with an increased risk of developing NAS after transplantation[21]. These results further reiterate the importance of limiting the use of advanced-age donor organs given the increased risk of post-operative complications. An interesting observation is the protective effect of higher (last) levels of donor alanine transaminase on the development of NAS. We believe this finding is of minimal clinical relevance given that a large majority of the cohort (91.3%) had relatively low ALT levels (<100 IU/L), which is classified as a mild elevation of transaminases[22].

The liver possesses a double afferent blood supply in which 75% to 80% of the blood entering the liver is supplied by the portal vein whilst the hepatic artery accounts for the

remaining 20% to 25%[23]. Both of these afferent systems merge at the hepatic sinusoidal bed and thus in the event of disrupted or absent flow in one of the systems, for instance, upon initial portal or arterial reperfusion during transplantation, the physiological shunts in the sinusoidal bed allow for widespread blood supply throughout the liver. This may perhaps explain why prolonged arterialization does not result in an increased risk of developing NAS. It is plausible that through the physiological shunting within the sinusoidal bed, the hepatic artery supply to the biliary tree is fed with oxygenated blood from the portal venous supply and thus minimizing the further ischemic insult to the bile ducts. Nevertheless, in order to accurately assess which reperfusion approach is most protective against the development of NAS, and whether this also results in higher rates of graft and patient survival, a randomized controlled trial in which DCD liver recipients are randomly assigned to either undergoing liver transplantation with an IPR or IAR approach is necessary. In the meantime however, a retrospective cohort study in which DCD-LT procedures revascularized with IAR approach are compared with matched IPR cases is currently ongoing. Perhaps this study may bring forth more insight into the most protective reperfusion approach.

This study admittedly has a few limitations. Firstly, the retrospective design of the study limits the validity of the results and thus definite conclusions cannot be drawn. Moreover, the time of arterial reperfusion or arterialization time was not always documented by the transplant surgeon or anesthesiologists. Therefore, a number of cases unfortunately were excluded from the analysis. Nonetheless, this study is strengthened by the fact that arterialization time was incorporated into the model as a continuous variable instead of a categorical variable with pre-specified cutoff points. The latter would have led to a significant loss of valuable information.

In conclusion, DCD liver transplantation in which an initial portal reperfusion approach is utilized has no significant effect on the development of non-anastomotic strictures, graft failure or patient death. However, since cold ischemia time is a significant risk factor for developing NAS, CIT should be kept as short as possible to minimize the risk of NAS. Further research comparing an initial portal reperfusion technique with an initial arterial

reperfusion technique is highly recommended in the DCD population, particularly in cases in which it is expected that the CIT will be prolonged.

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