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ORIGINAL ARTICLE

High peak alanine aminotransferase determines extra risk for nonanastomotic biliary strictures after liver transplantation with donation after circulatory death

A. Claire den Dulk,[1,†] Kerem Sebib Korkmaz,[1,†] Bert-Jan F. de Rooij,[1] Michael E. Sutton,[2] Andries E. Braat,[3] Akin Inderson,[1] Jeroen Dubbeld,[3] Hein W. Verspaget,[1] Robert J. Porte[2] and Bart van Hoek[1]

1 Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, the Netherlands
2 Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands
3 Department of Transplant Surgery, Leiden University Medical Center, Leiden, the Netherlands

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biliary strictures, ischemia, liver transplantation, nonanastomotic, reperfusion.

Correspondence
Prof. Dr. Bart van Hoek, MD, PhD, Department of Gastroenterology and Hepatology, C4-P, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands.
Tel.: 31 71 526 1852;
fax: 31 71 524 8115;
e-mail: B.van_Hoek@lumc.nl

†Contributed equally.

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Summary

Orthotopic liver transplantation (OLT) with donation after circulatory death (DCD) often leads to a higher first week peak alanine aminotransferase (ALT) and a higher rate of biliary nonanastomotic strictures (NAS) as compared to donation after brain death (DBD). This retrospective study was to evaluate whether an association exists between peak ALT and the development of NAS in OLT with livers from DBD (n = 399) or DCD (n = 97) from two transplantation centers. Optimal cutoff value of peak ALT for risk of development of NAS post-DCD-OLT was 1300 IU/l. The 4-year cumulative incidence of NAS after DCD-OLT was 49.5% in patients with a high ALT peak post-OLT, compared with 11.3% in patients with a low ALT peak. ($P < 0.001$). No relation between peak ALT and NAS was observed after DBD-OLT. Multivariate analysis revealed peak ALT ≥1300 IU/l [adjusted hazard ratio (aHR) = 3.71, confidence interval (CI) (1.26–10.91)] and donor age [aHR = 1.04, CI 1.00–1.07] to be independently associated with development of NAS post-DCD-OLT. A peak ALT of <1300 IU/l carries a risk for NAS similar to DBD-OLT. Thus, in DCD-OLT, but not in DBD-OLT, peak ALT discriminates patients at high or low risk for NAS.

Introduction

Orthotopic liver transplantation (OLT) has evolved into a routine treatment for advanced liver disease with excellent short- and long-term survival [1,2]. An increasing number of patients eligible for liver transplantation and a decreasing number of donors after brain death (DBD) have led to the extension of criteria for acceptance of potential liver grafts in the last decade [3,4]. OLT with livers from donation after circulatory death (DCD) has become common in the Netherlands and the United Kingdom. However, OLT with a liver from a DCD donor carries a high risk for development of nonanastomotic biliary strictures (NAS) [5]. NAS can occur in up to 13–34% after OLT with a DCD donor and is considered a major cause of morbidity and reduced graft survival [6–8]. Early recognition of an increased risk to develop NAS may be valuable for timely intervention.

Besides genetic factors, such as CCR5Δ32 and matrix-metalloproteinase 2 polymorphism, several ischemic parameters have been related to the development of NAS. For example, cold ischemia time (CIT) and warm ischemia times have been described as potential predictors of NAS in DCD and DBD donors, but clinical use of these parameters for predicting NAS has remained controversial [9–13].

Livers from DCD have an inevitable donor warm ischemia time (DWIT) between cardiac arrest and organ preservation, which may lead to a higher degree of ischemia–reperfusion injury (IRI) in the first week after OLT. It is likely that the higher incidence of NAS after OLT with DCD livers is largely the results of the additional ischemia–reperfusion injury due to the DWIT [14–16]. We therefore hypothesized that a relation may exist between injury of the liver parenchyma, reflected by first week post-OLT peak aminotransferases, and the risk for NAS development during follow-up, especially after DCD-OLT, as evaluated in a cohort from two independent centers.

Materials and methods

A total of 519 first consecutive OLTs for chronic liver diseases were performed in two liver transplantation centers. After exclusion of 23 patients with primary nonfunction or a minimum follow-up of <7 days, 496 patients were included in the analysis. From the Leiden University Medical Center (LUMC, center A), a total of 174 OLTs could be included in the time period of October 2001 until March 2011 next to 322 OLTs from the University Medical Center Groningen (UMCG, center B) performed in the time period of July 2000 until June 2012. This included OLTs using livers from DBD as well as DCD donors (Fig. 1). Patient follow-up was determined as time of transplantation until the first occurring event (i.e., development of NAS, retransplantation, or death) or in case of no event, until July 2012.

In 2001, a national protocol was introduced in the Netherlands regarding the acceptance of organs from donation after circulatory death [17]. In this protocol, only Maastricht category 3 donors below 55 years of age, with a DWIT below 30 min, a maximum of 15 min between respiratory withdrawal and cardiac arrest, a body mass index of <28, and a mean arterial pressure of <50 mm Hg for maximum 15 min before cardiac arrest were accepted. DCD donors were therefore selected in both centers according to this protocol.

Donor surgery

In case of DCD donors, a DWIT was measured, defined as the time between circulatory arrest and cold flush with preservation fluid in the donor. Cold ischemia time was defined as the time between cold flush with preservation fluid in the donor and removal of the liver from ice during the transplantation procedure. The recipient warm ischemia time was defined as the time of removal of the donor liver from ice until reperfusion of the donor liver in the recipient. In both centers, University of Wisconsin (UW) preservation fluid was used to flush out DBD liver grafts and in case of DCD liver grafts mainly histidine–tryptophan–ketoglutarate (HTK) was used.

Recipient surgery and routine follow-up

In both centers, OLT with standard technique of 'piggyback' cavo-caval anastomosis, porto-portal and hepatic artery to hepatic artery anastomosis was performed in most recipients. In some cases, the hepatic artery was anastomosed to the aorta via an iliac conduit. A duct-to-

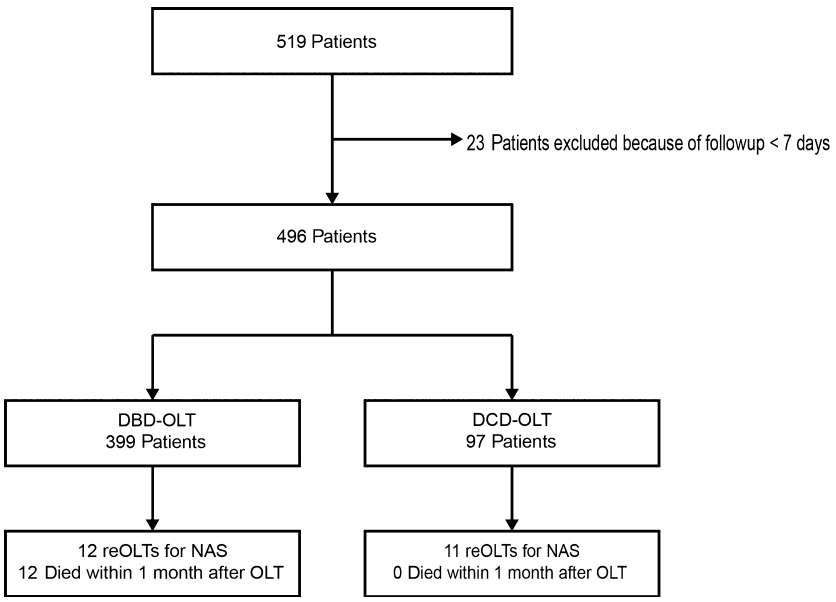

Figure 1 Flowchart of patient inclusion and exclusion.

duct biliary anastomosis—over a 8–12 Ch stent in center A, no stent in center B—was performed if possible. The biliary stent was removed endoscopically with endoscopic retrograde cholangiography (ERC) at 6 weeks or earlier as indicated. In general, post-transplantation care for both centers was comparable. Centers only differed in the use of prophylactic antibiotics, as was previously described [18]. In center A, a combination of gentamicin, cefuroxime, penicillin G, and metronidazole was used with an additional 3 weeks of selective digestive tract decontamination (polymyxin/neomycin, norfloxacin and amfotericin B). In center B, patients received prophylactic amoxicillin–clavulanate and ciprofloxacin. In the first year, blood liver biochemistry was performed daily in the first 2 weeks, weekly in the following 2 weeks, monthly thereafter in the first year, and then every 3 months. In both cohorts, ultrasound (US) was performed routinely on day 0, 1, and 7, and subsequently at 3, 6, 12 months, and yearly after OLT. ERC or magnetic resonance cholangiography (MRC) and other imaging studies were performed when indicated. A liver biopsy was performed per protocol at 6 months in center A and further as indicated in both centers. Pre-OLT baseline parameters, including laboratory model for end-stage liver disease (MELD) scores, were evaluated (n = 449). Due to missing variables, 49 MELD scores could not be computed (center A n = 7, center B n = 41).

IRI and NAS

The degree of both ischemic and reperfusion hepatocellular injury was evaluated by postoperative serum levels of alanine aminotransferase (ALT). Serum ALT was determined during the seven consecutive days after OLT and measured by routine biochemical methods. The highest level of the first peak ALT was evaluated individually. As there is no clear definition of NAS in the literature, we have used the definition with the most clinical relevance. NAS was defined as follows: any *treated* stricture or irregularity of the intra- or extrahepatic bile ducts occurring at least 1 cm above the anastomosis post-OLT. Analysis of NAS development was performed in both the combined cohort as well as the individual cohorts. For timing, the first endoscopic or percutaneous treatment for NAS, that is, balloon dilatation and/or a performed stenting procedure, was used. Nonanastomotic biliary strictures that did not require intervention and anastomotic strictures were not included in the definition of NAS for the current study. Because of a different pathophysiological mechanisms, biliary strictures as a result from hepatic artery thrombosis or arterial complications were not considered NAS and were excluded.

Statistical analyses

Statistical analysis was performed using SPSS 20.0 (IBM, Armonk, NY, USA). A Mann–Whitney U-test was used to calculate differences in medians, and Fisher's exact test was performed for categorized variables. The optimal cutoff value for peak ALT is defined as the point with the most significant split for association with NAS or no NAS as determined by log-rank test. Using the calculated cutoff value, a peak ALT below this value was considered as mild IRI, whereas a peak ALT above this value was considered as severe IRI. Cumulative incidence curves were established using one minus survival incidence rates according to the Kaplan–Meier method, and risk factor analysis was performed using univariate and multivariate stepwise forward Cox regression analysis. In case of a P-value of <0.20 in the univariate analysis, the parameter was taken into account in the multivariate analysis. A P-value of <0.05 was considered statistically significant.

Retrospective studies are approved by the institutional review board by legislation, and the study was performed according to the guidelines of the Helsinki and the Istanbul declaration.

Results

A total number of 496 OLTs with a minimum follow-up of 7 days were performed in both centers, with 399 DBD donor livers and 97 DCD donor livers. Median follow-up from OLT until development of NAS was 4.4 months (range 0.3–58). Donor and recipient variables are presented in Table 1.

Donor and surgical variables

Median donor age for DCD donors was significantly lower compared with DBD donors in the combined cohort (44 vs. 50 years, respectively, $P < 0.001$). CIT was shorter for DCD donors than for DBD donors (DCD 460 min vs. DBD 503 min; $P = 0.003$), but recipient warm ischemia time (RWIT) did not differ for both types of donors ($P = 0.44$). In case of DCD-OLT, median donor warm ischemia time from cardiac arrest to flush (DWIT) was 17 min. As DWIT is considered a risk factor for the development of NAS, DWIT was evaluated separately for patients who developed NAS and patients without NAS; however, this was not significantly different (NAS 17 min vs. no NAS 16 min, $P = 0.62$).

To evaluate injury of the liver parenchyma, peak ALT was evaluated. The median time point of the first peak ALT was found to be at 1 day after OLT. Median peak ALT was significantly higher after OLT using DCD donors than after DBD-OLT (DCD 1525 IU/l vs. DBD 719 IU/l; $P < 0.001$).

Table 1. Baseline characteristics. Data presented as median (range) for continuous variables and percentage (number) for categorized variables.

Characteristic	DBD (n = 399)	DCD (n = 97)	P
Donor age (median, range)	50 (14–86)	44 (14–65)	**<0.01**
Donor gender % (n)			
Male	49.4 (197)	60.8 (59)	**<0.05**
Female	50.6 (202)	39.2 (38)	
Recipient age (median, range)	53 (17–70)	54 (19–69)	0.62
Recipient gender % (n)			
Male	60.9 (243)	69.1 (67)	0.16
Female	39.1 (156)	30.9 (30)	
MELD (median, range)	16 (6–40)	14 (6–40)	0.42
Diagnosis pre-OLT % (n)			
ALD	16.8 (67)	15.5 (15)	0.87
HCC	10.5 (42)	11.3 (11)	
PSC	16.8 (67)	17.5 (17)	
PBC	4.8 (19)	7.2 (7)	
HBV	5.5 (22)	2.1 (2)	
HCV	9.5 (38)	11.3 (11)	
AIH	6.3 (25)	4.1 (4)	
Metabolic	7.3 (29)	7.2 (7)	
Other	22.6 (90)	23.7 (23)	
NAS % (n)	13.3 (53)	30.9 (30)	**<0.01**
CIT (median, 25–75% range)	503 (420–619)	460 (407–517)	**<0.01**
RWIT (median, 25–75% range)	40 (34–49)	40 (34–47)	0.44
DWIT (median, 25–75% range)	–	17 (14–20)	
Peak AST (median, range)	1006 (64–14750)	2557 (200–19590)	**<0.01**
Peak ALT (median, range)	719 (69–8242)	1525 (106–11105)	**<0.01**

DBD, donation after brain death; DCD, donation after circulatory death; MELD, model for end-stage liver disease; OLT, orthotopic liver transplantation; ALD, alcoholic liver disease; HCC, hepatocellular carcinoma; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; HBV, hepatitis B virus; HCV, hepatitis C virus; AIH, auto-immune hepatitis; NAS, nonanastomotic biliary strictures; CIT, cold ischemia time (min); RWIT, recipient warm ischemia time (min); DWIT, donor warm ischemia time (min); AST, aspartate aminotransferase (IU/l); ALT, alanine aminotransferase (IU/l). Statistically significant p-values are printed in bold.

Similar results were observed for median peak aspartate aminotransferase (AST) (DCD 2557 IU/l vs. DBD 1006 IU/l; $P < 0.001$). For each cohort individually, similar results were obtained (data not shown). Because of the strong correlation between peak AST and peak ALT (Pearson's coefficient = 0.86, $P < 0.001$) and a more explicit cutoff value and higher AUC in the receiver operating characteristic (ROC) curve for peak ALT, further analysis was performed for peak ALT only.

Other serum markers, such as bilirubin, alkaline phosphatase, and gamma-glutamyltransferase, were not included in the analyses because no optimal cutoff value could be determined.

To determine the potential influence of blood transfusion and kidney function on peak ALT level, correlation tests were performed. However, no correlation was found between peak ALT and creatinine level ($P = 0.715$) or estimated glomerular filtration rate ($P = 0.400$) and between peak ALT and the volume of erythrocyte, fresh frozen plasma, and cellsaver transfusion ($P = 0.284$, $P = 0.173$, and $P = 0.241$, respectively).

IRI and NAS

In the combined cohorts, NAS developed in 31% after DCD-OLT and in 13% after DBD-OLT ($P < 0.001$). The incidence of NAS was not statistically different between center A and center B ($P = 0.37$). Optimal cutoff value of serum ALT for NAS was calculated using log-rank statistics and a ROC curve for DCD-OLT ($n = 97$) and was established at ≥1300 IU/l (Fig. 2a and b). Using the calculated cutoff value, a low peak ALT of <1300 IU/l was considered mild IRI, whereas a high peak ALT of ≥1300 IU/l was considered severe IRI. Using this cutoff point, sensitivity of peak ALT level to predict NAS was 87%, specificity 54%, the positive predictive value 46%, and the negative predictive value 90%. A cutoff value of ≥1300 IU/l corresponded with the highest Youden Index of 0.41 and a positive likelihood ratio of 1.87. After DCD-OLT, severe IRI preceded NAS development in 46% cases compared with 10% in the mild IRI group ($P < 0.001$) (Table 2). Four-year cumulative incidence of NAS development was 49.5% in case of severe IRI compared with 11.3% when mild IRI occurred (log rank $P = 0.001$; Fig. 3). In 11% of DCD-OLT cases, retransplantation for NAS was needed. When the cumulative incidence was calculated for each cohort individually, similar results were obtained. In both cohorts, severe IRI was significantly associated with NAS development after DCD-OLT. (Data not shown) No optimal cutoff value of peak ALT could be obtained for DBD-OLT. (Fig. 4) Therefore, the threshold for severe IRI of ≥1300 IU/l was also applied to the DBD cohort. However, no association could be found between peak ALT ≥1300 IU/l and NAS after DBD-OLT, neither in the individual cohorts nor in the combined group ($P = 0.74$). Based on the cutoff value determined for DCD-OLT, 4-year cumulative incidence of NAS after DBD-OLT in the combined group was 14.3% when peak ALT was ≥1300 IU/l compared with 13.7% when peak ALT was <1300 IU/l (log rank $P = 0.94$).

Besides higher incidences of NAS, a peak ALT of ≥1300 IU/l was also associated with worse patient and graft survival. Overall, mortality or retransplantation rate was

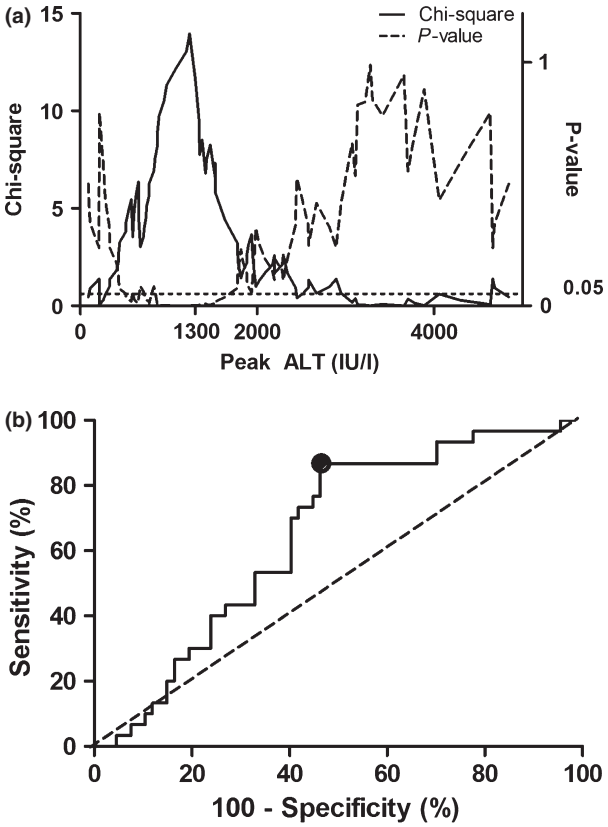

Figure 2 (a) Optimal alanine aminotransferase (ALT) cutoff point for nonanastomotic strictures (NAS) after donation after circulatory death (DCD)-orthotopic liver transplantation (OLT). Calculation of the optimal ALT cutoff (≥1300 IU/l) for prediction of NAS in DCD-OLT using the combined cohorts. (b) receiver operating characteristic curve of peak ALT for NAS after DCD-OLT.

Table 2. Development of NAS respective of degree of IRI. Numbers are presented as % (*n*).

	Combined cohorts (center A and center B)			
	DBD		DCD	
Graft type	No NAS	NAS	No NAS	NAS
Mild IRI	86.2 (250)	13.8 (40)	90.0 (36)	10.0 (4)
Severe IRI	88.1 (96)	11.9 (13)	54.4 (31)	45.6 (26)
	$P = 0.74$		**$P < 0.001$**	

ALT, alanine aminotransferase; DBD, donation after brain death; DCD, donation after circulatory death; NAS, nonanastomotic biliary strictures; IRI, ischemia–reperfusion injury.
Mild IRI was defined as peak ALT ≤ 1300 IU/l and severe IRI as peak ALT > 1300 IU/l. Statistically significant p-values are printed in bold.

36% of the patients with severe IRI, as compared to 26% of the patients with mild IRI ($P = 0.03$). Twenty-three patients were previously excluded due to primary nonfunction of the graft, early mortality, and a follow-up of <7 days. This included 16 DBD-OLTs (3.9% of all DBD-OLT) and seven DCD-OLTs (6.7%). Ten of 16 DBD-OLTs and six of seven DCD-OLTs had a peak ALT of ≥1300 IU/l, indicating that severe IRI may also be associated with early graft loss. Mortality within 1 month was 1.5% for the patients with mild IRI and 4.2% for patients with severe IRI (all after DBD-OLT), which did not differ between the groups ($P = 0.12$).

Primary sclerosing cholangitis and NAS

Because radiodiagnostic features of NAS resemble the diagnostic criteria for primary sclerosing cholangitis (PSC), distinguishing NAS and recurrent PSC can be difficult, especially late occurrence of NAS might in fact be recurrent PSC. Therefore, the analysis was also performed after the exclusion of all patients with PSC ($n = 84$). After the exclusion of patients with PSC, the 4-year cumulative incidence of NAS after DCD-OLT was 52.4% in case of severe IRI compared with 13.5% when peak ALT was <1300 IU/l (log rank $P = 0.002$). For DBD-OLT, 4-year cumulative incidence was 9.0% when peak ALT was <1300 IU/l compared with 11.4% IU/l when peak ALT was ≥1300 IU/l (log rank $P = 0.49$).

Univariate and multivariate analysis

Cox regression analysis for risk of NAS development was performed for OLT with DCD and DBD donors separately. For DCD-OLT, donor age, MELD score, CIT, and peak ALT ≥1300 were significantly associated with NAS in the univariate analysis at the $P < 0.20$ value and were thus included in the multivariate analysis. PSC as indication for OLT, as well as the center at which OLT was performed, was not associated with development of NAS in the univariate analysis for DCD-OLT. Multivariate analysis showed peak ALT ≥1300 and donor age to be independently associated factor for the development of NAS after DCD-OLT, adjusted for MELD score and CIT (adjusted hazard ratio (aHR) for peak ALT ≥1300 = 3.71, confidence interval (CI) = 1.26–10.91, $P = 0.017$) (Table 3). Multivariate analysis revealed PSC as indication for OLT to be the only independently associated parameter for the development of NAS after DBD-OLT (aHR = 2.37, CI 1.32–4.26, $P = 0.004$) (Table 4).

Discussion

The present study describes a strong and independent association between a serum peak ALT of ≥1300 IU/l and the development of NAS after liver transplantation with donation after circulatory death (DCD-OLT). A higher donor

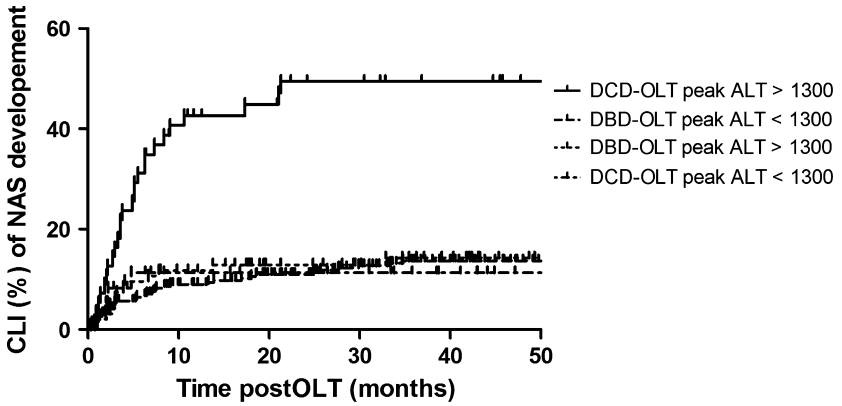

Figure 3 Development of nonanastomotic strictures (NAS) post-orthotopic liver transplantation (OLT). Cumulative incidence (CLI) of NAS development after OLT with livers from donation after cardiac death (DCD) reached 49.5% at 48 months when peak alanine aminotransferase (ALT) ≥1300 IU/l (n = 57) compared with 11.3% when peak ALT <1300 IU/l (n = 40) (P < 0.001). CLI rates of NAS after donation after brain death-OLT did not differ between recipients with mild or severe ischemia–reperfusion injury (IRI) or DCD-OLT with peak ALT <1300 IU/l/. CLI rates were calculated using one minus survival incidence rates with the Kaplan–Meier test and compared using log-rank test.

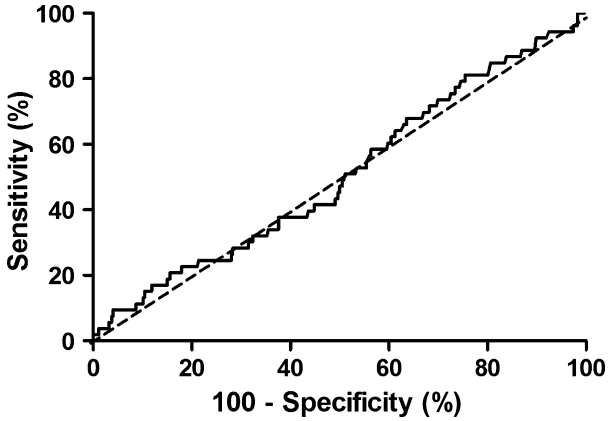

Figure 4 Receiver operating characteristic (ROC) curve of peak alanine aminotransferase (ALT) for nonanastomotic strictures (NAS) after donation after brain death (DBD)-orthotopic liver transplantation (OLT).

age was also independently associated with NAS after DCD-OLT.

In the last decade, transplantation of livers from DCD donors has become common in the Netherlands and United Kingdom to decrease wait-list mortality. However, these grafts are known to be associated with higher complications rates, such as primary nonfunction, ischemia–reperfusion injury, and nonhepatic complications such as the development of end-stage renal disease during follow-up after OLT [19,20]. It is therefore important to balance the risk for post-transplant complications after DCD-OLT and the survival benefit for an individual patient. In 2012, Jay and al. reported improvements in effectiveness for DCD-OLT when MELD scores were >15 as compared to lower MELD scores. However, for patients with a MELD score of 15–20, a high cost-effectiveness ratio, due to high direct medical costs, was reported [21]. The price of donation after circulatory death was further discussed by van der Hilst *et al.* [22]. That study described a significantly higher cost per life year for DCD grafts, mainly due to a higher complication rate and a longer ICU stay.

Similar patient survival was reported for DCD-OLT and DBD-OLT after a national protocol for accepting DCD organs was implemented in the Netherlands in 2001, with currently 30–40% of OLTs with a DCD donor [17].

Biliary complications are among the most frequent complications after DCD-OLT leading to considerable morbidity and mortality. A recent meta-analysis of O'Neill *et al.* [23] including 1619 patients with DCD-OLT showed an odds ratio for biliary complications of 2.4 as compared to DBD-OLT. NAS is considered the most challenging biliary complication because the strictures are often located beyond the scope of endoscopic treatment and resistant to therapy. It has been hypothesized that IRI may play an important role in the development of NAS. IRI is the combined result of ischemia, reperfusion, status of the graft (e.g., steatosis or not), and the immune reactions of the recipient during and after reperfusion. DCD grafts are known to be more prone to IRI due to an additional DWIT and to have more biliary complications after OLT than DBD grafts [24]. To reduce ischemic injury and hopefully compensate for DWIT-induced injury, ischemia times, especially cold ischemia time, are kept shorter for DCD-OLT compared with DBD-OLT. However, ischemia times are not indicative for injury induced during reperfusion, and there is evidence that most IRI-induced hepatic injury develops after restoration of blood flow, at least partially

Table 3. Univariate and multivariate analysis of risk factors for development of NAS after DCD-OLT.

		Univariate analysis		Multivariate analysis	
Variables DCD-OLT	%	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Donor age					
Continuous		1.04 (1.00–1.07)	**0.01**	1.04 (1.00–1.07)	**0.04**
Donor gender					
Male	60.8	1.36 (0.65–2.85)	0.42		
Female (reference)	39.2	1			
Donor ICU stay (days)					
Continuous		1.00 (0.85–1.18)	0.97		
Recipient age at OLT					
Continuous		1.01 (0.98–1.04)	0.65		
Recipient gender					
Male	69.1	1.21 (0.57–2.58)	0.62		
Female (reference)	30.9	1			
MELD score					
Continuous		1.03 (0.97–1.07)	0.17	1.03 (0.99–1.07)	0.22
Peak ALT					
Severe	58.8	4.90 (1.71–14.05)	**0.003**	3.71 (1.26–10.91)	**0.02**
Mild (reference)	41.2	1			
DWIT					
Continuous		1.01 (0.98–1.04)	0.49		
CIT					
Continuous		1.00 (1.00–1.01)	0.19	1.00 (1.00–1.01)	0.32
RWIT					
Continuous		0.98 (0.94–1.02)	0.28		
PSC as indication					
Other (reference)	82.5	1			
PSC	17.5	0.47 (0.14–1.55)	0.21		
Study Center					
Center B	60.8	0.66 (0.32–1.36)	0.26		
Center A (reference)	39.2	1			
Preservation solution					
UW	33.0	1.06 (0.50–2.27)	0.88		
HTK (reference)	67.0	1			
MAP $\leq$ 55 mmHg (during reperfusion)					
No	48.3	1.28 (0.54–3.02)	0.57		
Yes (reference)	51.7	1			

HR, hazard ratio; CI, confidence intervals; OLT, orthotopic liver transplantation; ICU, intensive care unit; MELD, model for end-stage liver disease; ALT, alanine aminotransferase; DWIT, donor warm ischemia time; RWIT, recipient warm ischemia time; CIT, cold ischemia time; PSC, primary sclerosing cholangitis; UW, University of Wisconsin; HTK, histidine–tryptophan–ketoglutarate; MAP, mean arterial pressure; DCD, donation after circulatory death; NAS, nonanastomotic strictures. Statistically significant p-values are printed in bold.

due to an excess of reactive oxygen species [25,26]. Serum peaks of transaminases within the first 7 days after OLT reflect, more than ischemia times, liver parenchyma injury as it includes both periods of ischemia and reperfusion.

Orthotopic liver transplantation with DCD donors have higher peak AST and ALT levels postoperatively than DBD-OLT. The current data show that severe IRI, defined as peak serum ALT ≥1300 IU/l post-OLT, is strongly associated with the increased incidence of NAS after DCD-OLT, with a 4-year cumulative incidence rate of NAS in this group of 49.5%. In the multivariate analysis, peak ALT ≥1300 IU/l was independently associated with the development of NAS after DCD-OLT. Furthermore, our study showed a higher donor age to be an independent risk factor for the development of NAS after DCD-OLT. This is an important finding as advancing age is generally accepted to be associated with more hepatic steatosis, whereas Baccarani *et al.* [27] described hepatic steatosis to be a risk factor for post-transplant biliary complications. However, donor age as a risk factor remains subject to debate in previous studies. Whereas some studies showed an advancing age to be associated with higher incidences of NAS, others have reported similar results for DCD-

Table 4. Univariate and multivariate analysis of risk factors for development of NAS after DBD-OLT.

		Univariate analysis		Multivariate analysis	
Variables DBD-OLT	%	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Donor age					
Continuous		1.01 (0.99–1.03)	0.38		
Donor gender					
Male	49.4	0.95 (0.55–1.62)	0.85		
Female (reference)	50.6	1			
Recipient age at OLT					
Continuous		0.99 (0.97–1.01)	0.15	0.99 (0.97–1.01)	0.25
Recipient gender					
Male	60.9	1.89 (1.02–3.47)	**0.04**	1.82 (0.98–3.39)	0.06
Female (reference)	39.1	1			
MELD score					
Continuous		1.00 (0.97–1.04)	0.89		
Peak ALT					
Mild (reference)	72.7	1			
Severe	27.3	1.03 (0.55–1.92)	0.94		
CIT					
Continuous		1.00 (1.00–1.00)	0.77		
RWIT					
Continuous		1.01 (0.99–1.04)	0.34		
PSC as indication					
Other (reference)	83.2	1			
PSC	16.8	2.71 (1.53–4.78)	**0.001**	2.37 (1.32–4.26)	**0.004**
Study Center					
Center B	65.9	1.29 (0.71–2.34)	0.41		
Center A(reference)	34.1	1			

HR, hazard ratio; CI, confidence intervals; OLT, orthotopic liver transplantation; MELD, model for end-stage liver disease; ALT, alanine aminotransferase; DWIT, donor warm ischemia time; RWIT, recipient warm ischemia time; CIT, cold ischemia time; PSC, primary sclerosing cholangitis; DBD, donation after brain death; NAS, nonanastomotic strictures. Statistically significant p-values are printed in bold.

OLT when younger and older donors were compared [7,8,28,29].

In literature, DWIT has also been associated with the development of NAS. This may be the result of continued energy consumption of the graft at body temperature during DWIT. Due to an inadequate tissue perfusion, this energy consumption may lead to adenosine triphosphate depletion and a shift from aerobic to an anaerobic metabolism, which may result in a more injured graft. In the present study, likely due to a low variability of DWIT between the patients, DWIT was not an independent risk factor. DWIT was defined as the time between circulatory arrest and cold flush with preservation fluid in the donor. This is slightly different than some other studies where DWIT is defined as the time between cessation of cardiopulmonary support and cold flush [30]. However, the absolute time period of DWIT is not the only risk factor of DWIT for the development of NAS. Because of different characteristics of the graft, such as steatosis and immune responses of the donor and recipient, some grafts may be more prone to injury from DWIT than others and the actual effect of DWIT on the graft may be different even though the time period is not different. Furthermore, Op den Dries *et al.* [31] recently evaluated peribiliary plexus injury. DBD livers showed a significantly higher percentage of grafts without any peribiliary vascular plexus injury than DCD livers (18% vs. 0%). Injury in the deep peribiliary glands was also more severe and more prevalent in patients that developed NAS, compared with patients without NAS.

We therefore hypothesize that the higher peak ALT and increased incidence of NAS in a subgroup of DCD-OLT patients are different presentations of the same ischemia–reperfusion injury due to the DWIT combined with warm ischemia between respiratory withdrawal and cardiac arrest in the donor.

After DBD-OLT, peak ALT was not associated with the development of NAS, whereas PSC was a risk factor in these patients. After DCD-OLT with peak ALT below 1300 IU/l, the risk of NAS was similar to DBD-OLT. Therefore, a peak ALT below 1300 IU/l could be used as a negative predicting factor in the extra risk for development of NAS after DCD-OLT. Mangus *et al.* [32]. have recently demonstrated that the pretransplant donor peak ALT is not related to post-transplant ALT levels. Moreover, a higher donor peak ALT

was not indicative for early graft function and 1-year survival. Close monitoring of recipient peak ALT may therefore be an important first marker to predict clinical patient and graft outcome after transplantation and allow a more intensive follow-up.

Reducing IRI in DCD-OLT to the extent that peak ALT is below 1300 IU/l will probably diminish the incidence of NAS to the incidence after DBD-OLT (14%), but may not completely eliminate NAS, as other pathophysiological mechanisms might be involved. This is consistent with the idea that NAS is most likely the result of a complex mechanism involving ischemic, immunologic, and toxic processes which all affect the biliary tree or its vascular supply [33–35]. Current preservation solutions and techniques may be insufficient [36]. Several attempts are being made to improve preservation and reduce IRI of liver grafts using machine liver perfusion and/or abdominal regional perfusion, but also fibrinolytic agents are used to dissolve possible microthrombi in the donor liver [37–39].

The current study has certain limitations. The sample size is relatively small. It is clear that these novel findings need to be replicated in larger cohorts. Furthermore, we used only ALT and not AST as marker for IRI occurring in the liver, as AST is derived from mitochondria in liver cells but is also produced in heart, skeletal muscles, and brain cells. After surgery, AST can also be elevated due to damage of the abdominal muscles during surgery, and this makes it less specific as a parameter of IRI after OLT. However, most cases of NAS developed within the first 6 months after OLT. Whether a more intensive follow-up and earlier intervention in patients with high peak ALT after DCD-OLT might prevent retransplantation remains to be established.

In conclusion, our data show that serum peak ALT ≥1300 IU/l and a higher donor age are strongly and independently associated with the development of clinically relevant NAS after DCD-OLT, with peak ALT below 1300 IU/l predicting a risk similar to DBD-OLT. In DCD-OLT, it can thus be used in classifying patients as high risk or low risk (similar to DBD-OLT) for developing NAS. The current data indicate that the higher risk of NAS after DCD as compared to DBD is likely the result of cases of DCD-OLT with more severe IRI due to warm ischemia in the donor. This enables the use of peak ALT below 1300 IU/l as target for future interventions aimed at prevention of NAS and a peak ALT of ≥1300 IU/l as a justification for a more intensive follow-up in DCD-OLT.

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Authorship

ACdD and KSK: Participated in research design, writing of the paper, performance of the research, data analysis. B-JFdR and MES: Participated in performance of the research. AEB and AI: Participated in patient care and writing of the paper. JD: Participated in writing of the paper. HWV: Participated in research design, data analysis and writing of the paper. RJP: Participated in research design, writing of the paper. BvH: Participated in research design, writing of the paper, performance of the research, data analysis.

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