Liver Transplantation with Grafts Donated after Circulatory Death

Current insights and new perspectives

Marjolein van Reeven

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Liver Transplantation with Grafts Donated after Circulatory Death
Current insights and new perspectives

Levertransplantatie met organen gedoneerd na circulatiestilstand
Huidige inzichten en toekomstige perspectieven

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Vul de dagen van je leven met je eigen kleuren in.
Elke bladzij is weer anders, elke dag een nieuw begin.

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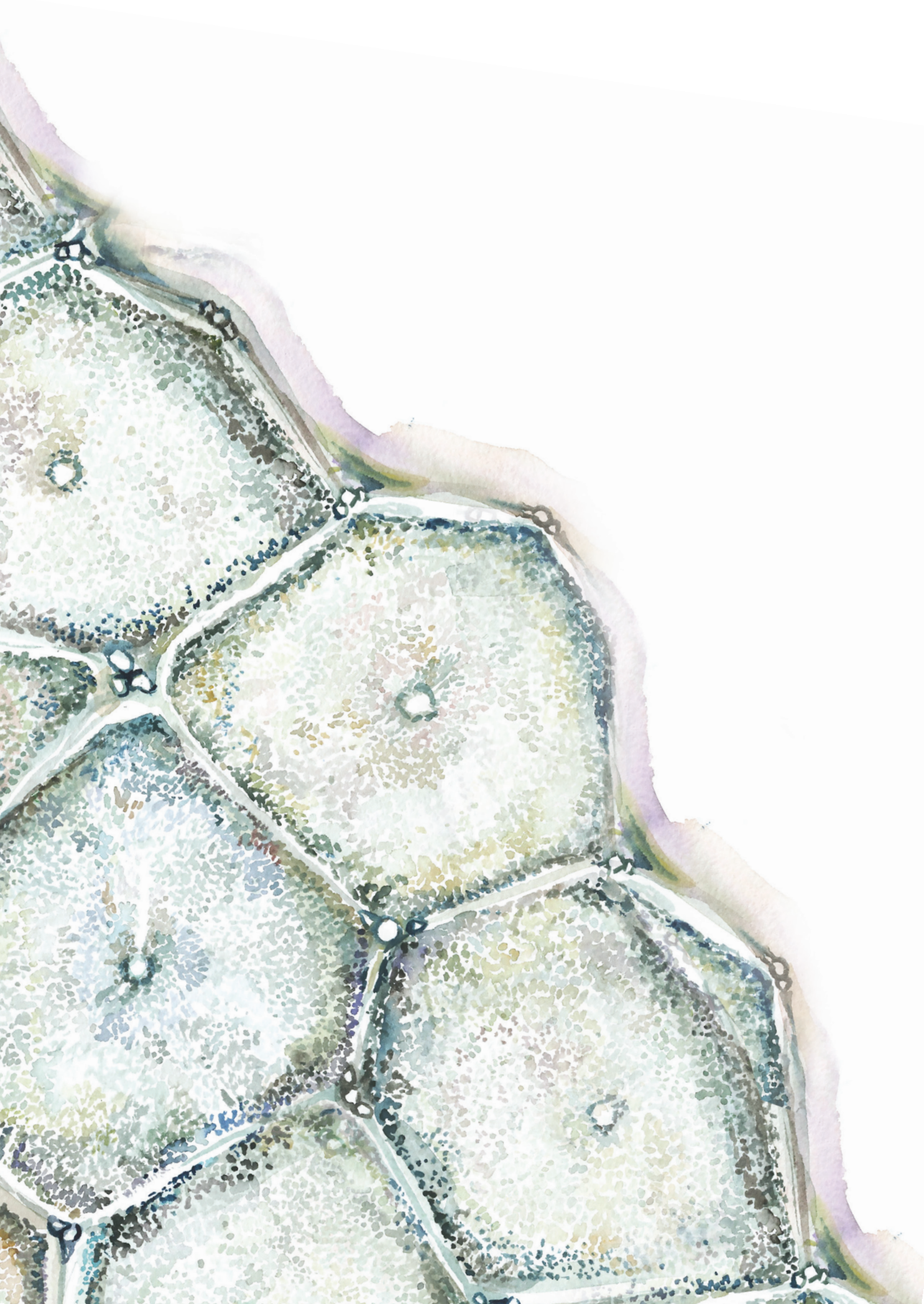

CHAPTER 1

General introduction

History of liver transplantation

Solid organ transplantation has been one of the most important advances in the field of medicine over the past 60 years. For many patients with end-stage organ failure, this still is the only curative treatment option. On April 3rd 1933, Dr. Yurii Voronoy, a Soviet surgeon, performed the first solid allograft transplantation: a cadaveric kidney was transplanted into a 26-year-old recipient who suffered from a mercury intoxication. Unfortunately, the recipient died one day after the procedure. (1) In 1963, Dr. Thomas Starzl performed a series of five human orthotopic liver transplantations. Sadly, all recipients died within 23 days after transplantation, three of which due to a pulmonary embolism. (2) A few years later, in 1967, Dr. Starzl and his team were the first to perform a successful liver transplantation with the recipient survival exceeding one year. (3) Dr. Jean Demirleau carried out the first liver transplantation in Europe in 1964. Unfortunately, the recipient died a few hours after transplantation due to fibrinolysis. (4) The first successful liver transplantation in Europe was performed in 1968 by Sir Roy Calne in Cambridge, United Kingdom. (5) Since then, improvements in surgical techniques, peri-operative care and intensive care medicine have contributed to the success of liver transplantation. Finally, with the development of several immunosuppressive agents, liver transplantation has become a well-established medical treatment with one-year survival rates of 90%. (6)

Different types of post-mortem organ donation

The first solid organ transplantations made use of grafts procured from donors who had died after cardiac arrest. In 1968, a multidisciplinary committee at Harvard Medical School developed the concept of brain death. (7) With the introduction of the so-called Harvard criteria, transplantation of organs from brain death donors (DBD) became the gold standard. In the 1980s and 1990s, donation after circulatory death (DCD) gained renewed interest as a result of the growing shortage of organs for transplantation. (8, 9) The Netherlands had a leading role in this, with their use of DCD grafts for kidney transplantation. (10) Nowadays, the Netherlands is one of the countries with the highest proportion of liver transplantations using DCD grafts: of the 162 deceased-donor liver transplantations performed in 2020, almost half (N = 75) have been procured with controlled DCD grafts. (11)

Towards the end of the 20th century, a clear definition of the DCD donor was still lacking. At the first International Workshop on non-heartbeating donation in Maastricht, the Netherlands, in 1995 DCD was classified into four categories, known as the Maastricht classification. (12, 13) Category I and category II are both called uncontrolled procedures, reflecting that a circulatory arrest occurred

unexpectedly, either out of hospital or in hospital. Type III is a controlled approach, reflecting a scheduled withdrawal of all life-supporting treatment. Type IV refers to brain dead donors who suffer from a cardiac arrest; the approach is either controlled or uncontrolled. A fifth category was added in 2012: medically-assisted cardiac arrest (Table 1). (14)

In the Western world, the majority of DCD grafts for liver transplantion are obtained from Maastricht type III donation. Only a few countries perform liver transplantations with type II DCD grafts, of which Spain is a worldwide pioneer. Of note, the Spanish DCD type II liver transplantion program is successful because normothermic extracorporeal membrane oxygenation is used to maintain the donor oxygenated after circulatory arrest with the solely aim of organ perfusion. This approach is not legally permitted in every country. (15, 16)

Table 1: Modified Maastricht classification of donation after circulatory death
Adapted from Thuong et al., Transplant International, 2016 (17)

Category	
Category I Uncontrolled	***Found dead*** Category IA: Out-of-hospital Category IB: In-hospital
Category II Uncontrolled	***Witnessed cardiac arrest*** Category IIA: Out-of-hospital Category IIB: In-hospital
Category III Controlled	***Withdrawal of life-sustaining therapy***
Category IV Either uncontrolled or controlled	***Cardiac arrest while brain dead***
Category V Controlled	***Medically assisted cardiac arrest (euthanasia)***

Donor warm ischemia time: inherent to donation after circulatory death

Brain dead organ donors have an intact circulation, and thus have adequately perfused organs until the start of cold perfusion at the operating room. Then, static cold storage reduces to a minimum the anaerobic tissue metabolism of the target organs. In contrast, by definition grafts from DCD donors are characterized by a period of inadequate organ perfusion in which the target organs have not yet been cooled sufficiently to minimize anaerobic metabolism. As a result, potentially toxic metabolites, including reactive oxygen species and inflammatory cytokines will have accumulated. The period of time in which this happens is called the donor warm ischemia time (dWIT). Roughly two phases can be distinguished: the agonal phase (from withdrawal of life-supporting

treatments to circulatory arrest) and the asystolic phase (from circulatory arrest to start of cold perfusion). As a too long donor warm ischemia time has a negative impact on the outcomes of liver transplantation, (18-20) many countries have set a maximum duration in which a liver graft is eligible for transplantation. In the Netherlands, for example, it should not exceed 60 minutes. (21)

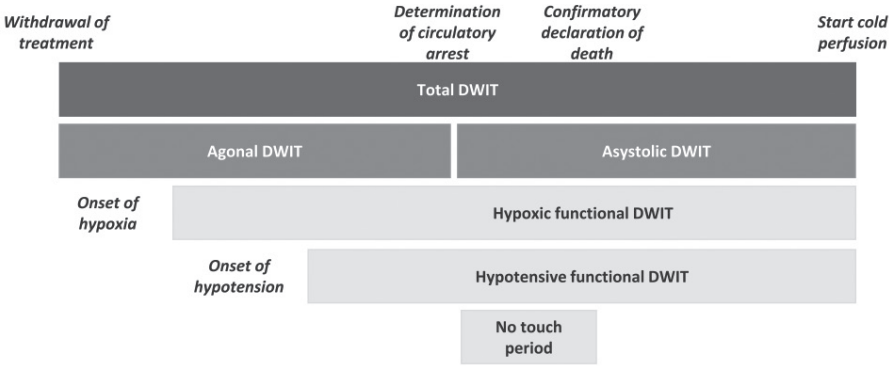

Figure 1: Components of donor warm ischemia time
From Kalisvaart et al., Transplantation, 2021 (22)
DWIT = donor warm ischemia time

Survival after DCD liver transplantation

In 2000, D'Alessandro and coworkers were the first to report outcomes of 19 liver transplantations with controlled DCD grafts (DCD-LT). Patient survival after DCD-LT was similar to that of recipients of DBD liver grafts (DBD-LT). However, as a result of a higher proportion of primary non-function, the allograft survival in recipients of DCD grafts was shorter than that of recipients of DBD grafts. (23) Since this report, many single-center studies on survival after DCD-LT have been published. The first report on nationwide outcomes of DCD-LT was published in 2004, based on data from the United Network of Organ Sharing. (24) The report concluded that graft survival of DCD-LT recipients was inferior to that of recipients of a DBD liver graft. A prolonged cold ischemia time (CIT; i.e. the period of time between the liver being stored on ice during the donor procedure and the liver being removed from ice during the implantation) proved to be an independent risk factor for poor outcome, especially among recipients of a DCD liver graft. Muiesan and coworkers evaluated the outcomes of 32 controlled DCD-LT in King's College Hospital, London, and found an overall patient survival of 87% and graft survival 84%. However, the authors pointed out that careful donor selection as well as a short CIT were essential to achieve these results. (25) Over the past years, several nationwide studies on DCD-LT have been performed in Europe. A Belgian nationwide study in 2010 concluded that controlled DCD-LT yielded inferior graft

survival when compared with DBD-LT, with 50% of the grafts being lost at three years post-transplant. Furthermore, both an agonal dWIT longer than 15 minutes and a prolonged CIT (over six hours) were associated with a higher risk of patient death. (26) Dubbeld and coworkers reported the first national results on DCD-LT in the Netherlands; i.e. patient and graft survival rates at 3-years of 80% and 68%, respectively. (27) These rates did not differ significantly from the corresponding rated for DBD-LT. Both dWIT and CIT were found independent risk factors for graft loss after DCD-LT.

Overall, these reported outcomes of DCD-LT are quite heterogeneous. Of note, none of the aforementioned studies have used matching to reduce any potential bias. Matching could especially be of benefit when comparing DCD-LT and DBD-LT, as the donor and recipient acceptance criteria substantially differ between DCD-LT and DBD-LT. Several studies have used a matched control group of DBD-LT to evaluate the outcomes of DCD-LT. (28-32) The oldest of these studies concluded that recipients of a DCD liver graft had a significantly lower graft survival in comparison with the matched DBD-LT cohort. (28, 29, 32) However, the more recently conducted studies have shown similar patient and graft survival rates among DCD-LT and DBD-LT recipients. (30, 31)

Biliary complications: the Achilles heel of DCD liver transplantation

Complications of the biliary tract after liver transplantation are still common. These can roughly be divided into three categories: biliary leakage, anastomotic strictures, and non-anastomotic strictures. Biliary leakages can originate at different sites of the biliary tract, such as at the surgical anastomosis, the remnants of the cystic duct, or at the dissection plane in case of a split liver graft or graft from a living donor. Most cases can be treated through endoscopic stenting or percutaneous drainage. (33)

Anastomotic strictures are by definition located at the site of the biliary anastomosis and can occur in both duct-to-duct anastomosis and Roux-Y reconstruction. In the early post-operative phase (≤ 3 months), they are mainly the result of surgical-technical aspects, such as a size mismatch between donor and recipient common bile duct. Late anastomotic strictures are the result of (local) ischemia and subsequent formation of fibrotic tissue. Most of the anastomotic strictures occur within the first year post-transplant. Endoscopic balloon dilatation and/or stenting is the current treatment of choice. (33-35)

Non-anastomotic strictures – also known as ischemic type biliary lesions or ischemic cholangiopathy – are defined as any stricture of the biliary tree, except

at the anastomosis site, in the presence of a patent hepatic artery. (36, 37) The origin is thought to be multifactorial. One of the underlying mechanisms is ischemia-induced destruction of the biliary epithelium without sufficient regenerating capacity. Regeneration of the lining of the bile ducts depend on two structures: the peribiliary glands and the peribiliary vascular plexus. The former are found in the deeper layer of the extrahepatic and large intra-hepatic bile ducts and contain stem cells that can proliferate to biliary epithelium. For blood supply, peribiliary glands rely mainly on the peribiliary vascular plexus. Any injury to either structure may, therefore, result in impaired regeneration of biliary epithelium, which itself is a great risk factor for the development of ischemic cholangiopathy. (38) A second important factor in the development of ischemic cholangiopathy is cytotoxic injury due to hydrophobic bile salts. Normally, the cytotoxic effect of hydrophobic bile salts is neutralized by complex formation with phospholipids and cholesterol. In the early post-transplant phase, however, sufficient phospholipids to neutralize the biliary salts are lacking, resulting in a higher cytotoxic effect on the biliary epithelium. (39) A third mechanism contributing to the formation of ischemic cholangiopathy is immune-mediated injury. Transplant physicians and surgeons consider non-anastomotic strictures the most detrimental of all biliary complications, complication by because of its high morbidity and mortality risk.

In 2003, Abt and coworkers were the first to report a higher incidence of biliary complications among recipients of a DCD liver graft when compared to DBD recipients. (40) Two meta-analyses, published in 2011 and 2014, respectively, both concluded that recipients of a DCD liver graft have a significantly higher risk of developing any biliary complication (odds ratio of 2.4 in both studies) and ischemic cholangiopathy (odds ratio from 10.5 to 10.8), when compared with DBD recipients. (41, 42) The additional donor warm ischemia time and subsequent ischemia reperfusion injury were indicated as the main cause for the higher incidence of biliary complications in DCD-LT.

Machine perfusion: new kid on the block

Many efforts have been made to optimize the quality of liver grafts from donation after circulatory death as well as from other types of extended criteria donors. Probably the largest effort is the development of dynamic preservation techniques. Currently, three types of machine perfusion are being used clinically: normothermic regional perfusion in the donor, ex-situ normothermic machine perfusion of the liver graft, and ex-situ hypothermic machine perfusion.

Spain is the worldwide pioneer in normothermic regional perfusion. As mentioned earlier, this used to be a well-established technique to safely transplant liver grafts

from uncontrolled DCD donors (type II according to the Maastricht classification). More recently, the use of normothermic regional perfusion in controlled DCD-LT has received attention. When compared with the commonly performed static cold storage method, the use of normothermic regional perfusion in controlled DCD-LT was found to be associated with a significant lower odds of biliary complications and graft loss. (43)

During ex-situ normothermic machine perfusion, the liver is perfused with oxygenated blood at a temperature of 35-37 degrees Celsius, to which medications and nutrients can be added. The rationale is to restore normal cellular processes of the liver after a period of ischemia (either warm ischemia in the donor as well as cold ischemia during static cold storage). By restoring its normal metabolism, the liver is to some degree able to recover from ischemia reperfusion injury. (44) Furthermore, this technique allows assessment of the viability of the liver graft prior to implantation – for example by measuring the clearance of lactate or the amount of bile being produced. (45) Besides several retrospective studies, one randomized controlled trial has evaluated the benefit of normothermic machine perfusion in liver transplantation. This trial demonstrated a significantly lower post-transplant peak level of aspartate transaminase and rate of early allograft dysfunction after reconditioning of both DCD and DBD liver grafts with the use of normothermic machine perfusion. The authors labeled this as clinically relevant, as aspartate transaminase and early allograft dysfunction are well-established biomarkers for long-term survival after liver transplantation. (46) Unfortunately, this multicenter RCT was statistically underpowered to assess whether normothermic machine perfusion was associated with a lower risk of post-transplant biliary complications.

Liver grafts on ex-situ hypothermic machine perfusion are perfused with oxygenated artificial fluids at a temperature of 0-4 degrees Celsius. This low temperature slows down the cellular metabolism, whereupon the graft is able to restore its energy levels. Furthermore, oxygenation of a liver graft in the cold has been found associated with a lower release of radical oxidative species by mitochondria. (47) During this procedure, viability testing by observing bile production and lactate clearance is not possible. However, several markers of hepatic injury can be measured in the perfusate, such as the transaminases and lactate dehydrogenase. (48) Van Rijn and colleagues recently published the results of a multicenter randomized controlled trial on the use of dual hypothermic oxygenated perfusion in DCD liver grafts (DHOPE trial). The primary outcome of this study was the incidence of non-anastomotic strictures post-transplant. Recipients of a DCD liver graft that had been restored by this form of hypothermic machine perfusion had a significant lower risk (risk ratio 0.36) of developing non-anastomotic strictures in comparison with recipients of a

DCD liver graft after conventional static cold storage. Furthermore, the number of interventions required to treat the non-anastomotic strictures was four times lower in the machine group. (49) In the Netherlands, therefore, end-ischemic DHOPE has been incorporated into the national procurement protocol.

Aims and outline of this thesis

Approximately 20 years since its renewed introduction, the landscape of DCD-LT has changed significantly: the number of countries implementing a (national) DCD-LT program has increased substantially, and over 400 scientific articles on DCD-LT have been published in the past two decades. Nowadays, DCD-LT is on the verge of a new era, in which the long-lasting gold standard of DCD-LT with conventional static cold storage will probably be replaced by either a combination of static cold storage with machine perfusion or solely machine perfusion.

This thesis is divided in three parts. The first part provides insight in the current status of DCD-LT in the Netherlands and in other countries in Europe and North America. As previously described, both donor warm ischemia time and cold ischemia time affect the outcomes of DCD-LT. However, several other phases of (relative) ischemia in either donor or recipient can be distinguished. This is the subject of part two. In part three, the focus is shifted to new ways to use the pool of DCD liver grafts to its full potential. For example, by organ donation after euthanasia.

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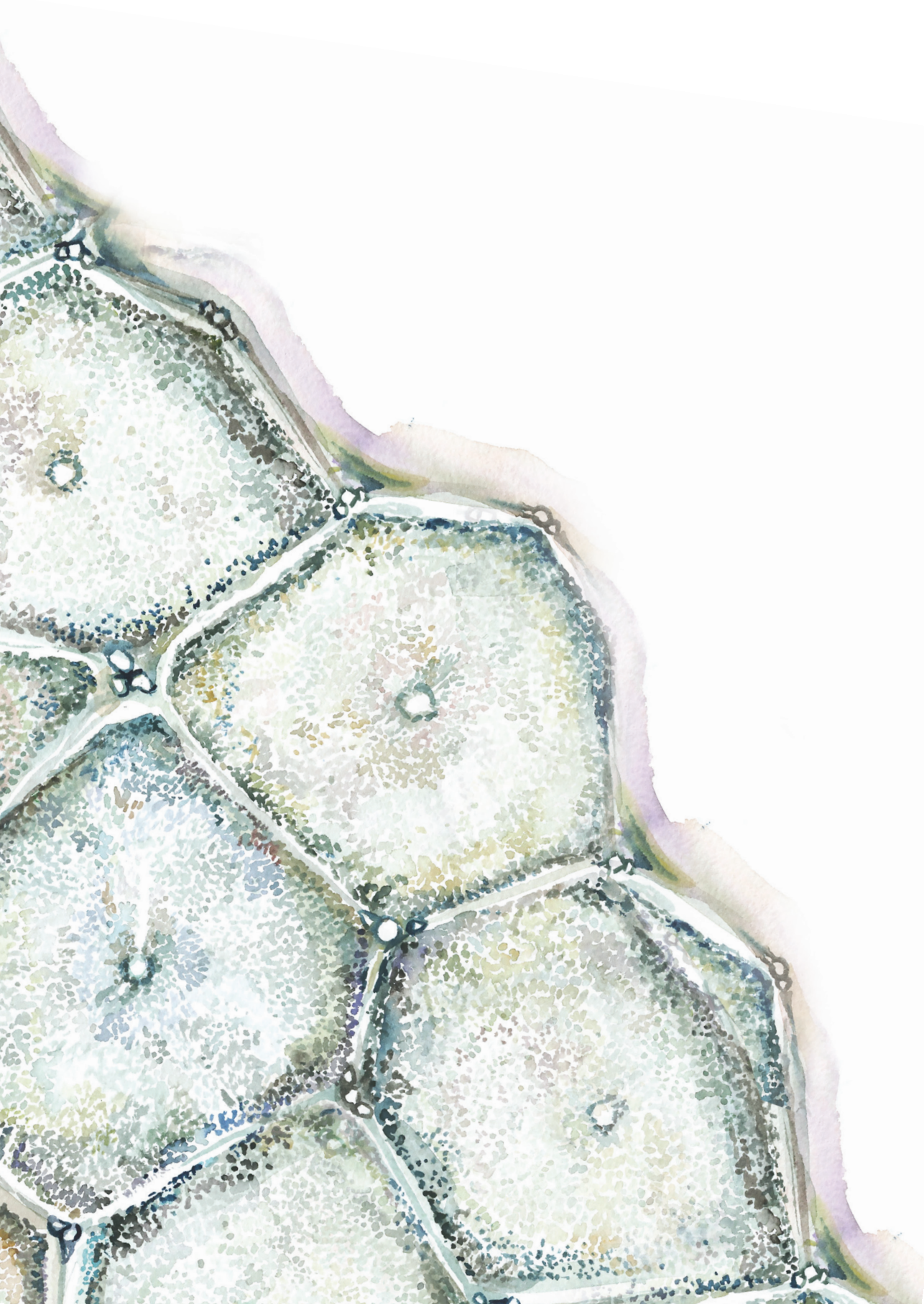

CHAPTER 2

Two decades of liver transplantation with grafts donated after circulatory death in the Netherlands

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In preparation

Abstract

Background: The field of liver transplantation (LT), especially that of donation after circulatory death (DCD), has evolved rapidly over the past years. This national study evaluated possible shifts in demographics and outcomes over two decades of DCD-LT.

Methods: Data of all DCD-LT performed in the Netherlands between start of the program on the 22nd of October 2001 and the first of July 2020, were included. Cases in which machine perfusion was involved, were excluded from statistical analysis. We distinguished three consecutive eras to assess possible changes over time.

Results: A total of 600 DCD-LT have been performed in the study period, including 98 cases in which machine perfusion was involved. Numbers of DCD-LT have increased substantially, accounting for 38% of all LT with post-mortem grafts in 2019. Changes over time were related to, among other things, donor cause of death, primary indication for LT, hepatectomy time and blood loss. The 1-year incidence of portal vein thrombosis and anastomotic stricture increased significantly over time. Patient and graft survival had not significantly changed.

Conclusions: During twenty years of DCD-LT, several patient and recipient characteristics had changed. However, these changes had not resulted in better survival rates post-transplant.

Introduction

Liver transplantation remains the only curative treatment option for end-stage liver disease. In 1963 and 1964, several attempts to successfully transplantation a human liver have failed. (1) In 1967, Starzl and colleagues were the first to perform a successful human orthotropic liver transplantation. (2)

The first organ transplantations with grafts from post-mortem donors have all been executed with grafts obtained from donors after circulatory death (DCD). (3-7) However, after a clear definition of the concept of brain death had been formulated in 1968, the use of organs donated after brain death became the worldwide gold standard. (8)

As a result of the growing shortage of donor organs, the use of DCD grafts for liver transplantation regained international interest in the 1990s. (9) In the Netherlands, the first liver transplantation with a DCD graft was performed in 2001 in the Leiden University Medical Center. In 2021, the number of these procedures had risen to 81, which accounted for 45% of all liver transplantations (both deceased donor transplantations and living donor transplantations) performed in the Netherlands. (10)

Since the introduction of liver transplantation with grafts donated after circulatory death (DCD-LT), the field of solid organ transplantation has evolved substantially. For example, machine perfusion has been introduced as a new preservation technique, and organ donation after euthanasia was added as fifth category to the Maastricht classification of donors after circulatory death. (11, 12) Furthermore, the knowledge on how to effectively use liver grafts from extended criteria donors, including DCD, has improved significantly, as can be concluded from several studies in which outcomes of DCD-LT were similar to that of LT with grafts donated after brain death. (13, 14)

In this national, retrospective cohort study, it was aimed to evaluate the outcomes of and changes among two decades of DCD-LT in the Netherlands.

Methods

All DCD-LT performed in adults between the start of DCD-LT in the Netherlands in 2001 and 1st July 2020 were included. Cases in which the liver graft had been preserved by normothermic regional perfusion or any form of ex-situ machine perfusion have been excluded from further statistical analysis, since most of these cases were part of a clinical trial on machine perfusion.

In the Netherlands, liver transplantation is performed at three university hospitals: the Leiden University Medical Center, the University Medical Center Groningen and the Erasmus MC University Medical Center Rotterdam. Most of the required data for this study could be retrieved from the local databases of these centers. Any additional information on the donors or recipients was retrieved from the Eurotransplant Donor Data app or individual medical records.

Donation and transplantation procedure in the Netherlands

In the Netherlands, the vast majority of DCD donation procedures are classified as DCD type III donation, which is defined as expected circulatory arrest after the withdrawal of life-supporting treatments. (15) Since 2012, organ donation after euthanasia, referred to as the fifth category of DCD donation, is commonly performed in the Netherlands. (12) The withdrawal of life-supporting treatments or euthanasia is performed at either the intensive care unit or a regular ward near the operating room. To ascertain irreversible circulatory arrest of a DCD donor, an obligatory no touch period of 5 minutes has been implemented during which the donor cannot be transported. The organs are retrieved by a super-rapid sternolaparotomy. Pre-mortem cannulation as well as administration of heparin prior to withdrawal of life support is prohibited by Dutch law. Implantation techniques of a liver graft generally include the piggyback technique for the anastomosis of the caval vein, and end-to-end anastomosis for both the portal vein and hepatic artery and a duct-to-duct biliary anastomosis. The use of a portocaval shunt during implantation is optional, and highly dependent on the surgeon's preference and experience.

Definitions

For the purpose of this study, the donor warm ischemia time is defined as the time elapsed between circulatory arrest of the donor and the start of the cold flush during the procurement. The time elapsed between start of cold flush in the donor and the liver being stored on ice is defined as the donor hepatectomy time. The liver being stored on ice is the starting point of the cold ischemia time, which ends when the liver is removed from ice immediately before implantation. The time elapsed between removal of the liver from ice and the reperfusion of the liver graft in the recipient is defined as the recipient warm ischemia time (regardless of the reperfusion sequence chosen by the surgeon). Regarding post-operative complications, non-anastomotic strictures are defined as any clinically relevant stricture of the biliary tree besides strictures at the site of the biliary anastomosis. For all post-operative complications, we calculated the incidence during the first-year post-transplant instead of the prevalence at the end of the follow-up period.

Evaluation of trends

To assess any trends over time regarding the donor and recipient populations, surgical techniques, post-transplant complications and post-transplant survival, we distinguished three eras: 2006-2010, 2011-2015 and 2016-2020. The era 2001-2006 was deliberately excluded from this analysis for two reasons. One, only few DCD-LT had been performed in this era; second, the MELD score based allocation of liver grafts was introduced not until 2006. To enable proper comparison between the three eras, multi-organ transplantations and cases in which the liver was preserved using machine perfusion, were excluded.

Statistics

Continuous variables are presented as median with interquartile range (IQR). A Kruskal-Wallis H test was used to compare continuous variables between the different eras. Bonferroni correction was used to counteract for multiple testing. Categorical variables are presented as frequency with valid percentage and are compared between the eras using a Chi-Square test. Patient and graft survival were analyzed using the Kaplan Meier method. Survival curves were compared between groups using a log rank test. All statistical tests were performed in SPSS, version 25 (SPSS Inc.). A two-sided p-value below 0.05 was considered statistically significant.

Results

Six hundred DCD-LT had been performed during the period under study. The annual number of DCD-LT performed has increased over time (Figure 1). Simultaneously, the proportion of DCD-LT among the total number of deceased LT increased substantially. In 2012, DCD-LT accounted for 23% of all liver transplantations with grafts from deceased donors. In 2019 this proportion was 38%, versus a mean annual proportion of 33% since 2015. Data of 98 cases have been excluded from further statistical analysis because these involved any form of machine perfusion.

Donor and procurement

Table 1 provides the donor and procurement characteristics. Throughout the period under study we see a male predominance, and no significant differences in donor median age and BMI across the three eras. Regarding the donor cause of death, however, significant shifts occurred: the proportion of donors who had died as a result of a cerebrovascular accident decreased over the years, whereas the proportion of donors who died from anoxia increased (overall p-value = 0.04). Considering the procurement of DCD liver grafts, the hepatectomy time has decreased significantly over time, especially during the third era. Although the

overall p-value for total donor warm ischemia time indicated a significant change over time, post-hoc analysis showed only borderline significant differences between era 1 and 3 as well as between era 2 and 3.

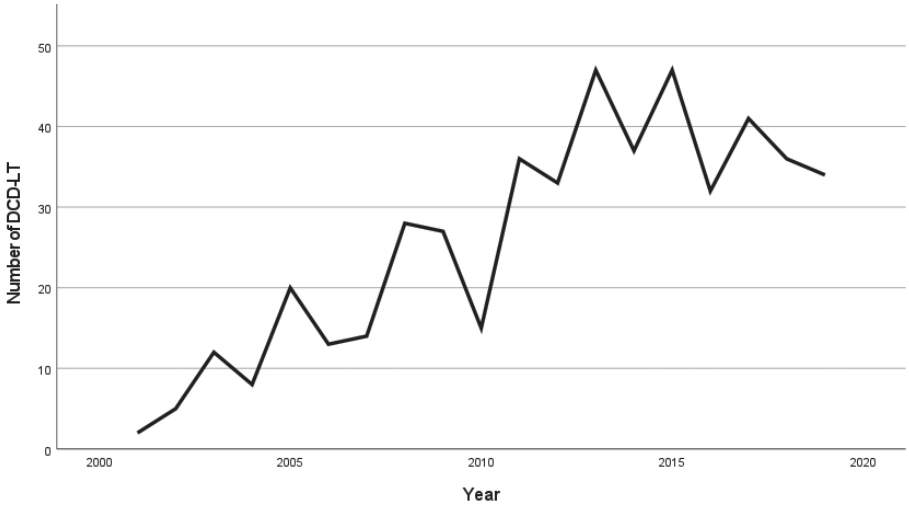

Figure 1: annual number of liver transplantations performed with grafts donated after circulatory death

Recipient and implantation

Table 2 provides the recipient and implantation-related characteristics. Across all three eras, almost 70% of the recipients of a DCD liver graft were male. The recipients' median age at time of transplantation was 56 years. The indication for DCD-LT has changed over time (overall p-value = 0.035). In all three eras, a hepatocellular carcinoma (HCC) was the most common indication. Still, the proportion of DCD-LT performed in recipients with HCC as primary indication for transplantation increased from 29.9% in era 1 to 43.0% in era 3. The proportions of patients with either a chronic viral hepatitis (i.e., cirrhosis due to a chronic hepatitis B or C infection) or alcoholic liver disease had decreased over the eras.

Across all three eras, most of the biliary anastomosis procedures were of the duct-to-duct type (p-value = 0.448). The initial arterial reperfusion approach was the most frequent reperfusion technique in era 2 (overall p-value < 0.001). The cold ischemia time, the recipient warm ischemia time, and the arterialization time had not significantly changed across the three eras. Analysis revealed a trend towards less blood loss during the implantation in era 1 and era 3 – with a median blood loss of 4000 ml and 3000ml, respectively (p-value = 0.006).

Table 1: Donor and procurement characteristics

	Total cohort 2001-2020 (n=502)	Era 1 2006-2010 (n=97)	Era 2 2011-2016 (n=200)	Era 3 2017-July 2020 (n=158)	Overall p-value	p-value (era 1 vs. 2)	p-value (era 1 vs. 3)	p-value (era 2 vs. 3)
Donor								
Gender								
Male	282 (56.2)	50 (51.5)	116 (58.0)	90 (57.0)	0.562	0.293	0.399	0.844
Female	220 (43.8)	47 (48.5)	84 (42.0)	68 (43.0)				
Age (years)	46.50 (33.00-53.00)	44.00 (35.00-50.50)	48.00 (34.00-54.00)	48.00 (35.75-54.25)	0.092	0.379	0.089	1.000
BMI (kg/m²)	24.00 (22.00-26.00)	24.00 (22.00-26.00)	24.00 (22.00-26.00)	25.00 (22.00-27.25)	0.066	1.000	0.320	0.075
Cause of death								
Anoxia	158 (31.5)	22 (22.7)	64 (32.0)	63 (39.9)	0.04	0.162	0.004	0.328
CVA	199 (39.6)	49 (50.5)	80 (40.0)	50 (31.6)				
Trauma	128 (25.5)	25 (25.8)	49 (24.5)	38 (24.1)				
Other	17 (3.4)	1 (1.0)	7 (3.5)	7 (4.4)				
Days in hospital	3.00 (2.00-5.00)	3.00 (2.00-5.00)	3.00 (2.00-5.00)	3.00 (2.00-5.00)	0.597	0.945	1.000	1.000
Days in ICU	3.00 (2.00-5.00)	3.00 (1.50-5.50)	3.00 (2.00-5.75)	3.00 (2.00-5.00)	0.533	0.788	1.000	1.000
Last ALT (IU/l)	35 (21.00-63.00)	29.00 (18.50-54.50)	34.00 (20.00-62.00)	39.00 (19.00-79.25)	0.162	0.867	0.174	0.860
Last AST (IU/l)	50.00 (32.00-86.75)	47.00 (28.00-76.00)	46.00 (29.25-83.75)	52.00 (31.00-93.00)	0.491	1.000	1.000	0.753
Last gGT (IU/l)	32.00 (18.00-61.75)	34.00 (19.00-60.00)	34.00 (20.00-67.50)	36.00 (19.00-70.25)	0.706	1.000	1.000	1.000
Procurement								
dWIT (min)	16.00 (13.00-18.00)	17.00 (13.50-19.00)	15.00 (12.00-18.00)	15.00 (13.00-18.00)	0.110	0.240	0.128	1.000
tdWIT (min)	31.00 (26.00-37.00)	32.50 (26.75-39.25)	32.00 (27.00-38.00)	29.00 (25.00-35.00)	0.023	1.000	0.060	0.061
Hepatectomy time (min)	59.00 (44.00-75.00)	63.00 (55.00-73.00)	62.50 (48.00-80.00)	47.00 (37.50-61.50)	<0.001	1.000	<0.001	<0.001

Data are shown as median (IQR) and frequency (valid percentages). Due to rounding, percentages may not add to 100%. dWIT is defined as time between circulatory arrest and cold perfusion. tdWIT is defined as time between withdrawal of life supporting treatment and cold perfusion. Hepatectomy time is defined as time between start of cold perfusion and the liver being stored on ice. ALT: alanine transferase; AST: aspartate transferase; BMI: body mass index; CVA: cerebrovascular accidents; dWIT: donor warm ischemia time; gGT: gamma-glutamyltransferase; ICU: intensive care unit; tdWIT: total donor warm ischemia time.

Table 2: Recipient and implantation characteristics

	Total cohort 2001-2020 (n=502)	Era 1 2006-2010 (n=97)	Era 2 2011-2016 (n=200)	Era 3 2017-July 2020 (n=158)	Overall p-value	p-value (era 1 vs. 2)	p-value (era 1 vs. 3)	p-value (era 2 vs. 3)
Recipient								
Gender								
Male	348 (69.3)	66 (68.0)	136 (68.0)	109 (69.0)	0.978	0.994	0.874	0.842
Female	154 (30.7)	31 (32.0)	64 (32.0)	49 (31.0)				
Age (years)	56.00 (49.00-63.00)	56.00 (50.00-62.00)	54.00 (47.00-62.00)	58.50 (51.00-65.00)	0.004	1.000	0.135	0.004
Primary indication								
ALF/ACLF	12 (2.4)	4 (4.1)	3 (1.5)	5 (3.2)				
ALD	68 (13.5)	18 (18.6)	28 (14.0)	20 (12.7)				
AIH	11 (2.2)	3 (3.1)	4 (2.0)	1 (0.6)				
PBC/PSC	87 (17.3)	15 (15.5)	32 (16.0)	24 (15.2)				
CCa	9 (1.8)	2 (2.1)	3 (1.5)	4 (2.5)				
Cryptogenic liver cirrhosis	25 (5.0)	8 (8.2)	9 (4.5)	3 (1.9)				
HCC	163 (32.5)	29 (29.9)	61 (30.5)	68 (43.0)	0.035	0.292	0.024	0.101
NASH	29 (5.8)	4 (4.1)	17 (8.5)	8 (5.1)				
Polycystic liver disease	17 (3.4)	1 (1.0)	9 (4.5)	6 (3.8)				
ReLT	17 (3.4)	1 (1.0)	4 (2.0)	9 (5.7)				
Viral hepatitis cirrhosis	33 (6.6)	7 (7.2)	14 (7.0)	3 (1.9)				
Other	31 (6.2)	5 (5.1)	16 (8.0)	7 (4.5)				
Laboratory MELD	15.00 (10.00-21.00)	14.50 (10.00-19.00)	15.00 (10.00-21.00)	16.00 (10.00-22.00)	0.865	1.000	1.000	1.000

	Total cohort 2001-2020 (n=502)	Era 1 2006-2010 (n=97)	Era 2 2011-2016 (n=200)	Era 3 2017-July 2020 (n=158)	Overall p-value	p-value (era 1 vs. 2)	p-value (era 1 vs. 3)	p-value (era 2 vs. 3)
Transplantation								
Biliary anastomosis								
Duct-to-duct	432 (90.0)	80 (82.5)	180 (90.0)	141 (89.2)	0.448	0.326	0.984	0.244
Roux-Y	48 (10.0)	9 (9.3)	13 (6.5)	16 (10.1)				
Reperfusion technique								
Initial portal reperfusion	424 (85.8)	96 (100)	133 (66.8)	154 (97.5)	<0.001	<0.001	0.300	<0.001
Initial arterial reperfusion	69 (14.0)	-	65 (32.7)	4 (2.5)				
Simultaneous reperfusion	1 (0.2)	-	1 (0.5)	-				
Use of portalcaval shunt	80 (16.7)	1 (1.0)	56 (28.0)	22 (13.9)	<0.001	<0.001	<0.001	0.001
CIT (min)	341.00 (288.00-407.00)	352.00 (305.00-453.00)	369.00 (302.75-444.00)	365.00 (289.50-439.00)	0.685	1.000	1.000	1.000
rWIT (min)	30.00 (25.00-35.00)	33.00 (27.00-39.00)	31.00 (26.00-38.00)	31.00 (25.00-35.00)	0.370	1.000	0.691	0.797
Arterialization (min)	30.00 (23.00-40.00)	32.00 (26.00-45.00)	30.00 (24.00-40.00)	30.00 (23.00-42.25)	0.620	1.000	1.000	1.000
Blood loss	3500 (2000-5500)	4000 (2125-7195)	3700 (2000-5175)	3000 (1600-4475)	0.006	0.649	0.006	0.081

Data are shown as median (IQR) and frequency (valid percentages). Due to rounding, percentages may not add to 100%. CIT is defined as time between the liver being stored on ice in the donor hospital and the liver being removed from ice in the recipient hospital. rWIT is defined as the time between the liver being removed from ice and reperfusion. Arterialization time applies only to DCD-LT using an initial portal vein reperfusion approach and is defined as the time between portal reperfusion and completion of the arterial anastomosis. ACLF: acute on chronic liver failure; AIH: auto-immune hepatitis; ALD: alcoholic liver disease; ALF: acute liver failure; CCa: cholangiocarcinoma; CIT: cold ischemia time; HCC: hepatocellular carcinoma; NASH: non-alcoholic steato-hepatitis; PBC: primary biliary cirrhosis; PSC: primary sclerosing cholangitis; reLT: liver retransplant; rWIT: recipient warm ischemia time.

Table 3: Post-transplant outcomes and complications

	Total cohort 2001-2020 (n=502)	Era 1 2006-2010 (n=97)	Era 2 2011-2016 (n=200)	Era 3 2017-July 2020 (n=158)	Overall p-value	p-value (era 1 vs. 2)	p-value (era 1 vs. 3)	p-value (era 2 vs. 3)
Post-operative								
Bilirubin level day 7 (µmol/l)	35.00 (18.00-84.00)	43.00 (19.00-108.00)	32.00 (18.00-83.50)	30.00 (15.00-67.00)	0.090	1.000	0.112	0.365
Peak ALT day 1-7 (IU/l)	1021.50 (517.50-1935.25)	1316.00 (704.00-1949.00)	1015.00 (557.00-2005.00)	811.00 (404.00-1699.00)	0.004	0.435	0.004	0.074
Peak AST day 1-7 (IU/l)	1098.50 (545.75-2445.25)	1629.00 (676.00-2535.50)	1197.00 (580.00-2537.00)	911.00 (448.50-2052.00)	0.012	0.862	0.015	0.092
Peak creatinine day 1-7 (µmol/l)	112.00 (79.00-159.00)	116.00 (83.00-186.50)	110.00 (77.00-159.00)	109.00 (80.00-159.00)	0.456	0.643	1.000	1.000
Days in ICU	2.00 (1.00-5.00)	3.00 (2.00-7.00)	2.00 (1.00-4.00)	2.00 (1.00-4.00)	0.007	0.062	0.006	0.831
Days in hospital	18.00 (13.00-29.00)	22.00 (15.00-34.50)	17.00 (13.00-26.00)	14.00 (11.00-25.50)	<0.001	0.025	<0.001	0.007
Complications during first year post-transplant								
Primary non-function	13 (2.6)	6 (6.2)	3 (1.5)	3 (1.9)	0.047	0.063	0.088	0.770
Vascular								
HAT	29 (5.8)	5 (5.2)	11 (5.5)	12 (7.6)	0.643	0.902	0.448	0.422
PVT	12 (2.4)	1 (1.0)	2 (1.0)	8 (5.1)	0.028	1.000	0.159	0.025
Biliary								
Bile leak	61 (12.2)	11 (11.3)	26 (13.0)	20 (12.7)	0.919	0.685	0.755	0.924
Anastomotic strictures	124 (24.7)	15 (15.5)	54 (27.0)	45 (28.5)	0.046	0.027	0.017	0.756
Non-anastomotic strictures	107 (21.3)	19 (19.6)	44 (22.0)	36 (22.8)	0.830	0.633	0.547	0.860

Data are shown as median (IQR) and frequency (valid percentages). Due to rounding, percentages may not add to 100%. ALT: alanine transferase; AST: aspartate transferase; HAT: hepatic artery thrombosis; ICU: intensive care unit; PVT: portal vein thrombosis;

Post-transplant outcomes

Analysis revealed a trend towards lower ALT and AST peak levels post-transplant across the three eras (overall p-values of 0.004 and 0.012, respectively) (table 3). The post-operative hospital stay in era 3 was significantly shorter than that in era 1 (overall p-value < 0.001).

Although the overall p-value of 0.047 for the incidence of primary non-function indicated statistical significance, post-hoc comparisons between the eras showed no significant differences in this respect. The incidence of portal vein thrombosis in era 3 was significantly higher than that in the last era (p-value = 0.025). In both era 2 and era 3, significantly more patients were diagnosed with an anastomotic stricture within the first year post-transplant when compared to era 1 (p-value of 0.027 and 0.017, respectively). The 1-year incidences of hepatic artery thrombosis, bile leaks and non-anastomotic strictures had not changed across the eras.

Survival

The 30-days, 90-days and 1-year patient survival rates for the total cohort were 96%, 95% and 89%, respectively. The corresponding figures for graft survival were 91%, 88% and 79%, respectively. The survival rates per era are given in Table 4.

Table 4: Patient and graft survival

	Total cohort 2001-2020 (n=502)	Era 1 2006-2010 (n=97)	Era 2 2011-2016 (n=200)	Era 3 2017-July 2020 (n=158)
Patient survival				
30-days	96%	98%	95%	97%
90-days	95%	95%	94%	96%
1-year	89%	87%	90%	91%
Graft survival				
30-days	91%	88%	91%	92%
90-days	88%	86%	89%	90%
1-year	79%	73%	81%	81%

Survival rates were calculated using the Kaplan Meier method. Patient survival is defined as death of the recipient of a DCD liver graft, graft survival is defined as either death of the recipient or retransplantation.

Kaplan Meier survival curves of both patient and graft survival, stratified per era, are depicted in Figures 2 and 3. Both patient survival and graft survival did not differ between the three eras.

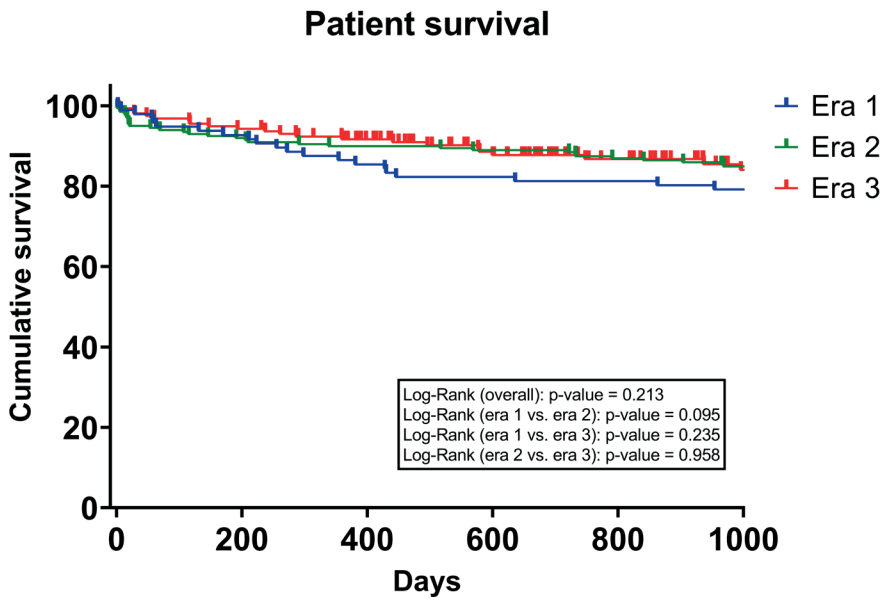

Figure 2: Patient survival after DCD-LT, stratified per era
Patient survival is defined as death (with or without functioning graft).

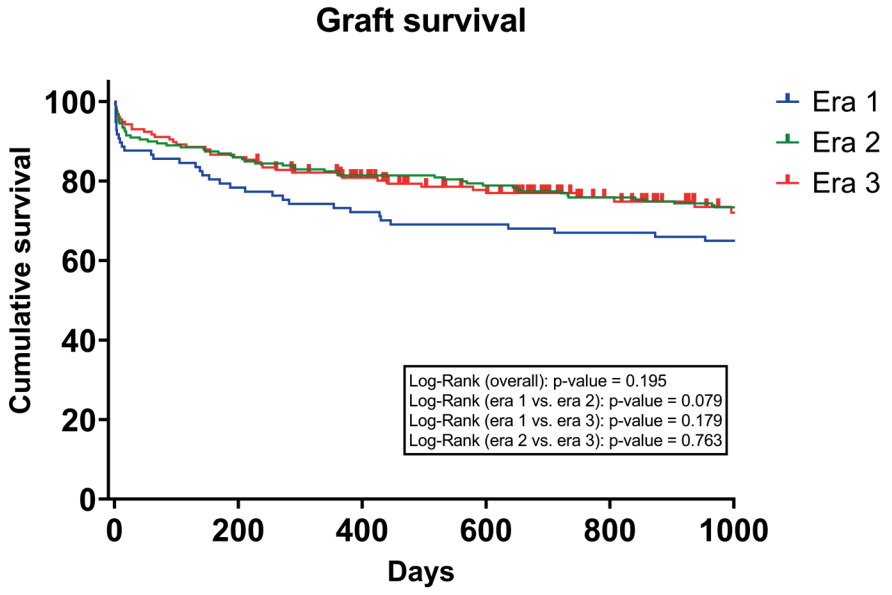

Figure 3: Graft survival after DCD-LT, stratified per era
Graft survival is defined as death (with or without functioning graft) or consecutive retransplantation.

Machine perfusion

In 98/600 cases, machine perfusion was applied for preservation, first in 2014 (era 2) in the University Medical Center Groningen. In most of these cases (n = 73), the type of machine perfusion was dual hypothermic oxygenated machine perfusion (DHOPE). The benefits of dual hypothermic oxygenated machine perfusion (DHOPE) over static cold storage were evaluated by an international, multicenter, randomized trial (DHOPE-DCD trial), that was coordinated by the University Medical Center Groningen and in which all three Dutch transplant centers have participated. (16) This study found that, when compared with static cold storage, DHOPE leads to a lower risk of non-anastomotic strictures after DCD-LT.

In sixteen DCD-LT included in the current study, the liver graft was preserved by normothermic ex-situ machine perfusion, preceded by DHOPE. Part of these sixteen grafts have been included in the DHOPE-COR-NMP trial (designed and executed by the University Medical Center Groningen). In the DHOPE-COR-NMP trial, DCD liver grafts that were nationwide declined for transplantation, were subjected to NMP in order to carefully assess the viability of the DCD graft. A substantial part of these grafts fulfilled predefined viability criteria and could therefore be successfully transplanted, increasing the number of transplantable livers by 20%. (17)

In 2018, the Erasmus MC University Medical Center en Leiden University Medical Center have started a trial on outcomes of DCD-LT with grafts that have been preserved by normothermic regional perfusion, in which extracorporeal circulation is used to restore the intra-abdominal blood flow in the DCD donor for a limited period of time. This trial is ongoing and results have yet to be presented.

Discussion

At the beginning of this century, as we witnessed a growing shortage of donor organs, the use of liver grafts from DCD donors regained interest in the Netherlands. Since then, the field of liver transplantation has evolved enormously. We are on the verge of a new era in which almost all DCD liver grafts will be preserved using a type of machine perfusion. To our knowledge, this is the first study that evaluated the experiences in the past two decades in the Netherlands.

We found that in the early years of liver transplantation with the use of grafts from DCD donors, the majority of the donors had died from a cerebrovascular accident, whereas later the most common cause of death was anoxia. A possible explanation for this shift is the improvement of primary and secondary prevention for cerebrovascular diseases as well as more evolved treatment modalities for these diseases. (18) Furthermore, donors who had died from anoxia were the ones who (a) suffered post-anoxic brain damage after, for example, cardiac arrest, hanging or drowning, and (b) donated their organs after euthanasia. Organ donation after euthanasia was introduced in the Netherlands in 2012, and since then over 60 persons have donated their organs after euthanasia. (12) This practice might have contributed to the shift in donor cause of death.

Research has shown that donor warm ischemia time is an important risk factor for inferior outcomes after DCD-LT. (19-21) The median donor warm ischemia time we found is substantially longer than that reported for other countries, such as Belgium. (22) This is probably the result of differences in national legislation and transplant protocols. For example, in Belgium withdrawal of the donor's life support takes place in the operating room, whereas in the Netherlands withdrawal of life support must take place at a regular ward or intensive care unit. The time needed to transport the donor to the operating room automatically leads to a longer donor warm ischemia time. The current legislation in the Netherlands prevents shortening the donor warm ischemia time. Still, the donor hepatectomy time – another risk factor for inferior outcomes – is highly dependent on the experience of the organ retrieval team and thus is modifiable. (23) In 2018, the Dutch Transplant Society, made transplant surgeons aware of the relatively long median hepatectomy time in the Netherlands. (24) The campaign was successful, as can be concluded from the significantly lower donor hepatectomy time in era 3 of the current study.

Not only the donor landscape has changed over time; characteristics of recipients of DCD liver graft have changed somewhat as well. An important finding is the decrease in the proportions of patients who required a transplantation because of a chronic viral hepatitis. This finding is supported by literature and is likely to be the

result of the introduction of direct-acting antivirals as treatment modality for with viral hepatitis C. (25) Surprisingly, after an increase in the proportion of recipients requiring a DCD liver graft because of non-alcoholic steato-hepatitis during era 2, this increase did not continue during era 3, but even decreased. This development is not in line with the literature suggesting that – due to the obesity epidemic in the Western world – non-alcoholic steato-hepatitis will become one of the most common indications for liver transplantation. (26-29) A possible explanation for our finding lies in the disease classification used. All cases of HCC, were assigned to the category HCC as primary indication, irrespective of the underlying liver disease. The proportion of HCC patients with underlying non-alcoholic steato-hepatitis might have grown over the three eras. The disease classification used in this study has inevitably led to a loss of data on the underlying diseases in the HCC category, as well as on secondary indications for DCD liver transplantation. In our opinion, this is the biggest limitation of the study.

Another interesting observation from the current study is that in era 2 significantly more transplantations were performed using an initial arterial reperfusion sequence, whereas a portal first reperfusion technique is the standard in the Netherlands. This observation is attributable to the fact that in the early 2010s the Leiden University Medical Center for research purposes applied initial arterial reperfusion approach in all liver transplantations. (30)

Regarding post-transplant complications, two striking observations are the increases in numbers of anastomotic strictures in eras 2 and 3 as well as the sudden increase in portal vein thrombosis in era 3. We do not have a clear explanation for these findings. We found that a high proportion of recipients had developed a non-anastomotic stricture. This finding deserves more careful evaluation, because non-anastomotic strictures are the Achilles' heel of DCD liver transplantation. Of note, however, the applied definition of non-anastomotic stricture resulted in substantial heterogeneity among individual cases: some were resolved completely after one endoscopic retrograde cholangiography, whereas other cases required multiple endoscopic/percutaneous interventions and eventually retransplantation. Recently, Croome and colleagues classified non-anastomotic stricture, also known as ischemic cholangiopathy into four distinct patters, each with its own clinical course. (31) For the purposes of further research, it would be helpful to classify all Dutch cases according to this classification.

Besides the disease classification mentioned above, an important limitation of this study is that we were unable to gain full insight in the donor and especially recipient characteristics of those cases in which machine perfusion had been used. It would be of great interest to know whether there are significant differences in baseline characteristics between recipients receiving a DCD liver

graft that has been preserved using static cold storage and those who received a graft preserved by machine perfusion. Presuming there are significant differences, excluding machine cases might have led to other findings.

In conclusion, in two decades of DCD liver transplantation in the Netherlands, several subtle occurred in characteristics of both the donor pool and recipient pool, as well as in transplant-related characteristics. However, these shifts have not resulted in significantly different survival rates.

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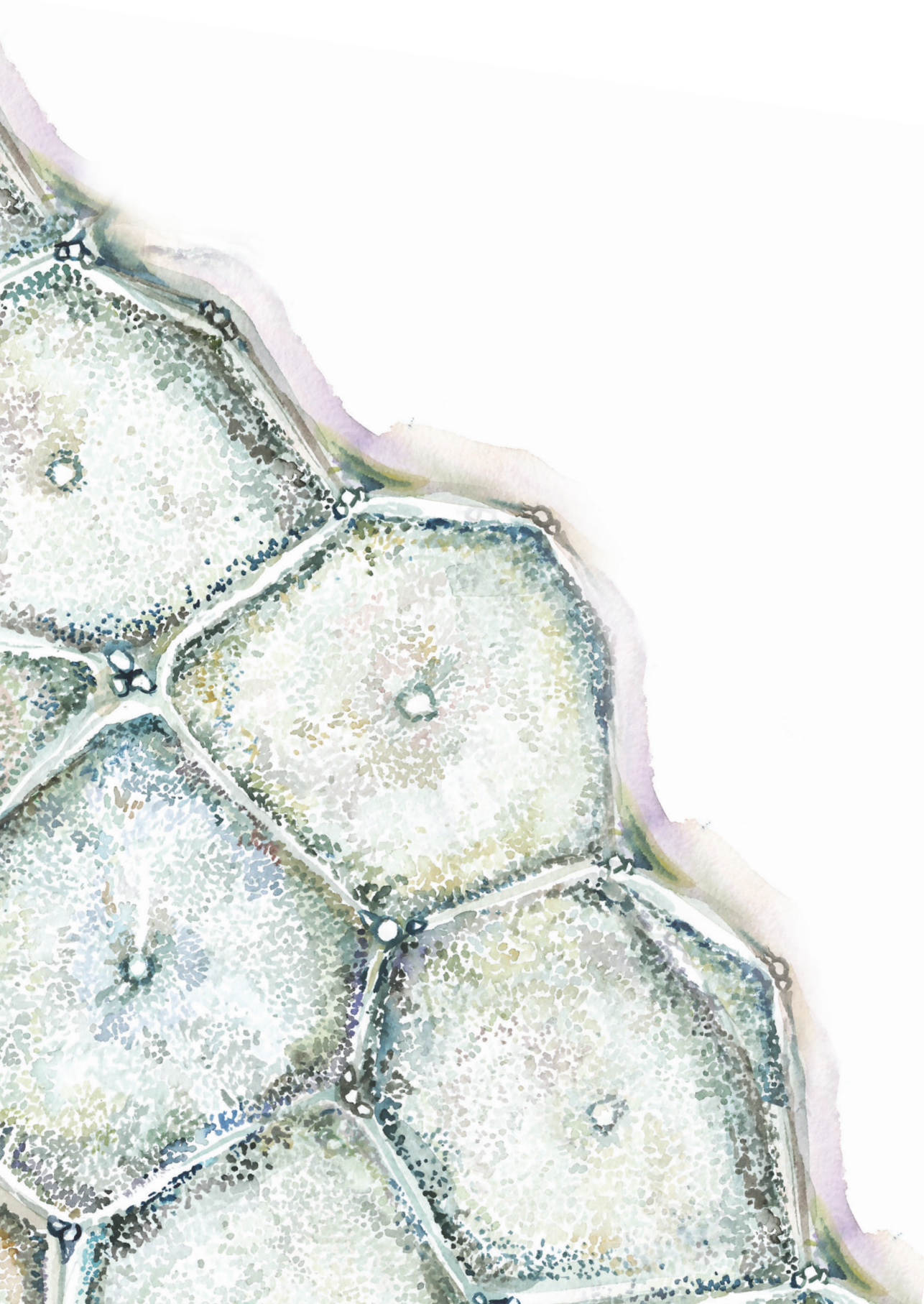

CHAPTER 3

Policies towards donation after circulatory death liver transplantation: the need for a guideline?

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Abstract

Background: Liver transplantation (LT) using grafts from donation after circulatory death (DCD) is evolving to standard of care in many countries. Various transplant centers have developed a protocol for DCD-LT. The existence of numerous protocols may cause inconsistencies. Knowledge of these differences may help improve the outcome of DCD-LT.

Methods: An internet-based survey was sent to 119 transplant surgeons among four countries: Belgium (BE), the Netherlands (NL), Spain (ES) and the United Kingdom (UK).

Results: Thirty-three percent of all respondents indicated having no specific age limit for DCD-LT donors, and if there was a limit, half of them ignored it. Calculation of donor warm ischemia time (dWIT) varied substantially between countries. In ES and the UK, the starting point of dWIT was defined as deterioration of saturation/blood pressure, while in NL, cardiac arrest was used as starting point. Seventy-eight percent of the respondents used a super-rapid sterno-laparotomy as procurement technique. Surgeons from NL and BE mainly used aortic perfusion (95% and 72%), while dual perfusion was more common in the UK (90%) and ES (91%).

Conclusions: This study demonstrates major differences in approach to DCD-LT. To assure both donors and recipients a consistent standard of care, a consensus meeting on DCD-LT is highly recommended.

Introduction

The growing disparity between the number of patients on the waiting list for liver transplantation (LT) and the number of available grafts has led to changed acceptance criteria for deceased donor grafts. To prevent a further increase of waiting list mortality, transplant physicians and surgeons are compelled to use grafts from donation after circulatory death (DCD). In 2017, the percentages of deceased donor LT performed with DCD grafts in the Netherlands (NL), Belgium (BE), the United Kingdom (UK) and Spain (ES) were 38%, 26%, 22% and 13%, respectively. (1-3)

The expansion of DCD practices is accompanied by the introduction of many different protocols. In the USA, 64 different DCD protocols have been reported. (4) Moreover, there is a considerable variation among DCD policies in American children's hospitals on important areas such as the declaration of death. (5) In Europe, different protocols exist regarding inclusion and exclusion criteria for donation and preservation methods, although exact numbers are not known. (6) In the case of DCD-LT, this is also the result of the absence of a European guideline.

A multitude of protocols may lead to inconsistencies and eventually to the inability to provide a consistent standard of care for a patient awaiting a liver graft. This is of major concern especially in DCD-LT, since DCD-LT is associated with lower patient and graft survival and a higher incidence of graft failure and biliary complications compared to LT performed with grafts from donation after brain death (DBD-LT). (7-12)

The aim of this study was to prepare an inventory of differences in policies towards DCD-LT in four European countries with extensive experience of DCD-LT and to assess the impact of these differences on the need to develop a European guideline on DCD-LT.

Methods

Study design

An internet-based survey was sent to transplant surgeons performing DCD-LT in four European countries: BE, ES, NL, and the UK. Table 1 depicts a global description of the liver transplant programs in these countries. We included only surgeons who were responsible for the acceptance of donor grafts. To guarantee that all participants were experienced surgeons, surgical residents and trainees were excluded from this study. Furthermore, participants who opted out of invitations by SurveyMonkey® (Palo Alto, CA, USA) were excluded. In BE, NL, and the UK, the surgical directors of the transplant programs assigned eligible participants to the study. The Sociedad Española de Transplante Hepático (SETH)

functioned as an intermediary to disseminate the survey in ES. The study was approved by the institutional review board of the Erasmus University Medical Center Rotterdam (MEC-2017-311).

Table 1: Global description of DCD liver transplant program per country

			BE	ES	NL	UK
Start DCD-LT program			2003	1995[a]	2001	2001
Duration of no touch period (min)			5	5	5	5
Number of centers performing LT in 2017			6 (6 DCD)	26 (20 DCD)	3 (3 DCD)	7 (7 DCD)
Number of DBD-LT 2017			214	1081	100	737
Number of DCD-LT 2017			71	166[b]	61	209[c]
Proportion DCD-LT of all deceased LT 2017			25%	14%	38%	22[c]
Patient survival	DBD-LT	1-year	89%[d]	90%[e]	89%[d]	91%[g]
		2-year	86%[d]	87%[e]	86%[d]	89%[g]
	DCD-LT	1-year	88%[d]	89%[f]	89%[d]	88%[g]
		2-year	84%[d]	85%[f]	87%[d]	83%[g]
Graft survival	DBD-LT	1-year	81%[d]	83%[e]	81%[d]	88%[g]
		2-year	77%[d]	79%[e]	77%[d]	85%[g]
	DCD-LT	1-year	78%[d]	82%[f]	77%[d]	81%[g]
		2-year	74%[d]	78%[f]	72%[d]	75%[g]

BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom. [a] Until 2012 only uncontrolled DCD-LT (Maastricht type 2). [b] Of which seven uncontrolled DCD-LT. [c] Time period 01.04.2016 - 31.03.2017. [d] Survival rates from national cohort of liver transplantations performed between 01.01.2010 - 31.12.2015, Source: Eurotransplant Survival Curves Application. [e] Survival rates from national cohort of all liver transplantations (consisting for 93% of DBD-LT) performed between 01.01.2013 - 31.12.2015. Source: *Registro Español de Transplante Hepático, Memoria de Resultados 2015*. Sociedad Española de Transplante Hepático. (29) [f]Survival rates from national cohort of either DCD type II or DCD type III liver transplantations performed between 01.01.2012 - 31.12.2015. Source: *Informe de actividad de donación y transplante de donantes en asistolia*, Organización Nacional de Trasplantes (ONT). (30) [g] Survival rates from cohort of LT performed in seven UK transplant centers between 01.01.2005 - 31.12.2010. Source Chistopher J. Callaghan et al. (11)

Survey design

The questionnaire contained 54 items, mostly multiple-choice questions, on four topics: donor and recipient characteristics as well as procurement and implantation (the full survey can be found in Appendix A). Transplant surgeons of the Erasmus University Medical Center Rotterdam tested and revised the questionnaire. All participants had the opportunity to make comments at the end of the survey.

Survey administration

The questionnaire was distributed by SurveyMonkey® between February 2016 (NL) and July 2017 (UK). Reminders were sent to all partial-responders and non-responders in NL, BE, and the UK. In consultation with SETH, only the chairmen of the Spanish transplant programs received reminders. If a participant filled out the survey more than once, only the first response was included in the analysis. Participation was voluntary, without any form of incentive.

Statistical analysis

Questionnaire data was extracted into an anonymous datasheet. All statistical analyses were performed using SPSS (IBM, Chicago, IL, USA). Medians (interquartile range (IQR)) or frequency (percentage) were used to describe numerical and categorical variables, respectively. The response rate varied substantially among countries; therefore, we did not perform any test to evaluate statistically significant differences between countries. Both completely and partially filled out questionnaires were included in the analysis. Proportions in the results section represent valid percentages (i.e. missing data excluded per question).

Results

One hundred and nineteen transplant surgeons were approached in 36 centers. Eighty-eight responses were obtained (response rate 74%). The response rate was the highest in ES (83%), followed by BE (80%), NL (76%), and the UK (59%). Two responses were excluded; one because the respondent was not responsible for graft acceptance, and one because the respondent was working in a center in which DCD grafts were not accepted. As four transplant surgeons responded twice, the true response rate was 69%.

Demographic characteristics

Seventy-two percent of the respondents performed both procurement and transplantation of DCD grafts, while 28% carried out only the implantation. Almost half worked in a transplant center in which at least 30% of LTs is performed with DCD grafts. The mean number of DCD-LT performed by one single transplant center over a period of 5 years was 86. The mean proportion of DCD-LT among all LT performed in a single center was 12% in ES, 26% in the UK, 27% in BE, and 40% in NL.

Donor-related questions

Thirty-three percent of surgeons reported that they do not use an upper age limit for a DCD liver donor in their center. The proportion of respondents having no age limit differed between the countries (Table 2). If an age limit was defined by a transplant center, 50% of the respondents stated having ignored this limit at some point, often when other risk factors in the donor were absent.

Table 2: Use of an upper age limit for a DCD liver donor

	BE n (%)	**ES n (%)**	**NL n (%)**	**UK n (%)**	**Total n (%)**
50 years	1 (5.3)	0	0	0	1 (1.3)
60 years	3 (15.8)	6 (46.2)	15 (71.4)	2 (8.7)	26 (34.2)
65 years	4 (21.1)	3 (23.1)	1 (4.8)	6 (26.1)	14 (18.4)
70 years	0	1 (7.7)	0	4 (17.4)	5 (6.6)
75 years	2 (10.5)	0	0	2 (8.7)	4 (5.3)
>75 years	1 (5.3)	0	0	0	1 (1.3)
No age limit	8 (42.1)	3 (23.1)	5 (23.8)	9 (39.1)	25 (32.9)

Data are shown as number (percentage). BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom.

As the starting point of donor warm ischemia time (dWIT) is not well-defined, one question focused on the use of this parameter. The majority of the respondents from the UK and ES indicated deterioration of saturation and/or blood pressure as the starting point of dWIT. The cut-off saturation used by the respondents varied between 50% and 90% SpO2. Regarding blood pressure, both mean arterial pressure (MAP) and systolic blood pressure (SBP) were used as a cut-off point and varied between a MAP of 50 mmHg and an SBP of 80 mmHg. In NL, the majority used cardiac arrest as the starting point of dWIT, while in BE more differences were noted (Figure 1). Fifty-three percent of the respondents accepted a maximum dWIT of 30 minutes, while 21% and 16% used a more strict limit of 20 and 15 minutes, respectively.

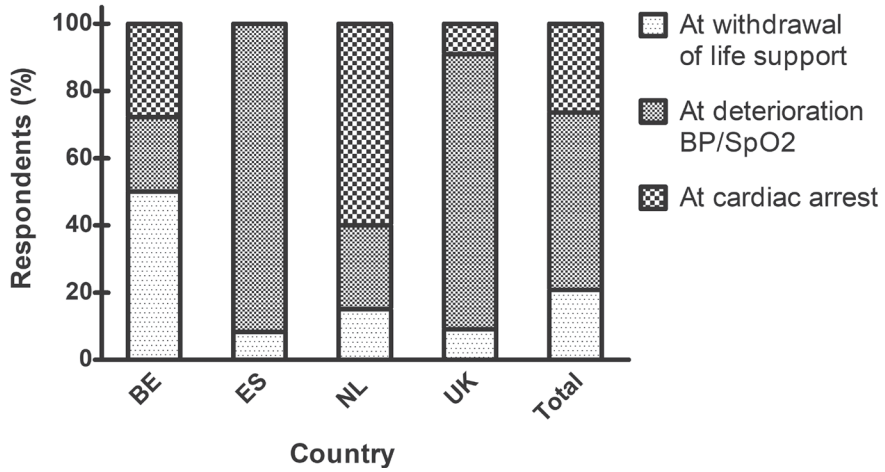

Figure 1: Starting point of donor warm ischemia time
BE Belgium, BP blood pressure, ES Spain, NL the Netherlands, SpO2 oxygen saturation, UK the United Kingdom

Besides the definition of dWIT, the location at which withdrawal of life-supporting treatment (WLST) of the donor takes place differed between the countries. All Dutch respondents and a majority of those from the UK answered that WLST took place in the intensive care unit, while in BE and ES, WLST was often performed in the operating room (Figure 2).

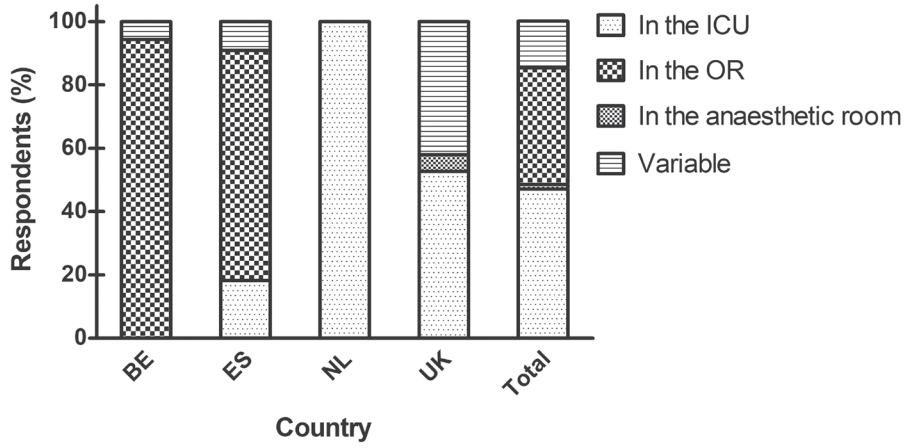

Figure 2: Location of withdrawal of life-supporting treatment
BE Belgium, ES Spain, ICU intensive care unit, NL the Netherlands, OR operating room, UK the United Kingdom

A majority of the surgeons stated that they perform a biopsy of the donor liver (BE 89%, ES 82%, NL 60%, UK 80%), either to have a baseline biopsy of the graft or when there was a suspicion of steatosis or fibrosis of the graft. Seventeen percent and 65% of the respondents accepted a maximum percentage of steatosis of 10% and 30%, respectively, while 7% did not accept any steatosis at all.

Procurement-related questions

A super-rapid procurement with sterno-laparotomy was the preferred technique of most Dutch, Belgian, and British surgeons. On the contrary, cannulation prior to laparotomy was more common among Spanish respondents (Table 3). Most respondents from BE and NL reported using only aortic perfusion (72% and 95%, respectively), while in the UK and ES, dual perfusion via the aorta and portal vein was preferred (90% and 91%, respectively).

Table 3: Procurement technique used in DCD liver donors

	BE n (%)	ES n (%)	NL n (%)	UK n (%)	Total n (%)
Super-rapid procurement with sterno-laparotomy	14 (77.8)	4 (36.4)	20 (100)	16 (80.0)	54 (78.3)
Cannulation prior to laparotomy	3 (16.7)	4 (36.4)[a]	0	2 (10.0)	9 (13.0)
Both	1 (5.6)	3 (27.3)	0	2 (10.0)	6 (8.7)

Data are shown as number (percentage). BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom. [a]Always as part of normothermic regional perfusion in Spain.

Since the role of machine perfusion in DCD-LT has evolved rapidly, two questions were focused on the use of machine perfusion by our respondents. Machine perfusion is used by 63% of the respondents (9% as standard of care, 54% only as part of clinical trials) (Figure S1, Appendix B). Regarding the type of machine perfusion, 47% used normothermic ex vivo machine perfusion whereas 53% used hypothermic oxygenated machine perfusion (Figure S2, Appendix B).

As many different perfusion solutions are available nowadays, respondents were asked what solution was used for DCD grafts. This varied substantially between the countries (Figure 3): University of Wisconsin (UW) was used by most Dutch and British surgeons (95% and 75%, respectively), 61% of the Belgian respondents used Institute George Lopez-1 solution (IGL-1), whereas in Spain Celsior was preferred (55%). The median amount of perfusion solution used was 5 and 10 l for aortic and portal perfusion, respectively. In 52%, heparin was added to the perfusion solution, and in 37%, heparin was administered to the donor directly after WLST.

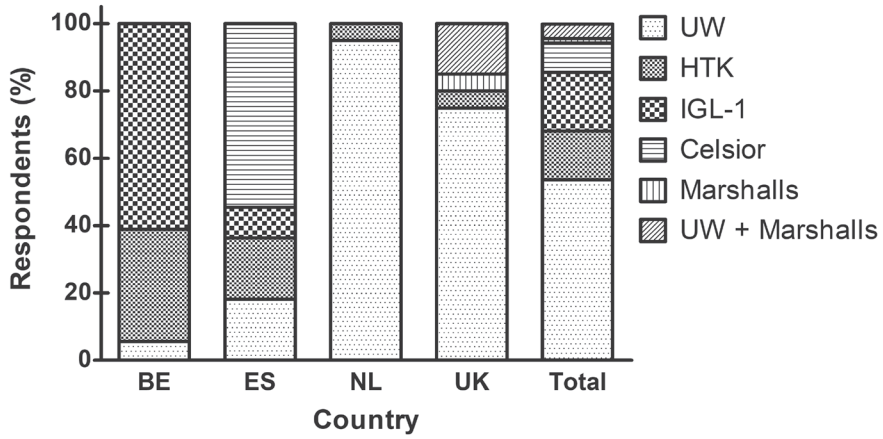

Figure 3: Preservation solution used during procurement of DCD liver graft
BE Belgium, ES Spain, HTK histidine-tryptophan-ketoglutarate, IGL-1 Institute George Lopez-1, NL the Netherlands, UK the United Kingdom, UW University of Wisconsin

Recipient-related questions

Half of the respondents indicated that their center has an age limit for a DCD liver recipient of 70 years, while 28% stated no age limit for their center. According to 76% of the surgeons, the age limit for a DCD recipient did not differ from that for a DBD recipient in their center. Surgeons also reported ignoring the age limit for DCD recipients – although the proportion (13%) was smaller than for DCD donors – mainly when the recipient was in good clinical condition or in case of urgent need for a graft (i.e. acute liver failure).

Lastly, the respondents were given a list of liver diseases, adapted from the 6th International Conference in Organ Donation held in Paris in 2013, and asked to report for each disease if it was an indication for receiving a DCD graft. The results differed widely between and within the countries (Table 4). There was also a great variety of possible contraindications (Table 5).

Implantation-related questions

According to the majority of Dutch, British, and Spanish respondents, the target cold ischemia time (CIT) was 8 h or less (65%, 63%, and 55%, respectively). In BE, 61% used a more strict limit of 6 h or even less. Finally, when questioned about what reperfusion technique was used, a portal reperfusion first approach is performed by 78%. Simultaneous perfusion was used by only 6% of the respondents, all of whom work in BE.

Table 4: Indications for the use of DCD liver grafts

		BE n (%)	ES n (%)	NL n (%)	UK n (%)	Total n (%)
Do you use DCD grafts for the following indications?						
ALF	Yes	13 (72.2)	5 (45.5)	11 (57.9)	8 (42.1)	37 (55.2)
	No	5 (27.8)	6 (54.5)	8 (42.1)	11 (57.9)	30 (44.8)
PSC	Yes	6 (33.3)	6 (54.5)	14 (73.7)	15 (78.9)	41 (61.2)
	No	12 (66.7)	5 (45.5)	5 (26.3)	4 (21.1)	26 (38.8)
PBC	Yes	8 (44.4)	6 (54.5)	14 (73.7)	18 (94.7)	46 (68.7)
	No	10 (55.6)	5 (45.5)	5 (26.3)	1 (5.3)	21 (31.3)
Alcoholic liver cirrhosis	Yes	17 (94.4)	11 (100)	19 (100)	19 (100)	66 (98.5)
	No	1 (5.6)	0	0	0	1 (1.5)
Cirrhosis due to chronic HBV	Yes	17 (94.4)	10 (90.9)	18 (94.7)	19 (100)	64 (95.5)
	No	1 (5.6)	1 (9.1)	1 (5.3)	0	3 (4.5)
Cirrhosis due to chronic HCV	Yes	17 (94.4)	10 (90.9)	18 (94.7)	19 (100)	64 (95.5)
	No	1 (5.6)	1 (9.1)	1 (5.3)	0	3 (4.5)
HCC	Yes	17 (94.4)	11 (100)	19 (100)	19 (100)	66 (98.5)
	No	1 (5.6)	0	0	0	1 (1.5)
Polycystic liver disease	Yes	7 (38.9)	8 (72.7)	13 (68.4)	9 (47.4)	37 (55.2)
	No	11 (61.1)	3 (27.3)	6 (31.6)	10 (52.6)	30 (44.8)
ReLT	Yes	7 (38.9)	4 (36.4)	6 (31.6)	3 (15.8)	20 (29.9)
	No	11 (61.1)	7 (63.6)	13 (68.4)	16 (84.2)	47 (70.1)

Data are shown as number (percentage). BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom; ALF, Acute Liver Failure; PSC, Primary Sclerosing Cholangitis; PBC, Primary Biliary Cirrhosis; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; HCC, Hepatocellular Carcinoma; ReLT, liver retransplantation;

Table 5: Contra-indications for the use of DCD liver grafts

		BE n (%)	ES n (%)	NL n (%)	UK n (%)	Total n (%)
Do you consider the following as a contra-indication for the use of DCD grafts?						
ALF	Yes	3 (16.7)	2 (18.2)	7 (36.8)	8 (42.1)	20 (29.9)
	No	15 (83.3)	9 (81.8)	12 (63.2)	11 (57.9)	47 (70.1)
ReLT	Yes	7 (38.9)	8 (72.7)	11 (57.9)	15 (78.9)	41 (61.2)
	No	11 (61.1)	3 (27.3)	8 (42.1)	4 (21.1)	26 (38.8)
PVT	Yes	3 (16.7)	5 (45.5)	7 (36.8)	10 (52.6)	25 (37.3)
	No	15 (83.3)	6 (54.5)	12 (63.2)	9 (47.4)	42 (62.7)
History of upper abdominal surgery	Yes	3 (16.7)	6 (54.5)	6 (31.6)	7 (36.8)	22 (32.8)
	No	15 (83.3)	5 (45.5)	13 (68.4)	12 (63.2)	45 (67.2)
History of SBP	Yes	1 (5.6)	3 (27.3)	4 (21.1)	3 (15.8)	11 (16.4)
	No	17 (94.4)	8 (72.7)	15 (78.9)	16 (84.2)	56 (83.6)
HPS	Yes	2 (11.1)	1 (9.1)	1 (5.3)	7 (36.8)	11 (16.4)
	No	16 (88.9)	10 (90.9)	18 (94.7)	12 (63.2)	56 (83.6)
Combined liver and kidney transplantation	Yes	4 (22.2)	7 (63.6)	10 (52.6)	7 (36.8)	28 (41.8)
	No	14 (77.8)	4 (36.4)	9 (47.4)	12 (63.2)	39 (58.2)

Data are shown as number (percentage). BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom; ALF, Acute Liver Failure; ReLT, liver retransplantation; PVT, portal vein thrombosis; SBP, spontaneous bacterial peritonitis; HPS, Hepato-Pulmonal Syndrome.

Discussion

This study is the first to give a general overview of the different approaches towards DCD-LT in four European countries with extensive experience of DCD-LT. The results show that there are major differences among and within countries. This is in accordance with a recently published study on the attitudes towards DCD-LT among transplant centers in the USA. (13) These major differences highlight the need for a consensus meeting and the development of a European protocol. Table 6 provides an overview of clinical questions arisen from the results of our survey that could be used as starting point for such a meeting.

Table 6: Topics to discuss during a consensus meeting

Unsolved topics in DCD-LT	
Donor related	Should all age limits for DCD-donors be rejected? Where should withdrawal of life supporting treatment in the donor take place? Standardization of the definition of total first WIT, functional first WIT and true first WIT.
Recipient related	Should all age limits for DCD-recipients be rejected? Are there any clear contra-indications for receiving a DCD liver graft?
Procurement and transplantation related	What type of perfusion can be best used in DCD-LT donors (dual or aortic only)? What perfusion solution can be best used in DCD-LT? What should be the role of machine perfusion and normothermic regional perfusion in DCD-LT?
Other	How can the risk of protocol violation be minimalized? What is the best interval for updating current protocols and guidelines?

DCD-LT, Liver Transplantation with grafts from Donation after Circulatory Death; DCD, Donation after Circulatory Death; WIT, Warm Ischemia Time;

The argument that differences in protocols are not that essential as the patient and graft survival after DCD-LT are almost equal in the four participating countries (Table 1) is invalid. The differences demonstrated in our study are very large, making plain comparisons of the results between – and sometimes even within – countries unjustifiable. Standardization of the definitions of donor and procurement characteristics is essential to make a proper comparison of the survival rates for a great variety of surgical approaches. Only then shall we be able to form a clear statement on what is the best standard of care in DCD-LT.

There are several possible explanations for the inconsistencies seen in our study. One was suggested by Manzini et al. in 2013. They concluded that most transplant centers base their choice of reperfusion technique on personal/institutional experience rather than on the available literature. (14) Based on the results of our

survey, this observation can be extended to other aspects of the DCD-LT protocols, for example the choice of perfusion solution used in the donor. Despite research stating that use of the perfusion solution histidine-tryptophan-ketoglutarate (HTK) is an independent risk factor for graft loss after LT, a substantial number of respondents in our survey reported using HTK. (15)

In our opinion, recommendations made in guidelines should never be based solely on personal or center experience. A thorough examination of current evidence for a certain intervention or approach is mandatory in order to guarantee an LT recipient the best standard of care possible. Although subjective values such as personal or expert opinions are definitely required to interpret the evidence, they should never by themselves be seen as evidence. (16) Transparency in the evidence underlying an expert opinion may enrich the value of a recommendation and should therefore be incorporated into new guidelines.

Another explanation for the large inconsistency among countries on certain aspects of DCD-LT is conflicting or inconsistent scientific literature. In our survey, this seems to be the case in the method of donor perfusion (single aortic versus dual aortic and portal perfusion) and the definition of dWIT. (17-21) International prospective cohort studies including many DCD-LTs should be performed to assess these topics with enough statistical power. Meanwhile, guideline developers have to consider other aspects when judging inconclusive evidence, such as – again – clinical experience, expert opinions, and the potential harm and benefits of a certain intervention. (22) Without a doubt, this will color the final recommendations made in the guideline. Whatever those final recommendations are, the rationale of this recommendation has to be stated clearly so that during implementation of a guideline, everyone is able to make their own judgement regarding the recommendations being made.

Some differences seen in our survey can be put into historical perspectives. For example, cannulation of the iliac artery and vein prior to donation is used more frequently in ES than in the other three countries, which is probably the result of the pioneering role of ES in the introduction of normothermic regional perfusion (NRP) in their protocol for uncontrolled DCD-LT (type 2 according to the Maastricht classification). (23) Further research is necessary to confirm whether the use of NRP is superior to standard procurement in controlled DCD-LT as well. Besides NRP, based on the results of our study many centers have used machine perfusion as part of a trial. The first results of these trials are currently being published.

Finally, legislation in some countries (e.g. in Germany and Portugal, DCD-LT is legally not allowed) and ethical dilemmas also seem to have an important influence on the current guidelines. For example, transplant centers in NL

perform WLST of a potential donor in the intensive care unit, although it has been shown that performing WLST in the operating room improves the outcomes of DCD-LT and reduces the incidence of ischemic cholangiopathy. (24) A further debate among the Dutch community on current donation procedures may be helpful to change this policy. However, it must be stressed that changes in donor procedures are only justifiable when legally and ethically well-regulated and without any negative impact for the donors and their relatives.

A striking observation is the high rate of respondents who reported to have violated the national or center-specific protocol on topics such as donor and recipient age limit. This could imply that the guidelines are no longer up to date. We therefore recommend regular protocol updates, at least every 3 years as suggested by Shekelle et al. (25) Protocol violation could also be the result of inaccurate implementation of guidelines. Several studies have shown that health-care professionals are often not familiar with the recommendations made in guidelines or are even unaware of the existence of a guideline. Furthermore, a professional's attitude towards and agreement with a guideline have reportedly played an important role in guideline implementation. (26-28) To minimalize the chance of protocol violation, health-care professionals should be directly and actively involved in the development and implementation of a guideline in order to create more awareness and acceptance.

Strengths and limitations

Our study has a few limitations. Based on the testing phase by the surgeons of the Erasmus University Medical Center Rotterdam, it was assumed that it would take a respondent approximately 8 minutes to complete the survey. However, based on the statistics provided by SurveyMonkey®, it took a mean of 15 minutes to finish the survey. This may explain the high rate of partially filled out surveys. Furthermore, only four of the 10 countries performing DCD-LT in Europe were included in this study. The rationale for this is that at the time of development of the survey in 2016 we only wanted to include those countries in which DCD-LT has been regularly performed for many years. We focused mainly on controlled DCD-LT. It might have been beneficial to create a separate survey for controlled and uncontrolled DCD-LT in order to disseminate the survey among more countries.

Conclusion

Donation after circulatory death grafts have a great potential to expand the donor pool for LT. Since a European guideline on DCD-LT is absent, many transplant centers have developed their own policy. This study is the first to show the enormous inconsistency regarding DCD-LT policies within and between

four European countries with extensive experience with DCD grafts. The medical community should minimize this inconsistency by creating a European consensus guideline based on both evidence-based medicine and expert opinions. Only then a legitimate comparison between the outcomes of DCD-LT in different countries can be made in order to assure consistent and optimal care for all potential LT recipients.

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Appendix A: Survey

General questions

1. In which country is your transplant centre located?
    a. Belgium
    b. The Netherlands
    c. United Kingdom
    d. Spain

2. Please fill out your name (this information will only be used to send a reminder for the survey if necessary)

3. How are you involved in the transplantation of DCD livers?
    a. I only perform the procurement of DCD livers
    b. I only perform the implantation of DCD livers
    c. I perform both procurement and implantation of DCD livers

4. How many DCD liver transplantations were approximately performed in your centre in 2015?

5. How many DCD liver transplantations were approximately performed in your centre in the last five years?

6. What is the current percentage of DCD liver transplantation in your centre, compared to DBD liver transplantation and living donor transplantations?

Donor characteristics

7. What is the upper age limit for a DCD liver donor in your centre?
    a. 50 years
    b. 60 years
    c. We do not have an upper age limit for a DCD liver
    d. We use another upper age limit, namely:

8. Has your centre in the past five years ever accepted a DCD liver from a donor who was older than the upper age limit in your centre?
    a. Yes
    b. No

9. (if the answer to question 8 was a) What was the reason to do so?

10. What is the upper BMI limit for a DCD donor in your centre?
    a. 28 kg/m2
    b. 30 kg/m2
    c. We do not have a BMI limit
    d. Other (please specify)

11. Has your centre in the past five years ever accepted a DCD liver from a donor whose BMI was higher than the upper limit in your centre?
    a. Yes
    b. No

12. (if the answer to question 11 was a) What was the reason to do so?

13. What is the cut-off level of transaminases in which you decide to accept a DCD liver?
    a. Normal levels
    b. 2x maximum
    c. 4x maximum
    d. Other (please specify)

14. Do you base your choice of rejecting a DCD liver on the last level of transaminases or on the trend of transaminases in time?
    a. On the last level
    b. On the trend
    c. Other (please specify)

15. Has your centre in the past five years ever accepted a DCD liver from a donor whose transaminases were above the cut-off point in your centre?
    a. Yes
    b. No

16. (if the answer to question 15 was a) What was the reason to do so?

17. What is the maximum time period between withdrawal of life support and cardiac arrest you accept for a DCD liver?
    a. 30 minutes
    b. 60 minutes
    c. Other (please specify)

18. What cut-off point in first Warm Ischemia Time do you accept for a DCD liver?
    a. 15 minutes
    b. 20 minutes
    c. 30 minutes
    d. Other (please specify)

19. At which point in time do you start counting the first Warm Ischemia Time?
    a. At the moment of withdrawal of life support
    b. When saturation and/or blood pressure deteriorate
    c. At cardiac arrest

20. (if the answer to question 19 was b) Which cut-off points for saturation and blood pressure do you use?

21. What is the percentage of steatosis of the liver that you accept for a DCD liver?
    a. < 10%
    b. < 30%
    c. We do not accept steatosis in a DCD liver
    d. Other (please specify)

22. Do you perform liver biopsy in a DCD liver?
    a. Yes, I do it routinely
    b. Yes, I do it on demand
    c. No, I never do it

23. (if answer to question 22 was a or b) What is the reason to perform a liver biopsy?

Surgical techniques of the procurement

24. Which technique is used in your centre to perform the liver procurement?
    a. A super-rapid procurement with sterno-laparotomy
    b. Cannulation of the iliac artery and vein with double-balloon triple-lumen catheter prior to laparotomy
    c. Other (please specify)

25. Which method of perfusion is used during the procurement?
    a. Aortic perfusion
    b. Dual perfusion (aortic + portal)

26. Which perfusion solution is used in your centre during procurement?
    a. UW
    b. HTK
    c. IGL-1
    d. Marshalls
    e. Other (please specify)

27. What is an average amount of perfusion solution used during procurement?
    a. Aortic (ml)
    b. Portal (ml)

28. Which form of additional perfusion is performed on the bench?
    a. Only arterial perfusion
    b. Only portal perfusion
    c. Both arterial and portal perfusion

29. Do you use the same perfusion solution for both the arterial and portal perfusion?
    a. Yes
    b. No

30. (if the answer to question 29 was a) Which perfusion solution do you use on the bench?
    a. UW
    b. HTK
    c. IGL-1
    d. Marshalls
    e. Other (please specify)

31. (if the answer to question 29 was b) Which solution do you use for the arterial perfusion on the bench?
    a. UW
    b. HTK
    c. IGL-1
    d. Marshalls
    e. Other (please specify)

32. (if the answer to question 29 was b) Which solution do you use for the portal perfusion on the bench?
    a. UW
    b. HTK
    c. IGL-1
    d. Marshalls
    e. Other (please specify)

33. Do you use machine perfusion in DCD liver transplantations?
    a. Machine perfusion is the standard in our center
    b. Only used in trials
    c. Never

34. (If the answer to question 33 was a or b) Which type of machine perfusion is used in your centre?
    a. Normothermic ex vivo machine perfusion
    b. Hypothermic oxygenated machine perfusion

35. How do you handle the bile duct during the liver procurement?
    a. In the same way as in DBD donors
    b. In a different way compared to DBD donors

36. (If the answer to question 35 was b) What is the difference in handling the bile duct between DBD and DCD livers in your centre?

Allocation and logistics

37. Which type of allocation is used by your country for DCD livers?
    a. National
    b. Regional
    c. Centre orientated
    d. Other (please specify)

38. Is the procurement of a DCD liver always performed by surgeons from your own transplant centre?
    a. Yes
    b. No

39. Where does the withdrawal of life support treatment in DCD take place?
    a. In the operating room
    b. In the anesthetic room
    c. In the intensive care unit
    d. Other (please specify)

40. When is heparin given to the donor?
    a. At the time of withdrawal of life supporting treatment
    b. After the withdrawal of life supporting treatment
    c. At the time of perfusion (heparin is in the preservation solution)
    d. Other (please specify)

Recipient characteristics

41. What is the upper age limit for a DCD recipient in your centre?
    a. 60 years
    b. 70 years
    c. Other (please specify)

42. Does the upper age limit for DCD recipients in your centre differ from that of DBD recipients?
    a. Yes
    b. No

43. (If the answer to question 42 was b) What is the upper age limit for a DBD recipient in your centre?

44. Has your centre in the past five years ever accepted a DCD liver for a recipient who was older than the upper age limit?
    a. Yes
    b. No

45. (If the answer to question 44 was a) What was the reason to do so?

46. What is the maximum MELD score at which a recipient is eligible for a DCD liver?

47. Has your centre in the past five years ever accepted a DCD liver for a recipient with a higher MELD score than the maximum mentioned in the previous question?
    a. Yes
    b. No

48. (If the answer to question 47 was a) What was the reason to do so?

49. Which of the following liver diseases are eligible for a DCD liver? (more than one answer is possible)
    a. Acute liver failure
    b. Primary Sclerosing Cholangitis (PSC)
    c. Primary Biliary Cirrhosis (PBC)
    d. Alcohol liver cirrhosis

e. Liver cirrhosis due to (chronic) Hepatitis B virus
f. Liver cirrhosis due to (chronic) Hepatitis C virus
g. Hepatocellular carcinoma
h. Polycystic liver disease
i. Retransplantation
j. I accept DCD livers for all indications for liver transplantation
k. Other (please specify)

50. Which of the following is a contra-indication for receiving a DCD liver?
a. Acute liver failure
b. Retransplantation
c. Portal vein thrombosis
d. History of upper abdominal surgery
e. Spontaneous bacterial peritonitis
f. Hepatopulmonary syndrome
g. Combined liver-kidney transplantation
h. Other (please specify)

51. What is the target Cold Ischaemia Time in your centre?
a. < 6 hours
b. < 8 hours
c. < 12 hours
d. Other (please specify)

Surgical techniques of the implantation

52. Do you use any thrombolytic agent (urokinase, r-TPA) at any time before reperfusion?
a. Yes
b. No

53. At what time do you use thrombolytic agents?
a. On the bench
b. Before reperfusion
c. Other (please specify)

54. What reperfusion technique is used for a DCD liver in your centre?
a. Arterial reperfusion first
b. Portal reperfusion first
c. Simultaneous reperfusion
d. Other (please specify)

Appendix B: Supplementary figures

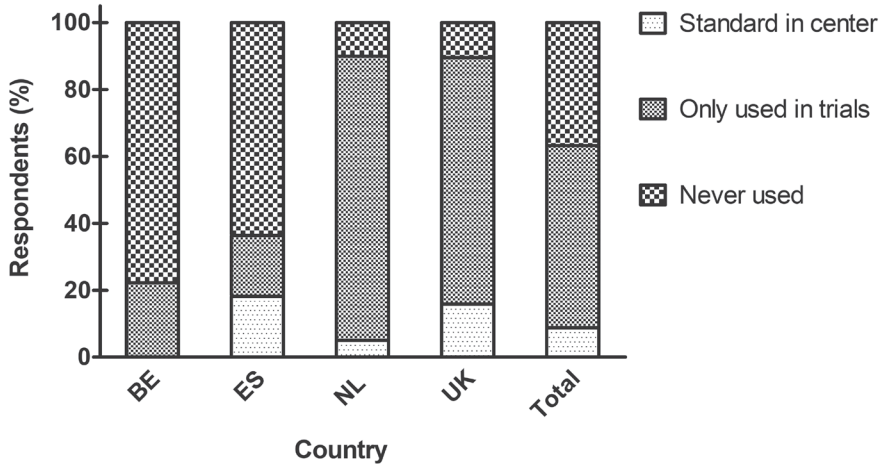


Figure S1: Use of machine perfusion for DCD liver grafts
BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom;

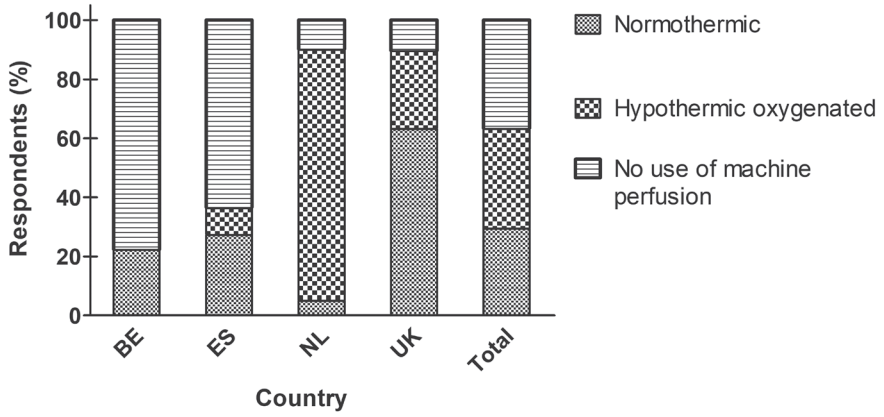
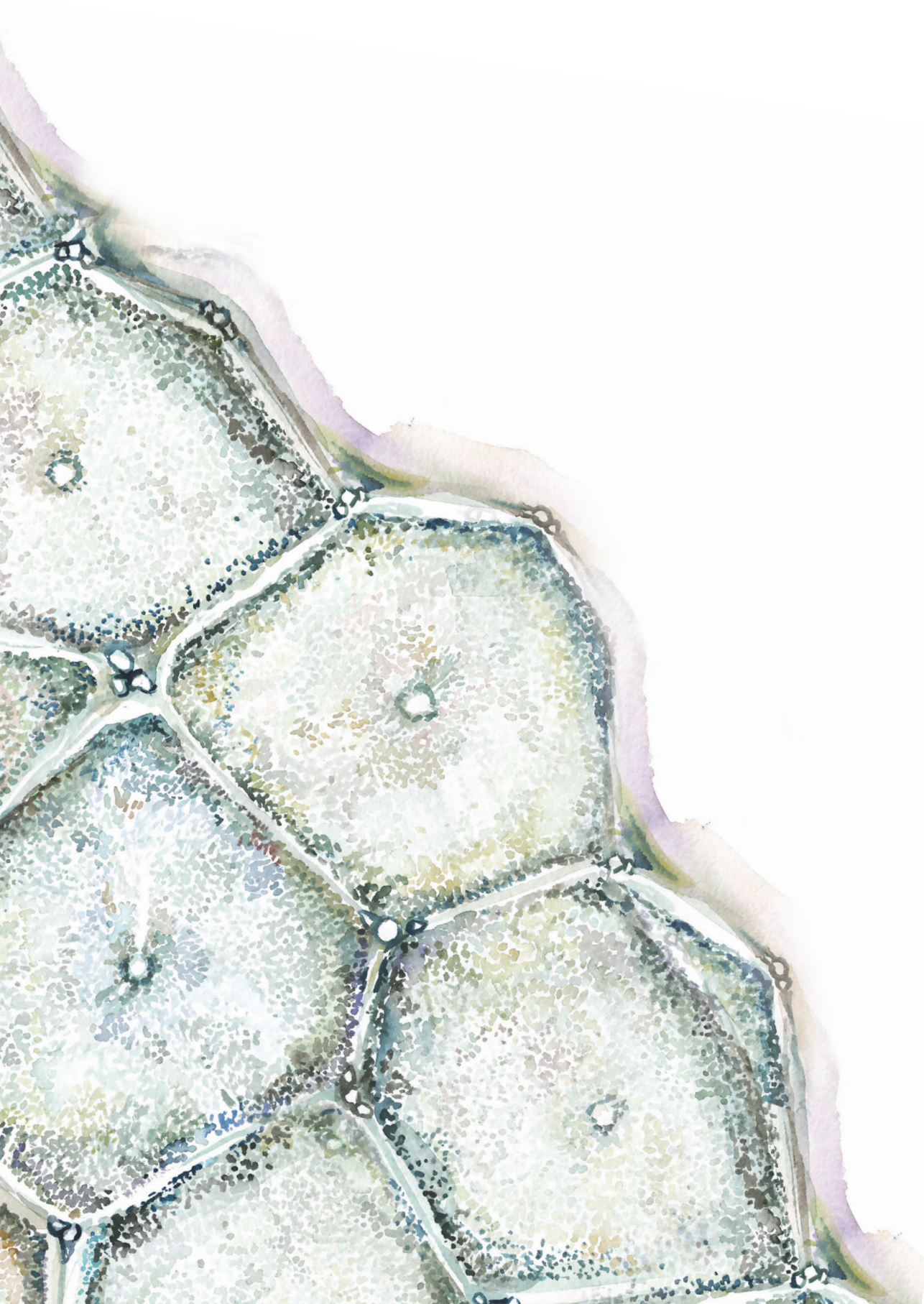


Figure S2: Type of machine perfusion used for DCD liver grafts
BE, Belgium; ES, Spain; NL, the Netherlands; UK, the United Kingdom;



CHAPTER 4

A multicentre outcome analysis to define global benchmarks for donation after circulatory death liver transplantation

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Abstract

Background: To identify the best possible outcomes in liver transplantation from donation after circulatory death donors (DCD) and to propose outcome values, which serve as reference for individual liver recipients or patient groups.

Methods: Based on 2219 controlled DCD liver transplantations, collected from 17 centres in North America and Europe, we identified 1012 low-risk, primary, adult liver transplantations with a laboratory MELD of ≤ 20 points, receiving a DCD liver with a total donor warm ischemia time of ≤ 30 minutes and asystolic donor warm ischemia time of ≤ 15 minutes. Clinically relevant outcomes were selected and complications were reported according to the Clavien-Dindo Grading and the Comprehensive Complication Index (CCI). Corresponding benchmark cutoffs were based on median values of each centre, where the 75th percentile was considered.

Results: Benchmark cases represented between 19.7% and 75% of DCD transplantations in participating centers. The one-year retransplantation and mortality rate was 5.23% and 9.01%, respectively. Within the first year of follow-up, 51.1% of recipients developed at least one major complication (≥ Clavien-Dindo-Grade-III). Benchmark cut-offs were ≤ 3 days and ≤ 16 days for ICU and hospital stay, ≤ 66% for severe recipient complications (≥ Grade-III), ≤ 16.8% for ischemic cholangiopathy, and ≤ 38.9 CCI points at one-year post-transplant. Comparisons with higher risk groups showed more complications and impaired graft survival, outside the benchmark cut-offs. Organ perfusion techniques reduced the complications to values below benchmark cut-offs, despite higher graft risk.

Conclusions: Despite excellent 1-year survival, morbidity in benchmark cases remains high with more than half of recipients developing severe complications during 1-year follow-up. Benchmark cutoffs targeting morbidity parameters offer a valid tool to assess the protective value of new preservation technologies in higher risk groups, and provide a valid comparator cohort for future clinical trials.

Introduction

For patients with acute liver failure, end-stage liver disease and malignant liver tumor, liver transplantation (LT) remains the only curative treatment option. Over the past decades, improved surgical techniques, anesthesiologic and medical treatment have significantly improved the outcome after LT. (1) Based on this success story, there is an increasing imbalance between available liver grafts and candidates, which forces transplant centres to increasingly utilize marginal grafts, including livers from donation after circulatory death (DCD) donors. (2, 3)

In context of different donor risk profiles in various countries and centres, outcomes were inconsistently reported, and results after LT from DCD donors were found equally good or inferior, compared to organs from donation after brain death (DBD) donors. (4–7) National and centre-specific guidelines, and surgeons experience with DCD grafts contributed significantly to the selection of DCD donors and related outcomes. (8) A recent systematic review and meta-analysis demonstrated with 3-39% a highly variable incidence of ischemic cholangiopathy (IC) after DCD liver transplantation. (9)

Such heterogenous outcomes found in multiple retrospective single centre studies, are the result of a very different risk profile accepted by each centre. In order to identify the best possible outcomes in deceased liver transplantation from DBD donors, the concept of Benchmarking has been introduced in the field of transplant surgery. (10) Based on a multicentre data collection, involving 17 transplant centres worldwide, Muller et al. have defined the specific donor-recipient risk in DBD transplantations, which leads to the best achievable outcomes and serves as reference values. (11) This study was based on 7492 DBD liver transplantations and authors identified more than half of the benchmark cases (e.g., cases with the lowest risk profile) with at least one severe complication, despite overall excellent one-year graft and patient survival rates. (11) The donor cohort after circulatory death, was however not considered for this study. Meanwhile, the Benchmark concept has also been established in various other surgical sub-specialties, including esophagectomy, bariatric and pancreatic surgery and major hepatectomies. (11-15)

The current study aims to define the most clinically relevant benchmark cut-offs, targeting the morbidity and mortality after transplantation from a low-risk cohort of DCD liver recipients from Europe and North America.

Methods

Participating Centres and Case selection

Liver transplant centres with experience in controlled DCD were screened. Corresponding centres were contacted and provided details of DCD liver transplantations at their center between 01.01.2000 and 31.12.2016. All cases included in the development of the benchmark parameters were primary, adult (≥ 18 years), whole Maastricht Type-III-DCD liver transplantations, performed with rapid retrieval, in-situ cooling and static cold storage (CS). To develop the benchmark values, the following exclusion criteria were applied: Any DBD organ, split, domino livers and combined transplantations; or living donors, any DCD liver procured with normothermic regional perfusion (NRP) or exposed to ex-situ machine perfusion (Supplementary Table 1).

Selection of the main study population and relevant variables

Paralleled by previous analyses (11, 12), the benchmark cases were identified in the DCD databases of the participating centres. To select the perfect DCD liver transplant cases, the waiting list mortality, and donor and recipient risk factors were considered. To obtain the most accurate duration of donor warm ischemia time (dWIT), total dWIT (from withdrawal of treatment to cold in-situ flush) and asystolic dWIT (from circulatory death to cold in-situ flush) were considered to define the benchmark cohort. (16, 17) Various cut-off values for both timings are discussed in the literature. In 2006, two large cohort studies found a higher incidence of graft loss with prolonged total dWIT of > 30 minutes. (18, 19)

This threshold was also recommended by the American Society of Transplant Surgeons (ASTS) in 2009 and is currently applied by the majority of centres to accept a DCD donor. (8, 20, 21) Taner et al. from the Mayo Clinic in Florida found a 16% odds-increase with each minute of asystolic dWIT. (17) Such earlier reports were confirmed by the Cox-regression analysis from our cohort. Both types of dWIT were found as strongest predictors for graft loss (Supplementary Figure 1). DCD liver transplantations were therefore allocated to the benchmark group, when their total and asystolic dWIT were ≤ 30 minutes and ≤ 15 minutes, respectively (Supplementary Table 1). Next, an increased laboratory Model of End-Stage Liver Disease of > 20 points is generally known to increase recipient mortality and graft loss, particularly in combination with additional donor risk. (22-24) With their national survey and outcome analysis, Sher et al. from the United States (US) have suggested to use DCD livers primarily for candidates with a laboratory MELD of ≤ 20 points. (21) In accordance with the recent Delphi consensus conference on Benchmarking, liver recipients with acute liver failure, or admitted to intensive care unit (ICU), or with the need for renal replacement therapy (RRT) or ventilation at the time of transplantation were excluded

from the developing cohort for benchmark parameters (Supplementary Table 1). (11, 25, 26)

Comparator cohort with higher risk

Three comparator cohorts with higher donor and recipient risk were identified to compare the benchmark outcomes. First, we considered a recipient cohort with higher laboratory MELD of > 20 points. Secondly, the benchmark cohort was compared to cases with a prolonged total and asystolic donor warm ischemia time of > 30 minutes and > 15 minutes, respectively. Finally, outcomes after DCD liver retransplantations (second graft) were assessed and compared to the benchmark group.

Impact of organ perfusion on outcomes in high-risk cohorts

To provide a practical example, how to use the benchmarking tool and to analyse the impact of organ perfusion, type-III DCD transplantations from countries with high donor risk, performed within the same time-period were collected. Italian transplant centres respect by law a 20 minute no touch period after circulatory arrest with subsequent long dWIT. Based on this, NRP is routinely performed. Livers are then cold stored with subsequent hypothermic oxygenated perfusion (HOPE). Additionally, DCD grafts in Switzerland suffer from prolonged dWIT with routine performance of endischemic HOPE-treatment before implantation. Such risky DCD livers with total and asystolic dWIT of > 30 minutes and > 15 minutes, were included in this comparator cohort, when procured with such organ perfusion protocols. Type-III DCD liver transplantations from an experienced centre in Spain, retrieved with NRP, served as control group with a similar low risk profile as the benchmark cohort. Despite several approaches, the number of DCD livers transplanted with > 30 minutes total and > 15 minutes asystolic donor warm ischemia time and normothermic machine perfusion was too limited to be compared with the other preservation techniques.

Data collection, follow-up and outcome

Investigators in participating centres collected risk factors and outcome parameters according to standardized definitions, which were summarized in an anonymous, password protected file. Well-known donor and recipient characteristics were included (Table 3, Supplementary Table 2 & 4). The functional dWIT was defined from saturation of < 70% or systolic blood pressure of < 50 mmHg to cold donor aortic flush. (7, 27)

In addition to various standard outcome measures collected after transplantation, the ClavienDindo-Classification (C-D; Grading 0-V) and the Comprehensive

Complication Index (CCI®; https://www.assessurgery.com) were used to describe post-transplant complications at four timepoints (in hospital, after 3, 6, and 12 months). (28, 29) Liver retransplantations were classified as Grade-IVa, unless a multiorgan failure (e.g. primary non-function = Grade-IVb) was evident, readmission to ICU and a newly developed renal failure with the need for RRT were both classified as Grade-IVa complication. Recipient death corresponds to Grade-V complication and a CCI® of 100points. (28, 29) Ischemic cholangiopathy (IC) was defined as irregularity or narrowing of the intra-or extrahepatic donor bile ducts (excluding the biliary anastomosis), detected by magnetic resonance cholangiography or any other type of cholangiography, combined with clinical symptoms including jaundice or signs of cholangitis or elevated parameters of cholestasis, in the absence of hepatic artery thrombosis (HAT) or stenosis. HAT was divided in early (within the first months after LT) and later (thereafter).

Statistical analysis and approval

Cases submitted by all centres were checked for completeness and correctness (AS, MvR). Narratively described complications were checked against completed variables, that capture this outcome measure in a dichotomous way. The overall cohort underwent descriptive analysis of donor-recipient risk factors and outcome parameters. Multivariate analyses were performed using a Cox-regression model. The impact of well-known risk factors on survivals was assessed and included: donor age, donor WIT, cold ischemia time (CIT), recipient age, recipient laboratory MELD (Supplementary Figure 1).

Benchmark Values

According to the predefined criteria, low and high-risk DCD donor-recipient combinations were extracted from the database. The benchmark metrics were obtained for the following outcome parameters: duration of transplantation, intraoperative blood transfusion, the need of RRT after LT, ICU and hospital stay, PNF, bleeding, anastomotic strictures, ischemic cholangiopathy, bile leak and HAT. Liver re-transplantation, graft and patient survival, any or mild (≤ Clavien-Dindo-Grade-II) and severe complications (≥ Clavien-Dindo-Grade-III) and the CCI® were presented with benchmark cut-offs within the first year after transplantation. To achieve the benchmark values, the median value for each indicator (continuous parameter) was calculated separately for each participating centre. For binary parameters, the proportion was established individually for each center. (26) Based on such median values (continuous parameter) or proportions (binary parameter), the 75th -percentile of each specific outcome parameter was calculated, which represents the benchmark cut-off value. (11, 12) Survival curves were calculated using the log-rank test comparing different cohorts. A p-value of < 0.05 was considered statistically significant.

Results

How are risk factors distributed in DCD liver transplantation?

Overall 17 centres (11 European, 6 North American) provided 2219 cases of Maastricht Type-III-DCD liver transplantations. (27) According to predefined criteria, 114 DCD cases were excluded (Figure 1A). In a first step, the overall DCD cohort (n = 2105) was analysed. During the study period, 1456 and 649 DCD transplantations were performed in European and North American centres. A detailed comparative analysis of such cases is presented in Suppl.Table 2 & 3 & Suppl.Fig. 2. Overall, 1012 DCD liver transplantations (45.6%) were identified as benchmark cases ranging between 19.7% and 75% among centres (Figure 1A & B). Typical risk factors describe the benchmark cohort with a short median total and asystolic dWIT of 22 minutes (IQR: 18-26) and 9 minutes (IQR: 8-11), respectively. The median laboratory MELD was 13 points (IQR: 9.5-16) and the median CS 6.13 hours (IQR: 5.05-7.42). To better understand how the risk profile and outcomes evolved over time, the overall and the benchmark cohort were both divided into three Eras (first: 2000-2005; second: 2006-2010, third: 2011-2016). While in the overall cohort slightly lower graft loss and retransplantation rates were seen in the most recent third Era, outcome parameters of the benchmark cohort remained similar throughout the three Eras (Supplementary Table 4 & 5).

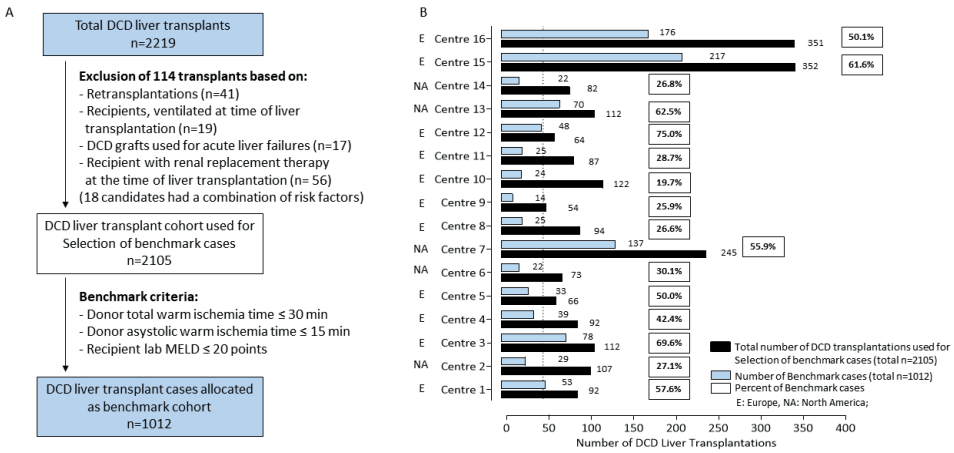


Figure 1: Selection and distribution of DCD liver transplantation benchmark cases among centres. Initially, liver retransplantation, recipients with acute liver failures or renal replacement therapy and ventilation were excluded. Based on available literature low risk benchmarking cases were defined and 1012 controlled DCD liver transplantations were identified.

What are the Benchmark Values in DCD liver transplantation?

The best possible outcomes in DCD liver transplantation were determined by the benchmark cut-off values, defined as 75th percentile of the median values of each benchmark parameter and each participating centre (Table 1 & 2, Supplementary Table 6-9). Specific perioperative parameters were set at the following benchmark cut-off values: ≤ 6.8 hours duration of transplant surgery, the need for ≤ 3 units RBCs, ≤ 9.6% RRT post-transplant, ≤ 3 and ≤ 16 days ICU and hospital stay, respectively. The benchmark cut-off values for severe complications (Clavien-Dindo ≥ Grade III, representing Grade IIIa, IIIb, IVa, IVb or V) during hospital stay, at 3, 6 and 12 months after DCD liver transplantation, were ≤ 43%, ≤ 56%, ≤ 60% and ≤ 66%, respectively. Correspondingly, minor complications (Clavien-Dindo of ≤ Grade-II) decreased with the following cut-offs: In hospital: ≤ 83%, after 3 months: ≤ 69%, and 6 months: ≤ 59% and 12 months: ≤ 58% (Table 1). The benchmark thresholds for IC, anastomotic strictures and biliary leakages were found at ≤ 16.8%, ≤ 28.4% and ≤ 8.3%. Of note, 31.5% of ICs in the benchmark cohort led to graft loss. The rate of PNF and post-transplant bleeding should ideally be found within 0 to ≤ 2.5% and in 0 to ≤ 10.3% of cases, respectively. The ideal DCD liver transplantation will develop an early HAT (within the first months) in ≤ 4.5%, while later HAT rates are slightly lower at a benchmark cut-off ≤ 2.3%. HAT-related graft loss was seen in 81.3% of benchmark cases (39 graft loss in 48 recipients). The benchmark values for the cumulative morbidity, were defined with a CCI® of ≤ 22.2 points, ≤ 30.8, ≤ 36.4 and ≤ 38.9 points in hospital, at 3, 6, and 12 months, respectively (Table 1). Centre size had no impact on the effective collection and number of low- or high-grade complications according to Clavien-Dindo. To assess the potential effect of the year of transplantation, Benchmark values were separately calculated for Era two (2006-2010) and three (2011-2016) and compared to those developed for the overall benchmark cohort. The calculated Benchmark cut-offs for the best possible outcomes were comparable (Supplementary Tablet 6-9).

Table 1: Benchmark cut-off's in DCD liver transplantation
Newly defined Benchmark cut-offs for the most relevant outcome measures are provided.

Benchmark Cases: controlled DCD liver transplantation: n=1012				
Perioperative Course				
Duration of Transplantation	≤ 6.8 hrs			
Intraoperative Blood transfusions	≤ 3 U RBC			
Renal Replacement Therapy	≤ 9.6 %			
ICU stay	≤ 3 days			
Hospital stay	≤ 16 days			
Key complications				
Primary Non-Function	≤ 2.5%			
Bleeding	≤ 10.3%			
Anastomic Strictures	≤ 28.4%			
Ischemic cholangiopathy	≤ 16.8%			
Bile leak	≤ 8.3%			
Hepatic Artery Thrombosis (HAT)*	≤ 4.5%			
Morbidity and Mortality	**Discharge**	**3 months**	**6 months**	**12 months**
Any complication	≤ 76%	≤ 90%	≤ 93%	≤ 95%
≤ **Grade II complication[§]**	≤ 83%	≤ 69%	≤ 59%	≤ 58%
≥ **Grade III complication[§]**	≤ 43%	≤ 56%	≤ 60%	≤ 66%
CCI ®	≤ 22.2 points	≤ 30.8 points	≤ 36.4 points	≤ 38.9 points
Graft loss	≤ 10.1%	≤ 13.3%	≤ 14.0%	≤ 14.4%
Re-transplantation	≤ 5.0%	≤ 6.4%	≤ 6.4%	≤ 6.9%
Mortality	≤ 6.5%	≤ 7.8%	≤ 7.8%	≤ 9.6%

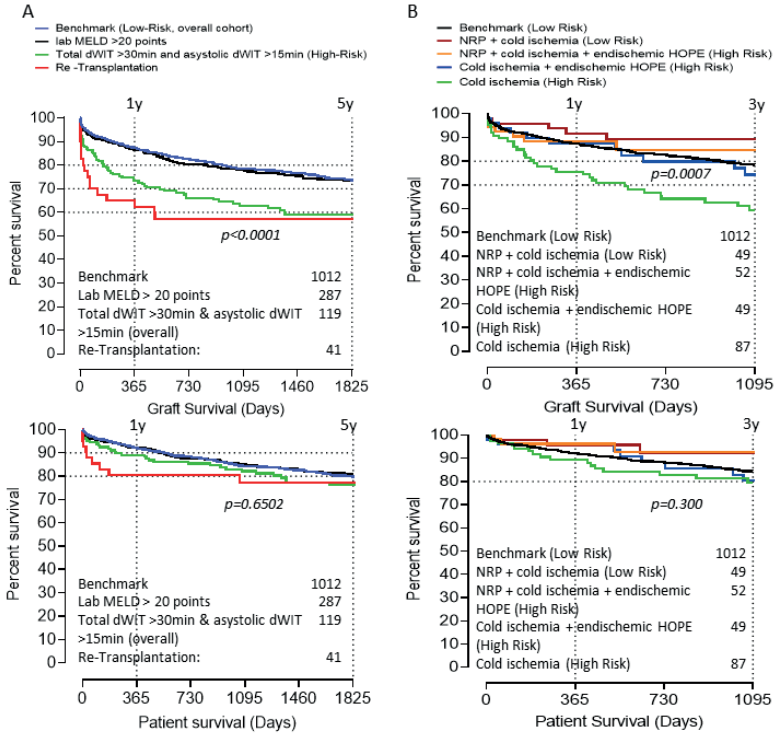
HAT* is early HAT within the first month after LT, the benchmark cut-offs for early and late HAT (after 1 month) are defined as ≤4.5% and ≤2.3%, respectively. Complications[§]: are the highest complications at that timepoint.

Table 2: Comparative outcome analysis after DCD liver transplantation with different risk profiles. Outcome parameters of the benchmark cohort and various high-risk cohorts are shown compared to suggested benchmark cut-offs.

Outcome parameter	Benchmark cases (n=1012) ¶	Total donor WIT>30min & asystolic donor WIT>15min (n=119) ¶¶	Recipient laboratory MELD >20 points (n=287)
Duration of Transplantation (hrs)	5.3 (4-6.7)	6.33 (4.75-7.54)	5.83 (4.69-6.8)
No. of RBC transfusions (U)	2 (0-6)	3 (0-5)	*4 (2-9)
ICU stay (days)	2 (1-4)	2 (1-5.5)	2 (1-4)
Hospital stay (days)	12 (8-18)	15 (11-23)	13 (8-22)
Renal replacement therapy (%)	12%	*13.4%	*10.14%
Any complication in 12 months (%)	74.41%	89.1%	75.96%
Primary non-function (%)	1.89%	2.5%	1.74%
Bleeding (%)	5.65%	10.08%	8.45%
Ischemic Cholangiopathy (%)	8.8%	*21.0%	7.22%
Anastomotic Strictures (%)	20.9%	22.7%	20.96%
Bile leak (%)	5.3%	8.4%	6.39%
Hepatic Artery Thrombosis (%)	4.74%	6.7%	1.74%
CCI ® until discharge (points)	8.7 (0-33.5)	*22.6 (0-4.7)	20.9 (0-33.7)
CCI ® 3 months (points)	20.9 (0-39.5)	*34.6 (20.9- 47.4)	24.2 (0-40.55)
CCI ® 6 months (points)	26.2 (0-42)	*40.5 (26.2- 53.2)	29.6 (0- 45.28)
CCI® 12 months (points)	29.6 (0-46.2)	*43.6 (28.1- 56.8)	32.15 (8.7-47.6)
Graft loss (12 month, %)	12.7%	*23.5%	9.76%
Retransplantation (12 months, %)	4.5%	*12.0%	2.11%
In Hospital Mortality (%)	3.26%	5.04%	2.44%
One-Year mortality (%)	8.39%	*13.44%	6.27%
Follow up (graft survival, days)	1386 (646.5-2277.8)	1096 (272-1847)	1499.5 (743.5-2327.0)
Follow up (patient survival, days)	1520 (822.75-2354.3)	1396 (716-2409.5)	1582 (849.5-2390.5)

Values presented as median and IQR (continuous parameter) and numbers or % (binary parameter); ¶: Benchmark cohort cases; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables); ¶¶: this group corresponds to the “cold storage” group (high-risk cohort) in Tables 3 & 4, Figure 2 and Supplementary Table 13; *: Value outside benchmark cut-off;

Retransplantation (n=41)	Benchmark Cut-off values (n=1012)	p-value (Benchmark¶ vs. long donor WIT¶¶)	p-value (Benchmark¶ vs. recipient laboratory MELD >20 points)	p-value (Benchmark¶ vs. Retransplantation)
5.48 (3.53-6.93)	≤ 6.8 hrs	< 0.0001	0.006	0.846
*5 (2-8)	≤ 3 U RBC	0.320	<0.0001	0.016
3 (2-6.75)	≤ 3 days	0.023	0.518	0.007
*25 (12.25-40.5)	≤ 16 days	< 0.0001	0.151	< 0.0001
*17.7%	≤ 9.6 %	0.7662	0.4637	0.3273
80.49%	≤ 95 %	0.0002	0.6443	0.4659
*12.5%	≤ 2.5 %	0.4937	1.0	0.0016
*17.5%	≤ 10.3 %	0.0665	0.0975	0.0095
9.37%	≤ 16.8 %	0.0001	0.7127	0.5700
12.5%	≤ 28.4 %	0.6353	1.0	0.2361
*15.6%	≤ 8.3 %	0.2037	0.559	0.025
12.2%	≤ 4.5 %	0.3679	0.0264	0.0502
*26.2 (0-48.45)	≤ 22.2	< 0.0001	0.235	0.003
*33.5 (8.7-50.7)	≤ 30.8	< 0.0001	0.277	0.016
35.7 (10.45- 54.25)	≤ 36.4	< 0.0001	0.251	0.009
*39.7 (10.45- 54.25)	≤ 38.9	< 0.0001	0.412	0.036
*36.6%	≤ 14.4	0.0029	0.1833	0.0001
*14.6%	≤ 6.9	0.0035	0.0618	0.0128
*14.6%	≤ 6.5	0.2897	0.5651	0.0030
*19.5%	≤ 9.6	0.0868	0.2668	0.0227
697.5 (54.25-3006.75)	-	0.001	0.288	0.050
1341 (465-3207)	-	0.460	0.568	0.868



DCD Transplantation with different risk categories

No. at Risk (graft survival)	Transplant	1y	2y	3y	4y	5y
Benchmark (overall)	1012	816	717	579	478	369
Lab MELD >20 points	287	366	319	269	222	174
Total dWIT>30min + asystolic dWIT >15min = Cold ischemia (High Risk)	119	106	89	76	60	41
Re-Transplantation	41	26	21	18	15	13

No. at Risk (patient survival)	Transplant	1y	2y	3y	4y	5y
Benchmark (overall)	1012	859	766	627	515	398
Lab MELD >20 points	287	387	343	291	243	192
Total dWIT>30min + asystolic dWIT >15min = Cold ischemia (High Risk)	119	128	113	96	77	55
Re-Transplantation	41	33	29	24	21	21

High- and low risk cohorts compared to organ perfusion

No. at Risk (graft survival)	Transplant	1y	2y	3y
Benchmark (overall)	1012	816	717	579
NRP + cold ischemia (Low Risk)	49	44	25	16
NRP + cold ischemia + endischemic HOPE (High Risk)	52	32	16	5
Cold ischemia + endischemic HOPE (High Risk)	49	42	32	28
Cold ischemia (High Risk)	87	64	48	37

No. at Risk (patient survival)	Transplant	1y	2y	3y
Benchmark (overall)	1012	859	766	627
NRP + cold ischemia (Low Risk)	49	45	26	17
NRP + cold ischemia + endischemic HOPE (High Risk)	52	36	18	2
Cold ischemia + endischemic HOPE (High Risk)	49	46	35	31
Cold ischemia (High Risk)	87	74	60	46

Comparisons made with Log Rank Test;

Figure 2: Impact of organ perfusion technology on outcomes after DCD liver transplantation in high-risk cohorts.
A) Five-year graft and patient survival comparing different risk classes with the benchmarking group. Allocation of DCD livers for retransplantation and the use of high-risk donor-recipient combinations with prolonged dWIT were found with impaired graft and patient survival. B) Novel organ perfusion technology demonstrated positive impact on graft and patient survivals in donors with prolonged warm ischemia time, when compared to standard cold storage.

How do high-risk DCD cohorts perform?

First, 119 DCD donors with a prolonged total and asystolic dWIT were compared with the benchmark cohort (Supplementary Table 5). Based on the higher graft injury, median peak transaminases within the first week were significantly higher in this cohort, compared to the benchmark group (AST: 1293 vs. 2671 U/L, p < 0.0001; ALT: 922 vs. 1714 U/L, p < 0.0001). The IC-rate was higher (21.0% vs. 8.8%, p=0.0001), exceeding benchmark thresholds (≤ 16.8%). Additionally, a higher median CCI was found at all timepoints after transplantation. First-year graft loss (23.5% vs. ≤ 14.4%) and retransplantation rate (12.2% vs. ≤ 6.9%) were both higher than the benchmark cut-off values (Table 2 & Figure 2A). The second high-risk cohort included 287 DCD recipients with a higher laboratort MELD of > 20 points. Subgroup analysis identified the majority between > 20 and ≤ 30 MELD points (n = 255, median 23 points; IQR: 22-27), while only 32 recipients were found with a laboratory MELD of > 30 points. Expectedly, such recipients presented slightly higher transfusion requirements and a higher median day-one INR, compared to the benchmark cohort. Of note, parameters collected during further follow-up appeared comparable to the benchmark cohort (Table 2 & Supplementary Table 10-12). Next, benchmark cases were compared to 41 DCD grafts utilized for retransplantations. Expectedly, more transfusions were required (5 vs. ≤ 3 units), and recipients were more frequently in need for RRT (17.7% vs. ≤ 9.6%). A higher rate of PNF`s (12.5% vs. 1.89%, p = 0.0016) and abdominal bleedings (17.5% vs. 5.65%, p = 0.0095) were found. Additionally, DCD liver utilisation for retransplantation led to a higher incidence of biliary leakages (15.6% vs. ≤ 8.3%). (23) The median posttransplant CCI® was higher and all survival endpoints were significantly impaired compared to the benchmark group and cut-off (Table 2 & Figure 2A).

Does novel organ perfusion technology improve outcomes in high-risk DCD liver transplantations?

We explored the impact of organ perfusion on outcomes in high-risk DCD cohorts with prolonged total and asystolic dWIT. Overall, 63 DCD grafts, retrieved with NRP, with subsequent cold storage during transport and endischemic HOPE-treatment were collected from Italian centres. Such cases were compared with 49 DCD livers from Switzerland, which underwent standard super-rapid procurement with immediate cold flush and cold storage with endischemic HOPE-treatment (Table 3 & 4). Such two cohorts were also compared with a DCD liver population procured and transplanted from an experienced centre in Spain. Of note, the donor and recipient risk in Spain is comparable to the benchmark cohort with short dWIT. The Spanish NRP-cohort showed similar results as seen in the benchmark group, with however lower anastomotic stricture- and IC-rates and more post-transplant bleedings. Both, the Italian (NRP-HOPE) and the Swiss cohort (HOPE) developed less DCD-specific and overall complications with better graft survival, compared to cold stored DCDs with prolonged dWIT. And the number of IC`s with subsequent graft loss decreased significantly (Table 4 & Supplementary Table 13; Figure 2B).

Table 3: Donor and recipient risk comparing high risk donor and recipient cohort with different preservation methods within the last 5 years
Currently accepted donor and recipient risk factors are highlighted here comparing different risk groups with the low-risk benchmarking cohort. Novel machine perfusion technology leads to a higher acceptance of risky DCD donors and grafts.

Risk factors	Low Risk: Total donor WIT ≤ 30min & asystolic donor WIT ≤15 min		High Risk: Total donor WIT >30 min & asystolic donor WIT >15 min			p-value	p-value
	Benchmark cases (n=1012) ¶	NRP + cold storage (n=49)	Cold storage (n=87)	Cold storage + HOPE (n=49)	NRP + cold storage + HOPE (n=63)	Cold storage vs. cold storage + HOPE	Cold storage vs. NRP + cold storage + HOPE
Donor age (years)	48 (34-57)	54 (45.5-67)	47 (36-55)	60 (52.9-71.2)	58 (51-63)	<0.001	<0.001
Donor BMI (kg/min²)	24.79 (22.35-28.0), n=589	25.95 (23.77-27.76)	25 (22.9- 29.18)	26.0 (23.7- 27.8)	26 (24.1-28)	0.626	0.130
Total donor warm ischemia time (min)	22 (18-26)	18 (13-22)	36 (33-41)	38 (34.5-41)	49 (39-67)	0.167	<0.001
Functional donor warm ischemia time (min)*	15 (12-20)	14 (9.5-17)	21 (18-23.5)	33 (31-36.5)	42 (35-55)	<0.001	<0.001
Asystolic donor warm ischemia time (min)	9 (8-11)	6 (5-7)	18 (17-22)	20 (18-21)	27 (24-34)	0.248	<0.001
Duration of NRP (hrs)	-	2	-	-	3.78 (2.8-4.75)	-	-
Cold ischemia time (hrs)	6.13 (5.05- 7.42)	6.7 (5.5-7.4)	7.34 (6.3-8.48)	3.92 (3.1-4.9)	5.5 (4.43-6.5)	0.0001	<0.001
Duration of HOPE / D-HOPE (hrs)	-	-	-	2.0 (1.5-2.5)	2.5 (1.8-3.5)	-	-
Total Preservation time (hrs)	6.13 (5.05- 7.42)	8.7 §	7.34 (6.3-8.48)	5.93 (5.2-7) ¶¶	11.48 (10.33- 13.45) ¶¶¶¶	<0.001	0.0001
Recipient age (years)	57 (51-62)	55 (50.5-63)	56.5 (49-63)	57.0 (51.4-65)	60 (55-64)	0.304	0.040
Recipient laboraoty MELD (points)	13 (9.5-16)	14 (9-20)	12 (9-15.3)	11 (8-15)	9 (8-13)	0.725	0.025
Recipient HCC (%)	43.44%	57.14%	47.1%	65.3%	71.4%	0.0496	0.0043

Median and IQR or numbers and proportions (%); *: fdWIT below a systolic blood pressure of 50mmHg or a saturation of >70% (n=452 in benchmark, n=8 in cold storage group, other groups complete), §: includes NRP and CS, duration of NRP according to Reference 44; ¶: Benchmark cohort cases, data shown as median and IQR or number and proportion (%); ¶¶: includes CIT and endischemic HOPE; ¶¶¶¶: includes duration of NRP, CIT and endischemic HOPE;

Table 4: Impact of machine perfusion on outcomes in high-risk DCD donor recipient combinations.
Machine perfusion concepts improve outcomes of high-risk donor-recipient combinations in controlled DCD liver transplantation. Particularly the occurrence of IC and subsequent graft loss was significantly reduced along with a better graft function.

Outcome parameter	Low Risk: Total donor WIT ≤ 30min & asystolic donor WIT ≤15 min		High Risk: Total donor WIT > 30 min & asystolic donor WIT > 15 min			Benchmark Cut-off values (n=1012)	p-value (Cold storage vs. cold storage + HOPE)	p-value (Cold Storage vs. NRP + cold storage + HOPE)
	Benchmark cases (n=1012) ¶	NRP + cold storage (n=49)	Cold storage (n=87)	Cold storage + HOPE (n=49)	NRP + cold storage + HOPE (n=63)			
Duration of Transplantation (hrs)	5.3 (4-6.7)	6 (5.2-6.8)	6.05 (4.3-7.42)	4.75 (3.71-5.88) #	7.8 (6.25-8.6)	≤ 6.8 hrs	0.002	< 0.001
No. of RBC transfusions (U)	2 (0-6)	0 (0-1.5)	2 (0-5.25)	0 (0-2)	3 (0-7)	≤ 3 U RBC	< 0.001	0.436
ICU stay (days)	2 (1-4)	4 (2-6)	2 (1-7)	3 (2-4.5)	3 (2-5)	≤ 3 days	0.713	0.546
Hospital stay (days)	12 (8-18)	13 (10.5-17)	15 (10-25)	17 (12.3-21.5)	14 (9-19)	≤ 16 days	0.822	0.562
Renal replacement therapy (%)	12%	2.04%	16.1%	16.3%	9.5%	≤ 9.6%	1.0	0.025
Any complication in 12 months (%)	74.41%	*95.9%	89.67%	89.8%	74.6%	≤ 95 %	1.0	0.025
Primary non-function (%)	1.8%	0%	3.45%	2%	3.2%	≤ 2.5%	1.0	1.0
Bleeding (%)	5.65%	22.45%	10.3%	4.1%	6.3%	≤ 10.3 %	0.3268	0.5587
Ischemic Cholangiopathy (%)	8.8%	0%	22.06%	4.1%	3.2%	≤ 16.8%	0.0071	0.0014
Anastomotic Strictures (%)	20.9%	10.2%	22.1%	26.5%	12.7%	≤ 28.4 %	0.6687	0.1855
Bile leak (%)	5.3%	14.29%	7.7%	6.1%	3.2%	≤ 8.3 %	1.0	0.2978

Outcome parameter	Low Risk: Total donor WIT ≤ 30min & asystolic donor WIT ≤15 min		High Risk: Total donor WIT > 30 min & asystolic donor WIT > 15 min			Benchmark Cut-off values (n=1012)	p-value (Cold storage vs. cold storage + HOPE)	p-value (Cold Storage vs. NRP + cold storage + HOPE)
	Benchmark cases (n=1012) ¶	NRP + cold storage (n=49)	Cold storage (n=87)	Cold storage + HOPE (n=49)	NRP + cold storage + HOPE (n=63)			
Hepatic Artery Thrombosis (%)	4.74%	2.04%	6.3%	4.1%	3.2%	≤ 4.5 %	0.7071	0.4629
CCI ® until discharge (points)	8.7 (0-33.5)	20.9 (8.7- 33.7)	25.1 (0- 46.5)	26.2 (20.9-41.5)	20.9 (0-33.7)	≤ 22.2	0.771	0.097
CCI ® 3 months (points)	20.9 (0-39.5)	29.6 (20.9-38.8)	33.7 (20.9-47.6)	33.5 (20.9-42.9)	20.9 (0-36.2)	≤ 30.8	0.412	0.028
CCI ® 6 months (points)	26.2 (0-42)	33.5 (20.9-43.35)	40.5 (22.6-54.2)	37.1 (20.9-47.8)	26.2 (0-41.8)	≤ 36.4	0.223	0.006
CCI® 12 months (points)	29.6 (0-46.2)	33.7 (20.9-47.6)	43.6 (26.2-55.8)	39.5 (20.9-54.7)	29.6 (0-41.8)	≤ 38.9	0.30	0.002
Graft loss (12 month, %)	12.7%	8.2%	24.13%	12.2%	11.1%	≤ 14.4	0.1188	0.0558
Retransplantation (12 months, %)	4.5%	4.1%	16.1%	8.2%	9.5%	≤ 6.9	0.2917	0.3316
In Hospital Mortality (%)	3.26%	4.1%	6.89%	4.1%	3.2%	≤ 6.5	0.7107	0.4687
One-Year mortality (%)	8.39%	8.2%	14.94%	4.1%	3.2%	≤ 9.6	0.0842	0.025
Follow up (graft survival, days)	1386 (646.5-2277.8)	729 (429- 1323)	892 (282-1491)	1160 (461-1922)	529 (281- 785)	-	0.081	<0.001
Follow up (patient survival, days)	1520 (822.75-2354.3)	738 (453- 1409)	1106 (531-1617)	1225 (526-1967)	541 (346- 858)	-	0.242	<0.001

Values presented as median and IQR for continuous parameter and % for binary parameter; comparisons made with MannWhitney-U test (continuous variables) or Fisher exact test (binary variables), ¶: Benchmark cohort cases, data shown as median and IQR or number/proportion (%); *Value outside benchmark cut-off; complication in 12 months=" highest-graded" in 1-year of follow up; #: transplantation technique is classic cava replacement, explaining the need for RRT; SP: Spain;

Discussion

This is the first international, multicentre study, which defines the best possible outcomes after DCD liver transplantation. Target cut-off values were presented for the most important key complications in DCD liver transplantation. When higher risk donors and recipients were assessed, prolonged donor WIT led to an increased morbidity, higher rates of IC and graft loss. Importantly, when organ perfusion techniques were applied in this high-risk DCD cohort, outcomes were comparable to the benchmark group. In the future, the identified benchmark cut-offs serve as useful quality control tool and to evaluate the impact of novel strategies to improve outcomes.

Benchmarking is an attractive economic concept, applied to establish a standard of excellence and to compare products and services of a specific company with the most successful – "the best in class" - in the corresponding industrial sector. (30) This concept was introduced in medicine 30 years ago, and was recently applied to various surgical procedures. (11-14, 26, 31, 32) Benchmarking is externally driven to encourage a healthcare provider to assess their own business and to compare to exemplar performances in the same field. (31) To successfully establish outcome-thresholds, centre selection appears as first step. Similarly to previous benchmark analyses, participating centres in our study were identified based on their DCD-experience with a case load of ≥ 50 DCD transplantations during the study period, specialized multidisciplinary teams and the existence of a prospectively maintained database. (11, 26) In context of the interconnection between transplant centres with DCD experience worldwide and in context of the available literature, the here selected centres are likely representative of the overall DCD transplant community. (21)

To identify the best possible outcomes with static cold storage, DCD livers, exposed to any sort of organ perfusion technology were excluded from the benchmark-development cohort. Because of these strict criteria, a number of centres could not contribute cases to the benchmark cohort (Italy, Spain, France, and Switzerland routinely use organ perfusion technology; Germany, Portugal, Australia, New Zealand, and Austria have none or limited experience with DCD). (33-36)

Benchmark cases represented a proportion of 45% in our DCD liver transplant cohort, ranging between 19.7% and 75%. A recent analysis to define benchmarks for LT from DBD donors, included a median of 27% benchmark cases from participating centres (8%-49%). (11) Similar case-mix proportions were found in other benchmark analyses in abdominal surgery, including 14% for bariatric surgery (4%-69%) (14), 32% for esophagectomies (15) and 38% for pancreatectomies (9%-93%). (13, 26)

Our analysis was performed according to recently introduced criteria for benchmarking in surgery. (10, 12, 26). Established risk factors in DCD liver transplantation were considered to allocate cases to the benchmark cohort and based on recommendations, from the international expert Delphi consensus conference on benchmarking. (26) To define a low-risk population, the recipient disease severity was taken into account through the laboratory MELD score. Our selected cut-off at 20 points is paralleled by the suggestion from the US-consortium to utilize DCD livers for low MELD candidates (≤ 20 points) to achieve optimal outcomes. (21-23) Limiting the laboratory MELD reduces additional risk factors, including the number of recipients admitted to ICU with the need for RRT or ventilation at the time of transplantation, known to contribute to more postoperative complications. (23, 24, 37, 38)

Most centres routinely avoid to allocate DCD grafts to recipients with an expected prolonged hepatectomy, due to a known portal vein thrombosis or liver retransplantation. This led to a small number of those potentially challenging recipient surgeries in our overall DCD population, which were excluded from the benchmark cohort.

The impact of type and duration of dWIT on various outcome measures is frequently discussed. (3, 23, 24, 39, 40) Here we used both, the total and asystolic dWIT, because such timings are clearly defined and uniformly reported by most centres. In contrast, the term functional dWIT, first considers various definitions as starting point, including a drop of donor saturation or the systolic and mean arterial blood pressures, and secondly this timing is less routinely considered in centres from North America. (4, 39, 41-44) The here selected cut-off for total dWIT (≤ 30 minutes) was based on the literature, where higher rates of graft loss were reported beyond this threshold, which was also adopted by the ASTS in 2009. (18–20) The national guidelines regarding the “stand-off” period have strong impact on the duration of dWIT with a wide range among countries, between 2 and 5 minutes in the US and 20 minutes in Italy. (33) The higher risk to develop an IC was described by Taner et al. with a 16% odds-increase for each additional minute of asystolic dWIT. (17) We therefore believe, that the two here selected cut-offs are of clinical relevance and widely accepted.

With recent cohort analyses, donor age as individual risk factor had no impact on outcome after DCD liver transplantation (beyond 60 or 70 years), given other risk parameters are kept low. (42,45) Donor age was therefore not selected as limiting parameter to identify the benchmark cohort, also because the median donor age of our entire cohort was only 48 years with a 75th percentile of 58 years. (42, 43)

Next, a continuously increasing recipient age was observed in the United States from 51 years in 2002 to 56 years in 2014. (46) Provided that other recipient risk factors, including the laboratory MELD are low, elderly recipients were found with similar one-year survival rates compared to younger cohorts. (47) Along with such population changes, the medical assessment prior to liver transplantation, particularly in context of cardiac complications, has evolved. Today, most centres pick older recipients selectively and multidisciplinary committee`s decide at the time of listing if a DCD graft is an appropriate source for an individual candidate. Based on this, the recent consensus conference on DCD liver transplantation did not suggest to apply any recipient age threshold for clinical DCD liver transplantation. (48) We adopted this strategy for our benchmarking concept and did not chose a specific recipient age cut-off to identify the benchmark cases.

Another important risk factor appears with cold ischemia time (CIT). The clinical impact of CIT was explored in several retrospective studies with the development of various thresholds ranging between ≤ 4 and ≤ 10hours. (4, 24, 49, 50) In context of todays optimized liver transport and modern communication, CIT is generally shorter and more accurately estimated. The majority of analyses interpret CIT therefore in combination with the cumulative donor and recipient risk aiming for liver implantation within ≤8 or ideally ≤ 6 hours. (5, 23, 38, 51) The median CIT in our overall DCD cohort was 6.25 hours (IQR:5.2-7.47 hours). Based on the lack of impact of CIT on outcomes in our cohort and the literature, CIT was not considered to select the benchmark cohort.

The identified benchmark values in our study were found very similar to results after optimal DBD liver transplantation. (11) This is paralleled by the clinical experience, that low-risk DCD donor livers transplanted in fairly healthy recipients, for example with an HCC, achieve excellent results. (8, 23, 52) Donor WIT appears at front with significant contribution to biliary and overall complications and graft loss. Our comparative analysis between benchmark cases and DCD transplantations with prolonged dWIT demonstrated the expected higher number of 21% ICs (benchmark cut-off ≤ 16.4%) and 23.5% graft loss (benchmark cut-off ≤ 14.4%) within the first year. These findings support previous literature, where all sorts of prolonged dWIT led to more biliary complications and impaired graft survival. (17–19, 23) Additionally, we have also seen, that an endischemic HOPE-perfusion or combinations of NRP and HOPE significantly reduces the number of biliary complications and graft loss, despite prolonged dWIT. Such results are further paralleled by the recent multicentre randomized controlled trial, where authors demonstrate significantly reduced IC rates with HOPE-treatment compared to cold storage. (53) We could however not assess, whether NRP alone would also reduce complications as the number of DCD transplantations with prolonged dWIT and procurement with NRP was very limited.

Although various benchmark analyses exist today, a few of the suggested steps to establish this tool in surgery are based on random decisions and lack external validation. (10, 11, 14, 26) Instead of analysing a few merged large national cohorts, we decided to collect the entire parameter set directly from the participating centres. Although the time frame of our benchmark analysis appears quite large, data collection, including overall post-transplant complications, was done meticulously and outcomes in the benchmarking groups did not change over time. Our study therefore provides data, otherwise not available in large national datasets. (54) To prevent interpretation issues with the cumulative collection of complications, the same two authors have checked and transformed all complications, narratively described into the Clavien-Dindo-Grading and the CCI. (28, 29, 55) Importantly, we did not observe any correlation between centre size and number or grade of Clavien-Dindo complications. Another limitation is the fact that we cannot account for some centre variations regarding patient management, including immunosuppression, transfusion regimen or criteria for liver retransplantation.

Benchmark studies provide useful information and compare centre and team performances in highly specialized medicine. Of note, the concept identifies the best possible way of treatment or operation and serves as reference for morbidity conferences and international meetings. Of particular interest in the field of DCD liver transplantation are complications contributing to costs.

In summary, the benchmarking concept is of high interest in DCD liver transplantation, to provide the best-possible outcomes achieved with the current standard treatment of a low-risk cohort. This tool also enables the more transparent risk and outcome analyses comparing centres and countries. Such analyses are of interest when a surgical team is allocated to a specific transplantation based on the donor and recipient risk profile. Liver transplant cases with lower overall and technical risk could be allocated to trainees with an additional opportunity to standardize the quality of surgical performance and training. The wider and routine application of benchmarking concepts will provide more objective comparisons between cohort studies, also in context of new organ perfusion technology.

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Appendix A: Supplementary Methods and Results

Development of inclusion and exclusion criteria

The Benchmark cohort was selected based on previous benchmarking analysis and on the typical risk factors available through multiple publications by all participating centres. Liver transplant centres with the required DCD experience (Supplementary Table 1) were considered Centres in a few western countries could not be included due to the lack of a relevant number of DCD liver transplant cases (see main manuscript). Risk factor thresholds were selected based on the current literature, suggestions from consortiums and large collaborations and based on the multivariable analysis of our overall cohort. Figure 1 demonstrates the selection pathway from the overall cases to the benchmark cohort with a specific overview of the participating centres.

Statistical analysis and approvals

The statistical analysis was performed with SPSS (IBM) Version 22 and GraphPad Prism V. 7.0. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the regulatory bodies in the individual centres (CARMS-13611; MEC-2017-1055; SRB_201810_201; S61718; CAPCR-ID:17-6219.0 (109370.0); NIG_59813102020; #2010.180.C; SRB2018_201-P2018/551, S61718).

Evaluation of obtained data and statistical analysis

Here we provide further details on the assessment of the DCD cohort. The dataset was entirely checked by the authors from Rotterdam and Birmingham. For example, when IC was narratively described as a cause of graft loss, the corresponding dichotomous variable (IC: yes:no), Clavien-Dindo classification (Grade IVa) and the CCI® were double checked for inclusion of the complication. A CCI® value of 100 points was double checked with the event of recipient death. And complications listed after the complication retransplantation were excluded from the quantification. Following the initial descriptive analysis, various multivariable analyses were performed to further understand the distribution of the data. Following exclusion of cases with retransplantation, candidates with ventilation, acute failure or RRT at transplantation, the remaining overall DCD cohort (n=2105) was used to perform detailed Cox regression analysis using a forward stepwise approach. The included variables are summarised in Supplementar Figure 1A & B and include: donor age, donor total and asystolic warm ischemia time, cold ischemia time, recipient age, recipient laboratory MELD score.

Supplementary Table 1: In- and exclusion Criteria to identify participating centres and Benchmark Cases

Transplant Centre Inclusion Criteria	DCD Liver Benchmark Inclusion Criteria	DCD Liver Benchmark Exclusion Criteria
Overall caseload of ≥ 50 DCD liver transplantations (Maastricht Type III=controlled) during the study period	Adult recipients, age ≥ 18 years	Donation after Brain death Donor (DBD)
Prospective Database available	Whole graft transplantation	DCD donors, other than Maastricht Type III
Centre with interest in outcome analysis after DCD liver transplantation (or national reference centres)	Standard cold storage as preservation method	Combined organ transplantation (e.g., liver and kidney transplant) or any partial graft
Specialized multidisciplinary team	Low risk recipient profile (laboratory MELD ≤20 points)	Redo-Liver transplantation
	Primary Liver transplantation	Acute Liver failure
	Documented follow up of at least 12 months	Recipient admitted to Intensive care unit at time of transplantation
		Recipient dialysis or haemofilter-dependent at time of transplantation
		Recipient ventilated at time of transplantation
		Total donor warm ischemia >30 min (time between treatment withdrawal and cold flush)
		Asystolic donor warm ischemia >15 min (time between donor circulatory death and cold flush)
		Recipient laboratory MELD > 20 points
		Organ preservation other than cold storage (e.g., normothermic regional perfusion, ex-situ normothermic or hypothermic liver perfusion, etc.)

The impact on graft survival was explored. Both donor warm ischemia times were repeatedly identified as main predictors for graft loss, when included as continuous or dichotomous variable. Other risk factors, including donor age, cold ischemia time and recipient age were not identified as predictors.

Results from the Cox regression analysis are presented below in Supplementary Figure 1A (Residual Chi Square = 8.089 with 4 df Sig. = 0.088) and B (Residual Chi Square = 11.053 with 4 df Sig. = 0.026).

A.

		Overall (score)			Change From Previous Block		
Step	-2 Log Likelihood	Chi-square	df	Sig.	Chi-square	df	Sig.
1	3365.009	17.338	1	<.001	15.177	1	<.001

Variables in the Equation

Step (Parameter)	B	SE	Wald	df	Sig	Exp (B)	95.0% CI for Exp (B)
1 (donor asystolic WIT (≤15 vs. >15min)	-.609	.148	16.812	1	<.001	.544	Lower: .407 Upper: .728

Variables not in the Equation

Step 1	Mean	Score	df	Sig.
Recipient age	56.021	.845	1	.358
Recipient lab MELD	15.504	.498	1	.480
Donor age	44.156	3.580	1	.058
Duration of cold storage	6.393	2.963	1	.085

B.

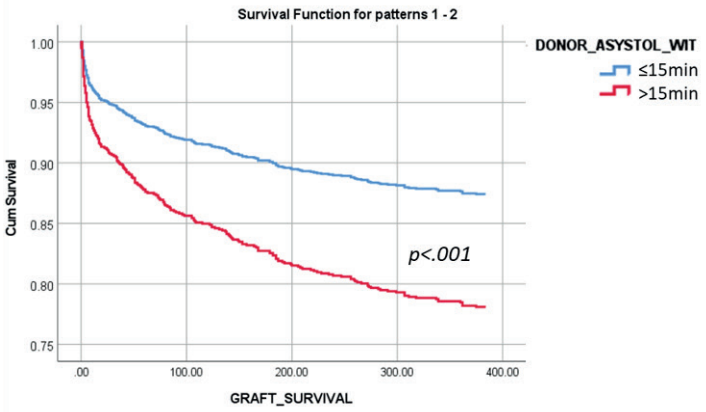
		Overall (score)			Change From Previous Block		
Step	-2 Log Likelihood	Chi-square	df	Sig.	Chi-square	df	Sig.
1	3084.984	10.483	1	.001	9.775	1	.002

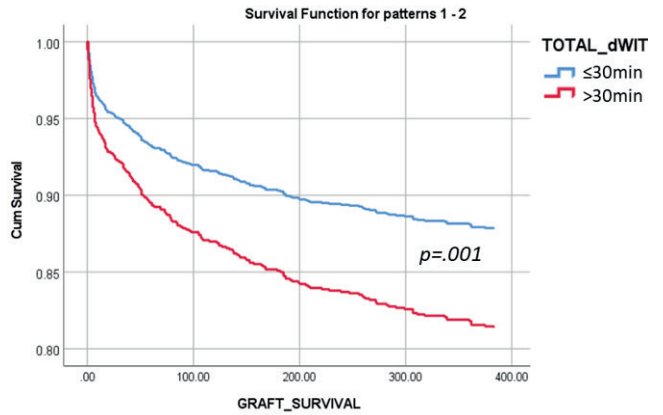
Variables in the Equation

Step (Parameter)	B	SE	Wald	df	Sig	Exp (B)	95.0% CI for Exp (B)
1 (total donor WIT (≤30 vs. >30min)	-.460	.143	10.300	1	.001	.631	Lower: .476 Upper: .836

Variables not in the Equation

Step 1	Mean	Score	df	Sig.
Recipient age	56.021	2.527	1	.112
Recipient lab MELD	15.504	1.949	1	.163
Donor age	44.156	3.200	1	.074
Duration of cold storage	6.393	3.383	1	.066





Supplementary Figure 1: Cox – Regression Model: Example One Year Graft Survival

A: Omnibus test of Model Coefficients (-2Log Likelihood: 3380.186), B: Omnibus test of Model Coefficients (-2Log Likelihood: 3380.186)

How were risk factors and outcome parameters distributed overall, in the benchmark cohort and comparing European and North American Centres

The overall 2219 controlled DCD liver transplantations were provided by 17 centres worldwide, mainly from North America and Europe. Based on defined criteria (Supplementary Table 1) 114 cases were excluded initially, resulting in an overall DCD cohort of 2105, which served as pool to select the benchmark cohort. Supplementary Table 2 presents donor and recipient risk factors. No cases from Asia, South America or Australia could be included due to the limited or non-existing source of DCD donors.

How are DCD liver transplantations in Europe and North America clinically managed?

The median duration required for a liver transplant surgery was comparable with 5.9 hours (Europe; IQR: 4.7-7.1) and 5.7 hours (North America; IQR: 4-6.7, $p = 0.005$). More red blood cell transfusions were administered in North American Centres, when compared to Europe (6 vs. 2 RBC, $p < 0.0001$). Overall, 90.5% and 81% of DCD livers were implanted using the piggyback technique in Europe and North America, respectively. The remaining cases were done with classic (cava replacement). A similar picture was seen regarding the connection of the biliary tree. The majority of recipients had a duct-to-duct biliary anastomosis with 90.4% in Europe and 96.2% in North America. Only 6.7% and 3.1% received a hepaticojejunostomy comparing Europe and North America, respectively.

While the median ICU stay was comparably short in both continents, the median hospital stay was significantly shorter in North America (9 vs. 15 days, $p < 0.001$), which is also related to a different discharge policy. The median INR one day after LT was comparable among all cohorts. In contrast, the median peak Aspartate Aminotransferase (AST) was found significant higher in recipients transplanted in North America (2259 vs. 1351 U/L, $p < 0.0001$). Slightly lower overall HAT rates were found in Europe compared to North American centres with 4.5% and 3.2%, respectively (Supplementary Table 3). The initial kidney function was better in North American recipients with a lower rate of RRT (8.9% vs. 14.3%, $p = 0.0006$). The median values for the CCI® were lower in North America in the first 12 months after LT. Slightly more grafts were lost during hospital stay in Europe, compared to North America with 7.3% and 4.6%. The overall 10-year graft survival was slightly inferior comparing Europe with North America (Supplementary Figure 1).

Supplementary Table 2: Donor and recipient risk comparing the overall cohort, the benchmark cohort and cases from Europe and North America
Paralleled by previous reports, DCD donors in North America were younger with shorter donor warm and cold ischemia times and a few more laboratory MELD points, when compared to Europe. While most recipient factors were comparable between the two continents, the laboratory MELD was a few points higher in North America (17 vs. 13 points, p < 0.0001) with more cases transplanted for Hepatitis C cirrhosis (40.1% vs. 17.6%, p = 0.0001). In the benchmark cohort, the recipient underlying diseases were found with 30.8% alcoholic liver cirrhosis, 25.9% were hepatis C and 4.4% hepatitis B cirrhosis. Overall, 43.4% of candidates in the benchmark group were also listed for hepatocellular carcinoma (HCC).

Risk Factors	Overall cohort (n=2105)	Benchmark cases (n=1012) ¶	European Centres (n=1456)	North American Centres (n=649)	p-value (Overall vs. Benchmark)	p-value (Europe vs. North America)
Donor age (years)	48 (32.8-57)	48 (34-57)	51 (37-60)	39 (26-50)	0.124	<0.0001
Donor BMI (kg/m²)	24.96 (22.3-28.09)	24.79 (22.35-28.0)	24.69 (22.36-27.5)	25.65 (22.23-29.6)	0.394	0.002
Total donor warm ischemia time (min)	25 (20-31)	22 (18-26)	26 (21-32)	23 (18-28)	<0.0001	<0.0001
Functional donor warm ischemia time (min)*	20 (14-33)	15 (12-20)	23 (15-36)	15 (10.75-19)	0.027	<0.0001
Asystolic donor warm ischemia time (min)	10 (8-13)	9 (8-11)	11 (9-14)	8 (7-11)	<0.0001	<0.0001
Cold ischemia time (hrs)	6.25 (5.2-7.47)	6.13 (5.05-7.42)	6.68 (5.55-7.83)	5.66 (4.91-6.62)	0.014	< 0.001
Recipient age (years)	57 (51-63)	57 (51-62)	57 (50-62.63)	58 (53-63)	0.477	<0.0001
Recipient BMI (kg/m²)	26.7 (23.76-30.15)	26.2 (23.6-30)	26.42 (23.71-29.91)	26.9 (23.9-30.9)	0.132	0.065
Recipient laboratory MELD (points)	14.2 (10-19)	13 (9.5-16)	13 (9.75-18)	17 (12.06-22)	<0.0001	<0.0001
Recipient HCC (%)	40.5%	43.4%	40.5%	40.5%	0.1302	1.0

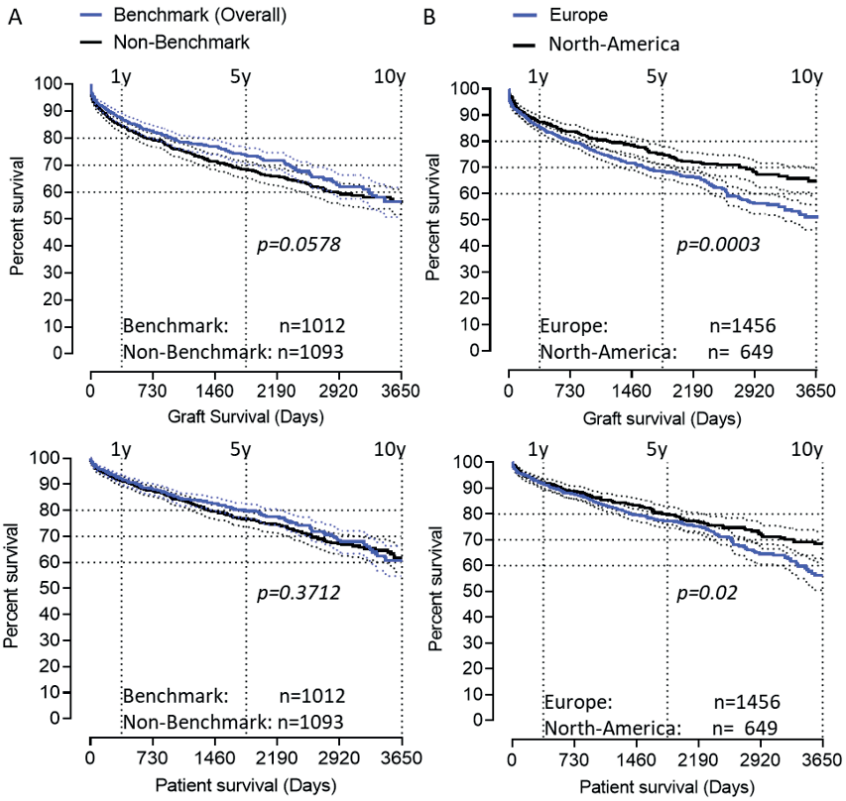
Median and IQR or n/%; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables); ¶: Benchmark cohort cases, data shown as median and IQR or number/proportion (%); *=fDWIT: systolic bloodpressure of < 50mmHg or saturation of < 70% to cold in situ flush (overall cohort: n=710, provided this information), n=452 in benchmark group, n=564 in Europe and n=146 in North American Centres). BMI; Body Mass Index, MELD; Model for End-Stage Liver Disease, HCC; Hepatocellular carcinoma.

Supplementary Table 3: Comparative outcome analysis of overall and benchmark DCD cohorts and livers transplanted in European and North American Centres

Outcome Parameter	Overall cohort (n=2105)	Benchmark cases (n=1012) ¶	European Centres (n=1456)	North American Centres (n=649)	p-value (Overall vs. Benchmark)	p-value (Europe vs. North America)
Duration of transplantation (hrs)	5.8 (4.4-6.9)	5.3 (4-6.7)	5.9 (4.7-7.1)	5.7 (4-6.7)	<0.0001	0.005
No. of RBC transfusions (U)	3 (1-6)	2 (0-6)	2 (0-4)	6 (2-11)	<0.0001	<0.0001
No. of FFP transfusions (U)	4 (1-8)	4 (0-8)	4 (0-7)	5 (2-10)	0.020	<0.0001
ICU stay (days)	2 (1-4)	2 (1-4)	3 (2-5)	2 (0-3)	0.001	<0.0001
Hospital stay (days)	13 (9-20)	12 (8-18)	15 (11-22)	9 (6-13)	<0.0001	<0.001
Renal replacement therapy (%)	12.67%	12%	14.33%	8.94%	0.6021	0.0006
Any complication (12 months) (%)	78.67%	74.41%	82.4%	68.77%	0.0300	0.0001
Grade II complications (12 months) (%)	17.9%	17.6%	18.45%	16.46%	0.8416	0.2952
Grade III complications (12 months) (%)	29.7%	27.27%	30.73%	27.54%	0.1642	0.1633
Grade IV complications (12 months) (%)	17%	15.4%	19.2%	12.16%	0.2792	0.0001
Primary non-function (%)	2.58%	1.89%	3.03%	1.55%	0.2569	0.0519
Bleeding (%)	7.13%	5.65%	6.42%	8.74%	0.1247	0.0539
Hepatic Artery Thrombosis (%)	4.133%	4.74%	4.53%	3.23%	0.4525	0.1924
Ischemic Cholangiopathy (%)	10.6%	8.8%	11.57%	8.59%	0.1262	0.0467
Anastomotic Strictures (%)	21.9%	20.9%	23.94%	17.51%	0.5153	0.0009
Bile leak (%)	5.9%	5.3%	5.29%	7.21%	0.5646	0.0882

Outcome Parameter	Overall cohort (n=2105)	Benchmark cases (n=1012) ¶	European Centres (n=1456)	North American Centres (n=649)	p-value (Overall vs. Benchmark)	p-value (Europe vs. North America)
CCI ® until discharge (points)	20.9 (0-36.2)	8.7 (0-33.5)	20.9 (0-40.5)	0 (0-29.6)	<0.0001	<0.0001
CCI ® 3 months (points)	26.2 (0-42.4)	20.9 (0-39.5)	27.6 (1.250-42.4)	20.9 (0-39.7)	<0.0001	<0.0001
CCI ® 6 months (points)	29.6 (8.7-46.2)	26.2 (0-42)	33.5 (8.7-46.2)	26.2 (0-44.9)	<0.0001	<0.0001
CCI® 12 months (points)	33.7 (8.7-49.1)	29.6 (0-46.2)	35.7 (20.9-49.9)	26.2 (0-47.6)	<0.0001	<0.0001
Graft loss (12 months, %)	13.9%	12.7%	14.62%	12.46%	0.3703	0.2199
Retransplantation (12 months, %)	5.23%	4.5%	5.7%	4.17%	0.4314	0.1677
Retransplantation overall (%)	10.29%	8.93%	10.46%	6.96%	0.2231	0.0118
In Hospital Mortality (%)	3.6%	3.26%	4.05%	3.08%	0.6776	0.3212
One-Year mortality (%)	9.01%	8.39%	8.85%	9.23%	0.5900	0.7410
Follow up (graft survival, days)	1347 (579-2252)	1386 (646.5-2277.8)	1207 (458.5-2086)	1649 (861.75-2642.5)	0.080	<0.0001
Follow up (patient survival, days)	1481 (788-2357)	1520 (822.75-2354.3)	1350 (683-2232)	1757 (985-2743)	0.350	<0.0001

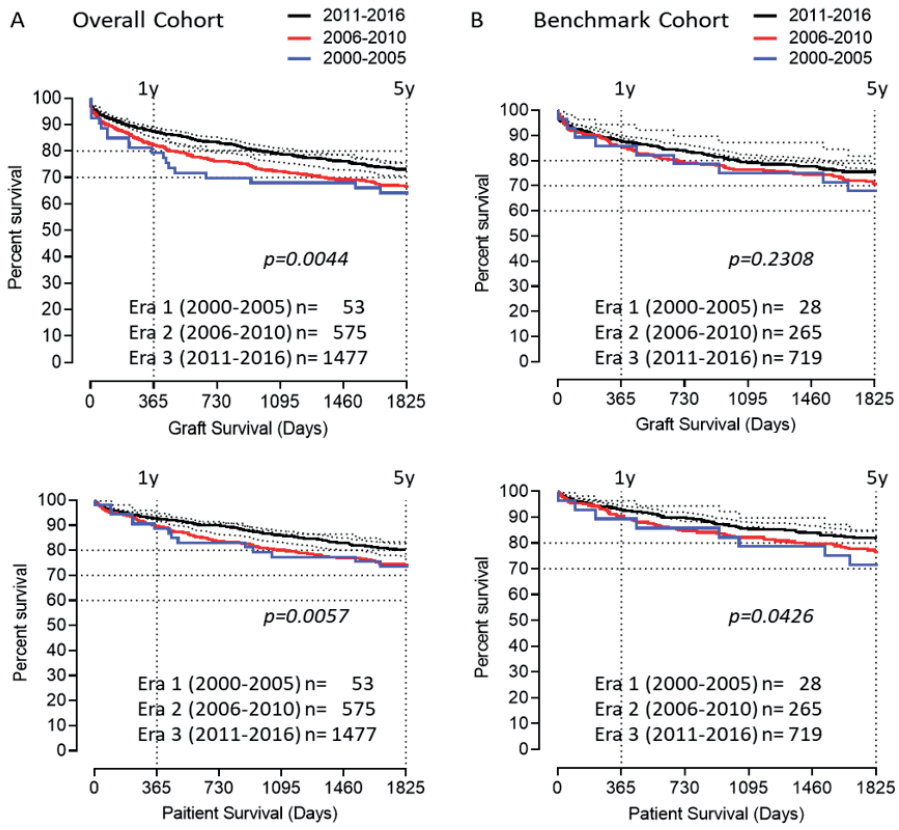
Values presented as median and IQR for continuous parameter and number/proportion (%) for binary parameter; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables), ¶: Benchmark cohort cases; complication in 12 months=highest in 1 year of follow up; RBC; Red Bloodcell Concetrate, FFP; Fresh Frozen Plasma; ICU; Intensive Care Unit, CCI; Comprehensive Complication Index.



Supplementary Figure 2: Ten-year graft and patient survival after DCD liver transplantation
Graft and patient survivals were slightly better in centres from North America compared to Europe. Such findings are somewhat expected and parallel the previous literature, based on the known lower donor risk in North America. Comparisons are made with the log-rank test. Following the initial exclusion of 114 cases (retransplantation, acute failures, ventilated recipient, renal replacement therapy), a total number of 2105 DCD transplantations was used as overall cohort. Benchmark and non-benchmark cases are compared in A. Supplementary Figure 1B compares outcomes from cases performed in North America and Europe.

Risk distribution and outcomes throughout the study period

To better describe the overall study population and to understand how risk factors and outcomes evolved over time, the overall and benchmark cohort were both divided into three subcohorts, representing three different Eras of time. The first one (early) from 2000-2005, second between 2006 and 2010, and the most recent third one from 2011-2016. Risk factors distribution and outcome analysis are presented in Supplementary Table 4 & 5 and Supplementary Figure 3.



Supplementary Figure 3: Five-year graft and patient survival after DCD liver transplantation according to different Eras

Overall and Benchmark cohort survivals according to the three different Eras. Comparisons were made with the Log-Rank-Test.

Development of Benchmark criteria

The overall benchmark cohort was split into three Eras: Era 1: 2000-2005 (total DCD cohort: n=53, benchmark cases: n=28), Era 2 (2006-2010) and Era 3 (2011-2016). Due to the low case load in the first Era, no benchmark values were developed and Suppl.Table 6 demonstrates Benchmark values of the overall cohort compared to Era 2 and 3 (same values as found in Table 1 main manuscript).

Supplementary Table 4: Donor and recipient risk comparing three different eras
Donor WIT largely remained stable and laboratory MELD scores slightly decreased from Era 2 to Era 3. Further parameters are detailed below:

Risk Factors	Overall cohort			
	All cases 2000-2016, n=2105	Era 1 (2000-2005), n=53	Era 2 (2006-2010), n=575	Era 3 (2011-2016), n=1477
Donor age (years)	48 (32.8-57)	43 (29-55)	44 (30-54)	49 (34-58)
Donor BMI (kg/min^2)	24.96 (22.3-28.09)	25.6 (22.2-29)	25.3 (22.9-28.6)	24.7 (22.2-28)
Total donor warm ischemia time (min)	25 (20-31)	26 (22-32)	25 (20-31)	25 (20-31)
Functional donor warm ischemia time (min)*	16 (12-21)	16 (11-23)	14 (11-20)	17 (12-21)
Asystolic donor warm ischemia time (min)	10 (8-13)	9 (8-11)	10 (8-13)	10 (8-13)
Cold ischemia time (hrs)	6.25 (5.2-7.47)	5.9 (4.86-7.08)	6.25 (5.17-7.44)	6.25 (5.25-7.495)
Recipient age (years)	57 (51-63)	54 (44.5-63)	56.8 (51-62)	57 (50-63)
Recipient BMI (kg/m²)	26.7 (23.76-30.15)	26.8 (24.2-30.9)	26.1 (23.4-30.6)	26.7 (23.9-30.09)
Recipient laboratory MELD (points)	14.2 (10-19)	14 (11-17)	16 (11-20)	14 (10-19)
Recipient HCC (%)	40.5%	37.7%	40.2%	40.56%

Median and IQR or n/%; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables); ¶: Benchmark cohort cases, data shown as median and IQR or %; *: fdWIT below a MAP of 50 or saturation of > 70% (overall cohort: n=710, era 1: n=11, era 2: n=247, era 3: n=778, benchmark cohort overall: n=452, era 1: n=6, era 2: n=133, era 3: n=422 provided this information). BMI; Body Mass Index, MELD; Model for End-Stage Liver Disaese, HCC; Hepatocellular Carcinoma.

Benchmark cases				P-value		
All Benchmark cases, n=1012 ¶	**Era 1 (2000-2005), n=28**	**Era 2 (2006-2010), n=265**	**Era 3 (2011-2016), n=719**	**Overall vs. Benchmark (all eras)**	**Overall cases Era 2 vs. Era 3**	**Benchmark cases Era 2 vs. Era 3**
48 (34-57)	39 (26.75-54.5)	45 (31-55)	49.5 (35-58.3)	0.124	<0.0001	0.004
24.79 (22.35-28.0)	25.6 (24.7-29)	26 (23.5-29)	24.3 (22-27.5)	0.394	0.030	<0.0001
22 (18-26)	20 (17-23)	22 (18-25)	22 (18-26)	0.0001	0.895	0.296
15 (12-20)	12 (8.5-17.25)	13 (11-17)	16 (12-20)	0.005	<0.0001	<0.0001
9 (8-11)	9 (8-10)	9 (8-11)	10 (8-12)	<0.0001	0.009	0.016
6.13 (5.05-7.42)	5.48 (4.78-7.017)	5.95 (4.95-7.217)	6.25 (5.1-7.492)	0.014	0.923	<0.0001
57 (51-62)	54 (42-60.75)	56 (51-62)	57 (51-62)	0.477	0.500	0.786
26.2 (23.6-30)	27.2 (23.9-31.6)	26.1 (24-30.1)	26.4 (23.4-30)	0.132	0.232	0.903
13 (9.5-16)	12.5 (10.3-16)	14 (10-17)	12.9 (9-16)	<0.0001	<0.0001	0.008
43.4%	35.7%	47.5%	41.9%	0.1302	0.8807	0.1115

Supplementary Table 5: Outcome analysis after DCD liver transplantation comparing three different eras
Expectedly, the outcomes did not change significantly for all parameters. In the overall cohort graft loss and retransplantation rate improved slightly between second and third Era. In contrast, the selection of low-risk cases with the benchmarking tool led to comparable outcomes throughout the entire study period and all three Eras. No significant differences in outcomes were seen comparing Era 2 and 3 in the benchmark cohort.

Outcome Parameter	Overall cohort			
	All cases (2000-2016), n=2105	Era 1 (2000-2005), n=53	Era 2 (2006-2010), n=575	Era 3 (2011-2016), n=1477
Duration of transplantation (hrs)	5.8 (4.4-6.9)	5 (3.62-5.49)	5.45 (3.93-6.88)	5.93 (4.67-7)
No. of RBC transfusions (U)	3 (1-6)	4 (3-6)	4 (2-8)	2 (0-5)
No. of FFP transfusions (U)	4 (1-8)	4.5 (2-11.5)	5 (2-10)	4 (0-8)
ICU stay (days)	2 (1-4)	2 (0-4.25)	2 (1-5)	2 (2-4)
Hospital stay (days)	13 (9-20)	14 (9-21.75)	14 (9-21)	13 (9-19)
Peak AST first week (U/L)	1507 (686-3152)	2105 (1499-3705)	1846 (905-3757)	1391.5 (632-2908.75)
Peak ALT first week (U/L)	980 (515-1917)	789 (545-1290)	860 (464-1741.5)	1053 (539-1951)
INR day 1	1.6 (1.4-2)	1.46 (1.21-1.66)	1.61 (1.4-2)	1.6 (1.4-2)
Peak Creatinine first week (µmol/l)	115 (77-173)	133 (79.58-168)	110.5 (72-168)	116 (78-175)
Renal replacement therapy (%)	12.67%	13.2%	12.69%	12.46%
Any complication (12 months) (%)	78.67%	50.94%	76.9%	79.8%
Grade II complications (12 months) (%)	17.9%	7.5%	16.9%	18.6%
Grade III complications (12 months) (%)	29.7%	13.2%	27.3%	31.3%
Grade IV complications (12 months) (%)	17%	15.1%	17.0%	17.1%
Primary non-function (%)	2.58%	5.66%	4%	1.89%
Bleeding (%)	7.13%	11.32%	8.9%	6.16%
Hepatic Artery Thrombosis (%)	4.133%	4.08%	5.04%	4.13%
Ischemic Cholangiopathy (%)	10.6%	11.32%	10.4%	10.08%
Anastomotic Strictures (%)	21.9%	20.75%	18.3%	22.7%
Bile leak (%)	5.9%	11.3%	5.6%	5.6%
CCI ® until discharge (points)	20.9 (0-36.2)	0 (0-29.6)	20.9 (0-34.6)	20.9 (0-36.2)
CCI ® 3 months (points)	26.2 (0-42.4)	0 (0-39.5)	26.2 (0-42.4)	26.2 (0-42.4)
CCI ® 6 months (points)	29.6 (8.7-46.2)	0 (0-42.4)	29.6 (0-46.2)	30.8 (8.7-46.2)
CCI® 12 months (points)	33.7 (8.7-49.1)	0 (8.7-46.2)	34.6 (8.7-50.18)	33.7 (20.9-48.1)
Graft loss (12 month, %)	13.9%	20.75%	17.56%	12.3%
Retransplantation (12 months, %)	5.23%	7.5%	7%	4.4%
Retransplantation overall (%)	10.29%	11.32	11.47%	8.46%
In Hospital Mortality (%)	3.6%	1.89%	4.17%	3.5%
One-Year mortality (%)	9.01%	11.3%	11.3%	7.98%
Follow up (graft survival, days)	1347 (579-2252)	2780 (445-4718)	2663 (712-3364)	1155 (553-1790)
Follow up (patient survival, days)	1481 (788-2357)	3273 (1602.5-4758.5)	2863 (1328-3455)	1241 (732-1845)

Values presented as median and IQR for continuous parameter and % for binary parameter; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables), ¶: Benchmark cohort cases, data shown as median and IQR or %; complication in 12 months=highest in 1 year of follow up; RBC; Red Bloodcell Concentrate, FFP; Fresh Frozen Plasma, ICU; Intensive Care Unit, AST; Aspartate Transaminase , ALT: Alanine Transaminase, INR; International Normalised Ratio, CCI: Comprehensive Complication Index.

Benchmark cases				P-value		
All Benchmark cases (n=1012)[1]	**Era 1 (2000-2005), n=28**	**Era 2 (2006-2010), n=265**	**Era 3 (2011-2016), n=719**	**Overall vs. Bench-mark**	**Overall cases Era 2 vs. Era 3**	**Bench-mark cases Era 2 vs. Era 3**
5.3 (4-6.7)	5 (3.44-5.42)	4.73 (3.52-6.33)	5.78 (4.34-6.87)	<0.0001	<0.0001	<0.0001
2 (0-6)	4 (3-6)	4 (1-7)	2 (0-5)	<0.0001	<0.0001	<0.0001
4 (0-8)	4 (1-9)	4 (2-9)	4 (0-7)	0.020	<0.0001	0.036
2 (1-4)	2 (0-4.5)	2 (1-4)	2 (2-4)	0.001	0.052	0.063
12 (8-18)	15.5 (8.25-23.5)	13 (8-20)	12 (8-17)	<0.0001	0.097	0.177
1293 (594.5-2737)	1901 (1285-3191)	1494 (740-3254)	1251 (497-2527)	<0.0001	<0.0001	0.001
922 (474.5-1743.5)	840 (457-1302)	701.5 (430.8-1579.8)	987 (491.8-1774.5)	0.001	0.002	0.032
1.6 (1.36-1.9)	1.34 (1.14-1.6)	1.6 (1.36-1.9)	1.6 (1.4-1.9)	<0.0001	0.613	0.134
106.1 (74-154.5)	92 (75-147)	104.5 (70.7-150.2)	106.98 (75.3-159.1)	<0.0001	0.109	0.245
12%	7.14%	11.698%	12.2%	0.6021	0.9409	0.9123
74.41%	50%	72.1%	75.7%	0.0300	0.1476	0.2487
17.6%	7.14%	16.2%	18.5%	0.8416	0.3723	0.4536
27.27%	14.29%	25.3%	28.5%	0.1642	0.0774	0.3356
15.4%	7.14%	13.96%	16.4%	0.2792	1.0	0.3759
1.89%	0	1.89%	1.95%	0.2569	0.0996	1.0
5.65%	0	6.42%	5.56%	0.1247	0.5841	0.6448
4.74%	7.14%	6.04%	4.45%	0.4525	0.4006	0.311
8.8%	10.71%	9.17%	8.56%	0.1262	0.8078	0.7896
20.9%	21.4%	19.1%	21.48%	0.5153	0.0310	0.4641
5.3%	14.29%	5.22%	4.88%	0.5646	1.0	0.8649
8.7 (0-33.5)	0 (0-20.9)	8.7 (0-33.5)	8.7 (0-33.7)	<0.0001	0.410	0.945
20.9 (0-39.5)	0 (0-33.5)	20.9 (0-39.7)	20.9 (0-39.7)	<0.0001	0.578	0.646
26.2 (0-42)	0 (0-33.5)	22.6 (0-42.6)	26.2 (0-42.4)	<0.0001	0.526	0.336
29.6 (0-46.2)	0 (0-39.5)	26.2 (0-47.3)	30.8 (0-46.2)	<0.0001	0.684	0.465
12.7%	14.29%	14.33%	12.1%	0.3703	0.0027	0.3879
4.5%	3.57%	4.91%	4.31%	0.4314	0.0251	0.7284
8.93%	7.14%	7.92%	8.1%	0.2231	0.042	1.0
3.26%	3.57%	4.15%	2.92%	0.6776	0.5153	0.3185
8.39%	10.71%	10.19%	7.5%	0.5900	0.0199	0.1911
1386 (646.5-2277.8)	2995 (1078-4617)	2800 (960-3397)	1154.9 (595-1833)	0.080	0.0001	<0.0001
1520 (822.75-2354.3)	2995 (1566-4617)	2981 (1561-3409)	1290 (771-1910)	0.350	0.0001	0.001

Supplementary Table 6a: Benchmark cut-off's in DCD liver transplantation comparing the overall cohort with era 2 and 3

Benchmark Cases: controlled DCD liver transplantation					
	Perioperative course				
	Duration of transplantation	Intra-operative Blood transfusions	Renal Replacement Therapy	ICU stay	Hospital stay
Overall (2000-2016); n=1012	≤ 6.8 hrs	≤ 3 U RBC	≤ 9.6 %	≤ 3 days	≤ 16 days
Era 3 (2011-2016); n=719	≤ 6.8 hrs	≤ 3 U RBC	≤ 10.7 %	≤ 3 days	≤ 14.5 days
Era 2 (2006-2010); n=265	≤ 6.3 hrs	≤ 6 U RBC	≤ 14.7 %	≤ 3 days	≤ 17 days

Two time frames are considered for HAT: early HAT within the first months after OLT, and late HAT, including all diagnosed HATs after one month after LT; DCD: Donation after circulatory death; ICU: Intensive care unit;

Supplementary Table 6b: Benchmark cut-off's in DCD liver transplantation comparing the overall cohort with era 2 and 3

Benchmark Cases: controlled DCD liver transplantation														
	Morbidity and Mortality													
	Any complication				≤ Grade II complication [§]				≥ Grade III complication [§]				CCI®	
	DC	3m	6m	12m	DC	3m	6m	12m	DC	3m	6m	12m	DC	3m
Overall (2000-2016); n=1012	≤ 76%	≤ 90%	≤ 93%	≤ 95%	≤ 83%	≤ 69%	≤ 59%	≤ 58%	≤ 43%	≤ 56%	≤ 60%	≤ 66%	≤ 22.2 points	≤ 30.8 points
Era 3 (2011-2016); n=719	≤ 77%	≤ 87 %	≤ 92%	≤ 92%	≤ 83%	≤ 68%	≤ 58%	≤ 58%	≤ 38%	≤ 53%	≤ 57%	≤ 62%	≤ 22.9 points	≤ 33.8 points
Era 2 (2006-2010); n=265	≤ 87%	≤ 98%	≤ 100%	≤ 100%	≤ 79%	≤ 68%	≤ 59%	≤ 60%	≤ 59%	≤ 75%	≤ 75%	≤ 76%	≤ 31.7 points	≤ 34.7 points

Complications [§]: are the highest complications, example: if a recipient has grade II in three month and then develops another grade IIIa afterwards within 6 months, his highest grade of complication changes from II to IIIa, explaining the decreasing rate of grade II complications throughout the first year after transplantation. DC: Discharge/ hospital stay after LT; 3m: three months; 6m: 6 months, 12m: 12 months;

The next three Tables show the individual benchmark value calculation for the overall and Era 2 and 3. Benchmarking outcome values were explored through calculation of median values for each parameter per centre (continuous parameter). The proportion of binary outcome parameters was established individually for each centre. The 75th percentile of the median values of each centre represents the Benchmark value and was calculated accordingly. Supple-mentary Table 7a-d shows the median and benchmark value for all outcome parameters (overall cohort 2000-2016).

Key complications						
Primary Non-Function	**Bleeding**	**Anastomotic Strictures**	**Ischemic Cholangio-pathy**	**Bile leak**	**Early Hepatic Artery Throm-bosis (HAT)**	**Late Hepatic Artery Throm-bosis (HAT)**
≤ 2.5 %	≤ 10.3 %	≤ 28.4 %	≤ 16.8 %	≤ 8.3 %	≤ 4.5 %	≤ 2.3 %
≤ 2.4 %	≤ 9 %	≤ 28.9 %	≤ 15 %	≤ 8.6 %	≤ 4.8 %	≤ 1.9 %
0 %	≤ 16.4 %	≤ 27.1 %	≤ 14.3 %	≤ 4.1 %	≤ 6 %	0 %

		Graft loss				Retransplantation				Mortality			
6m	**12m**	**DC**	**3m**	**6m**	**12m**	**DC**	**3m**	**6m**	**12m**	**DC**	**3m**	**6m**	**12m**
≤ 36.4 points	≤ 38.9 points	≤ 10.1%	≤ 13.3%	≤ 14.0%	≤ 14.4%	≤ 5%	≤ 6.4%	≤ 6.4%	≤ 6.9%	≤ 6.5%	≤ 7.8%	≤ 7.8%	≤ 9.6%
≤ 36.9 points	≤ 37.6 points	≤ 10.5%	≤ 10.7%	≤ 12.4%	≤ 14.7 %	≤ 5.2%	≤ 5.2%	≤ 5.8%	≤ 7%	≤ 5.1%	≤ 6.1%	≤ 7.6%	≤ 9.3%
≤ 36.5 points	≤ 46.3 points	≤ 15.6%	≤ 16.5%	≤ 18.2%	≤ 18.9%	≤ 4.3%	≤ 5.4%	≤ 6.9%	≤ 6.9%	≤ 14.7%	≤ 16.1%	≤ 16.1%	≤ 20%

Supplementary Table 7a: Benchmark value calculation (overall Benchmark cohort: 2000-2016)
In the column, for each centre, the median value of a specific outcome parameters is shown (cases from one centre were excluded because donor warm ischemia times were not available). The last two rows summarise such median values with the overall parameter median and the 75th percentile, which equals the Benchmark cut-off.

Centre	No. of DCD	% Bench-mark cases	PNF (%)	Bleeding (%)	Early HAT (%)	Late HAT (%)	IC (%)
1	88	60.2	7.55	15.09	4	4	0
2	97	29.9	0	10.71	0	3.6	0
3	107	72.9	0	10.26	4.1	0	6.94
4	88	44.3	0	2.56	2.8	0	16.67
5	61	54.1	6.67	0	0	0	17.24
6	68	32.4	0	4.55	5.3	0	0
7	243	56.4	0.74	4.88	0	2.4	9.45
8	94	26.6	0	8.33	4.2	0	33.3
9	54	25.9	0	7.14	16.7	0	30
10	122	19.7	4.17	12.5	13.04	0	0
11	87	28.7	0	0	0	2.2	4.55
12	64	75.0	0	10.42	0	1.54	0
13	112	62.5	0	1.43	1.54	0	1.54
14	82	26.8	0	4.55	9.5	1.49	4.76
15	342	63.5	3.23	3.23	0.99	5.33	5.42
16	348	50.6	2.26	5.08	3.6		17.37
Median	91	47.5	0	4.98	3.2	0	5.09
75th Percentile (Benchmark cut-off)	**n.a.**	**n.a.**	**2.5**	**10.3**	**4.5**	**2.3**	**16.8**

DCD: Donation after circulatory death; PNF: Primary non-function; HAT: Hepatic artery thrombosis; IC: Ischemic cholangiopathy; RRT: Renal Replacement Therapy; LT: Liver Transplantation; RBC: Red blood cell concentrates; ICU: Intensive care unit;

Anast. Stricture (%)	Bile leak (%)	RRT (%)	LT duration (min)	RBC (U)	ICU stay (days)	Hospital stay (days)
32.61	6.12	26.42	400	2	3	16.5
50	0	6.67	435.5	8	1.5	11
16.44	4.05	1.32	215.5	0	3	14.5
27.78	8.33	5.13	240	0	1	12
20.69	0	3.23	350	0	2	10.5
4.76	5	0	236	0	2	10
15.5	11.72	8.03	315	8	0	7
30.43	20.8	0	454	4	2	13
33.3	8.33	7.14	379	2	1.5	21
27.27	4.55	12.5	408	2	2	18
16.67	4.17	4	377	2	4	18
13.04	0	0	440	2	4	16
18.18	4.55	8.57	300	2	2	7
9.52	9.09	4.76	377	11	2	5
25.12	2.87	17.05	301	3	3	15
17.47	3.61	22.6	408	2	2	10
19.435	4.55	5.9	377	2	2	12.5
28.4	**8.3**	**9.6**	**408**	**3.2**	**3**	**16.1**

Supplementary Table 7b: Benchmark value calculation, part 2 (overall Benchmark cohort: 2000-2016)
In the column, for each centre, the median value of a specific outcome parameters is shown (cases from one centre were excluded because donor warm ischemia times were not available). The last two rows summarise such median values with the overall parameter median and the 75th percentile, which equals the Benchmark cut-off.

Centre	CCI at DC (points)	CCI at 3 months (points)	CCI at 6 months (points)	CCI at 12 months (points)
1	37.4	38.1	41.5	42.4
2	26.2	29.85	35.95	37.1
3	8.7	20.9	26.4	33.7
4	20.9	29.6	33.5	34.6
5	0	0	23.55	23.55
6	0	0	0	0
7	0	20.9	20.9	20.9
8	20.9	33.5	37.1	38.1
9	30.2	45.15	45.15	49.6
10	33.3	39.2	42.25	46.85
11	20.9	29.6	36.2	41.4
12	0	0	0	0
13	0	0	20.9	20.9
14	20.9	29.6	30.8	36.2
15	8.7	20.9	20.9	20.9
16	20.9	27.6	32.15	33.7
Median	20.9	28.6	31.475	34.15
75th Percentile (Benchmark cut-off)	**22.2**	**30.8**	**36.4**	**38.9**

CCI: Comprehensive Complication Index; DC: Discharge;

Supplementary Table 7c: Benchmark value calculation, part 3 (overall Benchmark cohort: 2000-2016)

In the column, for each centre, the median value of a specific outcome parameters is shown (cases from one centre were excluded because donor warm ischemia times were not available). The last two rows summarise such median values with the overall parameter median and the 75th percentile, which equals the Benchmark cut-off.

Centre	Any Complication (%)				Up to (and including) Grade II complication (%)				Grade III or higher complication (%) (Grade III, IV, V)			
	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months
1	84.91	90.57	92.45	100	43.39	35.84	35.85	30.19	56.6	64.15	64.15	69.8
2	73.3	80	86.7	86.7	50	40	30	26.67	50	60	70	73.3
3	53.8	65.38	80.77	85.9	76.92	69.23	56.4	46.15	23.08	35.9	43.59	53.85
4	66.67	89.7	94.9	94.9	64.1	46.15	41.03	38.46	35.89	53.85	59	61.54
5	24.24	45.45	51.52	51.52	90.91	66.67	57.58	57.58	9.09	33.33	42.42	42.42
6	13.63	18.18	18.18	18.18	90.91	86.36	81.82	81.82	9.09	13.64	18.18	18.18
7	35.04	57.66	57.66	60.58	84.67	68.61	64.96	62.04	15.33	31.39	35.04	38
8	60	72	80	88	56	36	28	20	44	64	72	80
9	92.86	92.86	100	100	57.14	28.57	14.29	12.29	42.86	71.43	85.7	85.7
10	91.67	91.67	95.83	95.83	54.17	54.17	45.83	41.67	45.83	45.83	54.17	58.33
11	92	96	96	96	88	64	52	40	12	36	48	60
12	22.92	37.5	45.83	47.92	79.17	64.58	56.25	54.17	20.83	35.42	43.75	45.83
13	27.14	48.57	54.29	54.29	82.86	70	61.43	60	17.14	30	38.57	40
14	68.18	77.27	86.4	90.91	72.73	59.09	54.55	45.5	27.27	40.91	45.45	54.55
15	52.07	56.22	57.6	65.44	74.19	70.05	69.59	59.91	25.35	29.49	30.41	40.09
16	72.88	84.18	88.13	90.96	57.06	45.76	42.37	35.6	42.94	54.24	57.63	64.41
Median	63.34	74.635	83.585	87.35	73.46	61.545	53.275	43.585	26.31	38.455	46.725	56.44
75th Percentile (Bench-mark cut-off)	**76.2**	**89.9**	**93.0**	**95.1**	**83.3**	**68.8**	**58.6**	**58.2**	**43.2**	**55.7**	**60.3**	**65.8**

DC: Discharge;

Supplementary Table 7d: Benchmark value calculation, part 4 (overall Benchmark cohort: 2000-2016)
In the column, for each centre, the median value of a specific outcome parameters is shown (cases from one centre were excluded because donor warm ischemia times were not available). The last two rows summarise such median values with the overall parameter median and the 75th percentile, which equals the Benchmark cut-off.

Centre	Graft Loss (%)				Redo-Liver transplantation (%)				Mortality (%)			
	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months
1	13.21	13.21	15.09	18.87	7.55	9.43	9.43	11.32	7.55	7.55	7.55	9.43
2	3.33	3.33	6.67	6.67	0	0	0	0	3.33	3.33	6.67	10
3	5.13	6.4	8.97	10.26	0	2.56	2.56	2.56	5.13	5.13	6.41	7.69
4	15.38	15.38	17.95	20.51	7.69	7.89	7.89	7.89	10.26	10.26	10.26	15.38
5	3.03	3.03	3.03	3.03	0	3.03	3.03	0	0	0	3.03	6.06
6	9.09	13.64	13.64	13.64	0	0	0	0	4.55	9.09	9.09	9.09
7	3.65	8.76	9.49	12.41	2.92	5.84	5.84	6.57	2.19	2.92	4.38	8.03
8	4	4	4	8	4	4	4	4	0	0	0	4
9	21.43	21.43	21.43	42.86	14.29	14.29	14.29	28.57	7.14	7.14	7.14	14.29
10	16.67	16.67	16.67	16.67	12.5	8.7	8.7	8.7	8.33	8.33	8.33	8.33
11	4	8	12	12	4	4	4	4	4	4	4	4
12	6.25	8.33	12.5	12.5	0	0	0	0	6.25	8.33	12.5	12.5
13	2.86	7.14	7.14	8.57	0	0	0	0	2.86	7.14	7.14	8.57
14	0	4.55	4.55	4.55	0	0	0	0	0	4.55	4.55	4.55
15	3.69	5.07	5.53	13.36	4.15	4.61	4.61	4.67	0	0.46	1.84	7.37
16	6.78	8.47	11.3	12.994	3.39	4.55	4.55	4.55	3.95	4.52	5.65	6.78
Median	4.565	8.165	10.395	12.455	3.155	4	4	4	3.975	4.84	6.54	8.18
75th Percentile (Benchmark cut-off)	**10.1**	**13.3**	**14.0**	**14.4**	**5**	**6.4**	**6.4**	**6.9**	**6.5**	**7.8**	**7.8**	**9.6**

DC: Discharge;

Supplementary Table 8a: Benchmark value calculation, Era 2 (2006-2010), part 1
Below the detailed calculations for the Benchmarking values during Era 2 are shown.

Centre	No. of DCD	% Bench-mark cases in Era 2	PNF (%)	Bleeding (%)	Early HAT (%)	Late HAT (%)	IC (%)	Anast. Stricture (%)	Bile leak (%)	RRT (%)	LT duration (min)	RBC (U)	ICU stay (days)	Hospital stay (days)
1	88	12.5%	0.00	18.18	9.09	9.09	0.00	62.50	0.00	27.27	320.00	6.00	3.00	18.00
2	97	9.3%	0.00	25.00	0.00	0.00	0.00	66.67	0.00	11.11	285.00	6.00	3.00	16.00
3	107	25.3%	0.00	7.69	0.00	0.00	4.00	8.00	3.85	3.85	205.00	0.00	3.00	16.00
4	88	10.2%	0.00	11.11	0.00	0.00	28.57	28.57	0.00	0.00	360.00	2.00	1.00	16.00
6	68	11.8%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	370.00	1.00	1.50	10.50
7	243	28.8%	1.45	2.90	0.00	4.29	15.38	15.15	10.77	8.57	229.00	8.00	0.00	7.00
8	94	3.2%	0.00	0.00	0.00	0.00	33.33	33.33	0.00	0.00	355.00	4.00	2.00	12.00
9	54	9.3%	0.00	20.00	40.00	0.00	0.00	0.00	0.00	0.00	457.00	2.00	3.00	31.50
10	122	4.1%	0.00	40.00	0.00	0.00	0.00	0.00	0.00	20.00	518.00	7.00	5.00	44.00
12	64	15.6%	0.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	380.00	4.50	3.50	15.00
13	112	3.6%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	310.00	0.50	3.00	5.00
14	82	12.3%	0.00	10.00	10.00	0.00	11.11	22.22	10.00	0.00	438.50	13.00	2.00	4.50
15	342	20.2%	5.80	4.35	1.45	2.89	4.62	22.72	4.48	15.94	300.00	3.00	2.00	17.00
16	348	7.5%	0.00	3.85	7.69	15.38	16.67	12.50	4.17	30.77	320.00	2.00	4.00	13.00
Median	91	11.0%	0.00	6.02	0.00	0.00	2.00	13.83	0.00	0.00	1.92	355.00	3.50	3.00
75th Percentile (Bench-mark cut-off)	**n.a.**	**n.a.**	**0.00**	**16.41**	**6.13**	**0.00**	**14.32**	**27.11**	**4.09**	**9.52**	**14.73**	**375.00**	**6.00**	**3.00**

DCD: Donation after circulatory death; PNF: Primary non-function; HAT: Hepatic artery thrombosis; IC: Ischemic cholangiopathy; RRT: Renal Replacement Therapy; LT: Liver Transplantation; RBC: Red blood cell concentrates; ICU: Intensive care unit;

Supplementary Table 8b: Benchmark value calculation, Era 2 (2006-2010), part 2
Below the detailed calculations for the Benchmarking values during Era 2 are shown.

Centre	CCI at DC (points)	CCI at 3 months (points)	CCI at 6 months (points)	CCI at 12 months (points)
1	44.30	49.90	55.60	55.60
2	26.20	26.20	26.20	26.20
3	20.90	20.90	28.25	34.25
4	33.50	33.70	34.80	44.90
6	0.00	0.00	0.00	0.00
7	0.00	20.90	20.90	21.75
8	0.00	26.20	37.10	38.10
9	44.90	49.50	53.70	72.10
10	44.90	52.00	56.70	64.30
12	0.00	0.00	10.45	10.45
13	0.00	0.00	0.00	0.00
14	20.90	34.55	34.55	46.80
15	8.70	8.70	8.70	20.90
16	23.55	34.80	34.80	37.10
Median	15.50	20.90	26.20	31.40
75th Percentile (Benchmark cut-off)	**16.75**	**31.68**	**34.74**	**36.53**

CCI: Comprehensive Complication Index; DC: Discharge;

Supplementary Table 8c: Benchmark value calculation, Era 2 (2006-2010), part 3
Below the detailed calculations for the Benchmarking values during Era 2 are shown.

Centre	Any Complication (%)				Up to (and including) Grade II complication (%)				Grade III or higher complication (%) (Grade III, IV, V)			
	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months
1	90.91	100.00	100.00	100.00	18.18	9.09	9.09	0.00	81.82	90.91	90.91	100.00
2	66.67	77.78	77.78	77.78	44.45	33.33	33.33	33.33	55.56	66.67	66.67	66.67
3	80.77	92.31	100.00	100.00	76.92	69.23	57.69	46.15	23.08	30.77	42.31	53.85
4	88.89	100.00	100.00	100.00	33.33	22.22	22.22	22.22	66.67	77.78	77.78	77.78
6	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
7	38.57	62.86	62.86	61.43	82.86	62.86	62.86	60.00	17.14	37.14	37.14	40.00
8	33.33	66.67	66.67	66.67	66.67	33.33	33.33	33.33	33.33	66.67	66.67	66.67
9	100.00	100.00	100.00	100.00	40.00	20.00	20.00	20.00	60.00	80.00	80.00	80.00
10	100.00	100.00	100.00	100.00	20.00	20.00	20.00	20.00	80.00	80.00	80.00	80.00
12	30.00	40.00	50.00	50.00	80.00	70.00	60.00	60.00	20.00	30.00	40.00	40.00
13	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00	0.00	0.00	0.00	0.00
14	60.00	70.00	80.00	90.00	70.00	50.00	50.00	30.00	30.00	50.00	50.00	70.00
15	52.17	53.62	55.07	65.22	76.81	50.00	50.00	62.32	23.19	50.00	50.00	37.68
16	73.08	92.31	92.31	96.16	53.85	38.46	38.46	30.77	46.15	61.54	61.54	69.23
Median	63.33	73.89	78.89	83.89	68.33	44.23	44.23	33.33	31.67	55.77	55.77	66.67
75th Percentile (Bench-mark cut-off)	**86.86**	**98.08**	**100.00**	**100.00**	**79.23**	**67.64**	**59.42**	**60.00**	**58.89**	**75.00**	**75.00**	**75.83**

DC: Discharge;

Supplementary Table 8d: Benchmark value calculation, Era 2 (2006-2010), part 4
Below the detailed calculations for the Benchmarking values during Era 2 are shown.

Centre	Graft Loss (%)				Redo-Liver transplantation (%)				Mortality (%)			
	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months
1	18.18	18.18	27.27	36.36	9.09	9.09	18.18	18.18	18.18	18.18	18.18	27.27
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	3.85	3.85	3.85	3.85	0.00	0.00	0.00	0.00	3.85	3.85	3.85	3.85
4	22.22	22.22	22.22	33.33	0.00	0.00	0.00	0.00	22.22	22.22	22.22	33.33
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	5.71	11.43	12.86	15.71	4.29	5.71	8.57	10.00	4.29	4.29	5.71	10.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	40.00	40.00	40.00	60.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	40.00
10	20.00	20.00	20.00	20.00	0.00	0.00	0.00	0.00	20.00	20.00	20.00	20.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	10.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	10.00	10.00	20.00
15	4.35	7.25	7.25	15.94	4.35	4.35	4.35	4.35	0.00	1.45	1.45	8.70
16	7.69	7.69	11.54	11.54	7.69	7.69	7.69	7.69	3.85	3.85	3.85	7.69
Median	4.10	7.47	8.62	10.77	0.00	0.00	0.00	0.00	1.92	3.85	3.85	8.19
75th Percentile (Bench-mark cut-off)	15.56	16.49	18.21	18.99	4.33	5.37	6.86	6.86	14.71	16.14	16.14	20.00

DC: Discharge;

Supplementary Table 9a: Benchmark value calculation, Era 3 (2011-2016), part 1
Below the detailed calculations for the Benchmarking values during Era 3 are shown.

Centre	No. of DCD	% Bench-mark cases in Era 3	PNF (%)	Bleeding (%)	Early HAT (%)	Late HAT (%)	IC (%)	Anast. Stricture (%)	Bile leak (%)	RRT (%)	LT duration (min)	RBC (U)	ICU stay (days)	Hospital stay (days)
1	88	47.73	9.52	14.29	2.38	2.38	0.00	26.32	7.50	26.19	415.00	1.00	3.00	16.00
2	97	20.62	0.00	5.26	0.00	5.00	0.00	38.89	0.00	5.00	435.50	8.00	1.00	11.00
3	107	47.66	0.00	11.76	5.88	0.00	8.70	21.28	4.26	0.00	235.50	0.50	3.00	13.00
4	88	32.95	0.00	0.00	3.45	0.00	14.29	28.57	10.70	6.90	350.00	0.00	1.00	12.00
6	61	54.10	6.67	0.00	0.00	0.00	17.24	20.69	0.00	3.23	240.00	0.00	2.00	10.50
7	68	20.59	0.00	7.14	7.14	0.00	0.00	7.69	8.30	0.00	350.00	0.00	2.00	9.50
8	243	23.87	0.00	8.89	0.00	0.00	3.77	16.67	9.26	8.62	245.50	8.00	0.00	7.00
9	94	23.40	0.00	9.52	4.55	0.00	33.33	30.00	23.80	0.00	307.00	3.00	2.00	13.00
10	54	16.67	0.00	0.00	0.00	0.00	42.86	44.44	11.11	11.11	451.00	2.00	1.00	13.00
12	122	15.57	5.26	5.26	15.79	0.00	0.00	33.33	5.56	10.53	372.00	1.00	2.00	18.00
13	87	28.74	0.00	0.00	0.00	0.00	4.55	16.67	4.17	4.00	408.00	2.00	4.00	18.00
14	64	59.38	0.00	13.16	0.00	2.63	0.00	13.89	0.00	0.00	375.00	2.00	4.00	16.00
15	112	58.93	0.00	1.52	1.52	1.52	1.64	19.35	4.84	9.09	380.00	2.00	3.00	7.00
16	82	14.63	0.00	0.00	8.33	0.00	0.00	0.00	8.33	9.09	461.00	11. 00	2.00	6.00
Median	342	38.30	2.29	3.05	0.76	2.29	4.07	25.40	1.59	18.32	330.00	3.00	2.00	14.00
75th Percentile (Bench-mark cut-off)	**348**	**43.10**	**2.67**	**5.33**	**2.67**	**3.33**	**16.90**	**17.73**	**3.55**	**21.33**	**372.00**	**1.00**	**3.00**	**9.00**

DCD: Donation after circulatory death; PNF: Primary non-function; HAT: Hepatic artery thrombosis; IC: Ischemic cholangiopathy; RRT: Renal Replacement Therapy; LT: Liver Transplantation; RBC: Red blood cell concentrates; ICU: Intensive care unit;

Supplementary Table 9b: Benchmark value calculation, Era 3 (2011-2016), part 2
Below the detailed calculations for the Benchmarking values during Era 3 are shown.

Centre	CCI at DC (points)	CCI at 3 months (points)	CCI at 6 months (points)	CCI at 12 months (points)
1	30.20	34.85	36.80	37.75
2	26.20	35.95	37.10	37.10
3	0.00	12.20	22.60	33.70
4	20.90	26.20	30.80	33.50
6	0.00	0.00	23.55	23.55
7	0.00	0.00	0.00	0.00
8	0.00	8.70	20.90	20.90
9	21.75	33.50	37.10	37.60
10	29.60	40.50	44.40	48.10
12	29.60	36.20	37.20	44.40
13	20.90	29.60	36.20	41.40
14	0.00	0.00	0.00	0.00
15	0.00	20.90	20.90	20.90
16	20.90	29.60	30.80	33.70
Median	20.90	20.90	20.90	20.90
75th Percentile (Benchmark cut-off)	**20.90**	**26.20**	**32.15**	**33.50**

CCI: Comprehensive Complication Index; DC: Discharge;

Supplementary Table 9c: Benchmark value calculation, Era 3 (2011-2016), part 3
Below the detailed calculations for the Benchmarking values during Era 3 are shown.

Centre	Any Complication (%)				Up to (and including) Grade II complication (%)				Grade III or higher complication (%) (Grade III, IV, V)			
	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months
1	83.33	88.10	88.10	90.47	50.00	42.86	42.86	38.09	50.00	57.14	57.14	61.91
2	75.00	80.00	90.00	90.00	50.00	45.00	30.00	25.00	50.00	55.00	70.00	75.00
3	41.18	52.90	72.54	80.39	76.47	68.62	54.90	45.09	23.53	31.38	45.10	54.91
4	58.62	86.21	93.10	93.10	75.86	55.17	48.28	44.83	24.14	44.83	51.73	55.17
5	18.18	45.45	51.52	51.52	90.91	66.67	57.58	57.58	9.09	33.33	42.43	42.43
6	21.43	28.57	28.57	28.57	85.71	78.57	71.43	71.43	14.29	21.43	28.57	28.57
7	32.76	53.45	56.90	60.35	86.21	75.86	67.24	63.79	13.79	24.14	32.76	36.21
8	63.64	72.73	81.82	90.91	54.55	36.36	27.27	18.18	45.46	63.64	72.73	81.82
9	88.89	88.89	100.00	100.00	66.67	33.33	11.11	11.11	33.33	66.67	88.89	88.89
10	89.47	89.47	94.73	94.73	63.16	63.16	52.63	47.36	36.84	36.84	47.37	52.64
11	92.00	96.00	96.00	96.00	88.00	64.00	52.00	40.00	12.00	36.00	48.00	60.00
12	21.05	36.84	44.73	47.36	78.95	63.16	55.26	52.63	21.05	36.84	44.74	47.37
13	28.79	51.52	57.57	57.57	81.82	68.18	59.09	57.58	18.18	31.82	40.91	42.43
14	66.67	83.33	91.67	91.67	75.00	66.67	58.33	58.33	25.00	33.33	41.67	41.67
15	54.19	60.30	61.83	68.70	72.52	67.93	65.65	57.25	27.48	32.07	34.35	42.75
16	72.00	83.33	88.00	90.67	57.33	47.33	43.33	36.67	42.67	52.67	56.67	63.33
Median	61.13	76.37	84.91	90.24	75.43	63.58	53.77	46.23	24.57	36.42	46.24	53.78
75th Percentile (Bench-mark cut-off)	**77.08**	**86.68**	**92.03**	**92.03**	**82.79**	**67.99**	**58.52**	**57.58**	**38.30**	**53.25**	**56.79**	**62.27**

DC: Discharge;

Supplementary Table 9d: Benchmark value calculation, Era 3 (2011-2016), part 4
Below the detailed calculations for the Benchmarking values during Era 3 are shown.

Centre	Graft Loss (%)				Redo-Liver transplantation (%)				Mortality (%)			
	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months	DC	At 3 months	At 6 months	At 12 months
1	18.18	18.18	27.27	36.36	9.09	9.09	18.18	18.18	18.18	18.18	18.18	27.27
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	3.85	3.85	3.85	3.85	0.00	0.00	0.00	0.00	3.85	3.85	3.85	3.85
4	22.22	22.22	22.22	33.33	0.00	0.00	0.00	0.00	22.22	22.22	22.22	33.33
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	5.71	11.43	12.86	15.71	4.29	5.71	8.57	10.00	4.29	4.29	5.71	10.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	40.00	40.00	40.00	60.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	40.00
10	20.00	20.00	20.00	20.00	0.00	0.00	0.00	0.00	20.00	20.00	20.00	20.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.00	10.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	10.00	10.00	20.00
15	4.35	7.25	7.25	15.94	4.35	4.35	4.35	4.35	0.00	1.45	1.45	8.70
16	7.69	7.69	11.54	11.54	7.69	7.69	7.69	7.69	3.85	3.85	3.85	7.69
Median	4.10	7.47	8.62	10.77	0.00	0.00	0.00	0.00	1.92	3.85	3.85	8.19
75th Percentile (Bench-mark cut-off)	**15.56**	**16.49**	**18.21**	**18.99**	**4.33**	**5.37**	**6.86**	**6.86**	**14.71**	**16.14**	**16.14**	**20.00**

DC: Discharge;

Supplementary Table 10: Donor and recipient Risk in different cumulative risk profiles

Risk Factors	Overall cohort (n=2105)	Bench-mark cases (n=1012) ¶	Total donor WIT > 30 min and asystolic donor WIT > 15 min (n=119) ¶¶	Recipient laboratory MELD > 20 points (n=287)	Retrans-plantation (n=41)	p-value (Bench-mark vs. long donor WIT)	p-value (Benchmark vs. recipient laboratory MELD >20 points)	p-value (Bench-mark vs. retrans-plantation)
Donor age (years)	48 (32.8-57)	48 (34-57)	46 (36-55)	43 (28-54)	39 (21.5-48)	0.125	<0.001	0.001
Donor BMI (kg/m²)	24.96 (22.3-28.09)	24.79 (22.35-28.0)	25 (22-29)	25.1 (21.9-29)	23.8 (21.7-25.9)	0.655	0.404	0.232
Total donor warm ischemia time (min)	25 (20-31)	22 (18-26)	37 (33-41)	22 (18-25)	23 (20-31)	<0.0001	0.484	0.002
Functional donor warm ischemia time (min)*	20 (14-33)	15 (12-20)	21.5 (20-24.5)	16 (12-22)	26 (18-36)	0.001	<0.001	0.006
Asystolic donor warm ischemia time (min)	10 (8-13)	9 (8-11)	18 (17-22)	8 (7-10)	10 (8-13)	<0.0001	<0.001	0.039
Cold ischemia time (hrs)	6.25 (5.2-7.47)	6.13 (5.05-7.42)	7.3 (6.28-8.22)	6 (5.12-6.8)	5.75 (3.95-8.45)	0.001	0.051	0.983
Recipient age (years)	57 (51-63)	57 (51-62)	56.75 (49-63.3)	57 (49-63)	48 (34.5-54.2)	0.279	0.868	<0.0001
Recipient BMI (kg/m²)	26.7 (23.76-30.15)	26.2 (23.6-30)	27.69 (24.41-31.21)	26.8 (23.6-30.3)	24.6 (21.2-30.1)	0.142	0.544	0.282
Recipient laboratory MELD (points)	14.2 (10-19)	13 (9.5-16)	12 (8.47-16)	23 (22-27)	19 (11.5-24.16)	0.002	0.0001	<0.0001
Recipient HCC (%)	40.5%	43.4%	47.9%	28.9%	19.5%	0.3797	0.0001	0.0020

Median and IQR or number/proportion (%); comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables); ¶: Benchmark cohort cases, data shown as median and IQR or number/proportion (%); ¶¶: this group corresponds to the "cold storage" group (high risk cohort) in Tables 3 & 4 and; total donor WIT: donor warm ischemia time from treatment withdrawal to cold flush; *: fdWIT: systolic bloodpressure of < 50 mmHg or saturation of < 70% to cold in situ flush (n=710, provided this information), n=452 in benchmark group, n=135 in laboratory MELD >20 points group, n=10 in prolonged donor warm ischemia time cohort, and n=27 in retransplant cohort); BMI; Body Mass Index, MELD; Model for End-Stage Liver Disease, HCC; Hepatocellular carcinoma.

What are donor-recipient constellations with higher risk

In a next step, high risk donor and recipient combinations were filtered out based on clinically relevant donor and recipient risk factors. Supplementary Table 10 shows the donor and recipient risk comparing the best possible and lowest cumulative donor risk – represented by the benchmark cohort – with higher risk donors (prolonged total and asystolic donor warm ischemia time), recipients with higher laboratory MELD of > 20 points and liver retransplantation. Expectedly, increased donor risk with longer warm ischemia time led to impaired outcomes. Graft loss and liver retransplantation is required more often and creates significant cumulative complications and costs.

How do DCD transplantations perform with different laboratory MELD score?

Non-Benchmark cases were explored according to their laboratory MELD of more than 20 points in different categories (Supplementary Table 11). Furthermore, such MELD groups were compared to the benchmark cohort. Only a few DCD livers were transplanted into recipients with a laboratory MELD of > 20. Only 32 recipients had a MELD of > 30 points at the time of transplantation.

Supplementary Table 11: Risk factor analysis according to different laboratory MELD categories. Accoording to the defined criteria only the laboratory MELD was increased while other donor and recipient risk factors were kept low to explore the individual impact of the laboratory MELD on outcomes. Expectedly most risk factors remained stable. Donor WIT and CIT decreased slightly throughout the laboratory MELD groups.

Risk Factors	Bench-mark cases (n=1012) ¶	MELD >20 – 25 (n=195)	MELD >25 – 30 (n=60)	MELD >30 – 35 (n=22)	MELD >35 (n=10)
Donor age (years)	48 (34-57)	45 (28.7-54)	38 (27-52.8)	43 (28.8-52.3)	40.02 (24-65)
Donor BMI (kg/m²)	24.79 (22.35-28.0)	25 (21.4-29.5)	26.9 (23.8-29.4)	23.1 (21.8-24.3)	26.9 (21-31)
Total donor warm ischemia time (min)	22 (18-26)	22 (18-25)	22.5 (19-25.8)	21.5 (17.8-24)	23 (18-26.3)
Functional donor warm ischemia time (min)	15 (12-20)	13 (10-17)	15 (11-16.5)	19.5 (14.5-20.8)	13.5 (9-18)
Asystolic donor warm ischemia time (min)	9 (8-11)	8 (7-10)	8 (7-10)	8.5 (7-11.3)	7 (4.75-9)
Cold ischemia time (hrs)	6.13 (5.05-7.42)	6.1 (5.25-6.81)	5.7 (4.56-6.7)	5.7 (5.02-6.7)	5.2 (3.7-5.9)
Recipient age (years)	57 (51-62)	57 (51-6)	54.5 (46.3-62)	55.9 (48-62.2)	58.7 (45-63)
Recipient BMI (kg/m²)	26.2 (23.6-30)	26.9 (23.9-30.4)	27.4 (23.8-31.6)	23.1 (22.3-25.9)	26 (24.5-26.7)
Recipient laboratory MELD (points)	13 (9.5-16)	22 (21-23)	28 (26.1-29)	32 (31-33.1)	38.5 (37-40.3)
Recipient HCC (%)	43.4%	33.3%	18.3%	27.3%	20%

Median and IQR or number/proportion (%); MELD; Model for End-Stage Liver Disease, BMI; Body Mass Index, HCC; Hepatocellular carcinoma.

Supplementary Table 12: Outcomes of DCD recipients according to different laboratory MELD categories

Expectedly higher laboratory MELD candidates had prolonged hospital stays and more post-transplant bleedings.

Risk Factors	Benchmark cases (n=1012) ¶	MELD >20 – 25 (n=195)	MELD >25 – 30 (n=60)	MELD >30 – 35 (n=22)	MELD >35 (n=10)
Duration of Transplantation (hrs)	5.3 (4-6.7)	6 (4.9-7)	5.04 (4.16-6)	6.13 (5.67-6.79)	5.58 (3.49-6.04)
No. of RBC transfusions (U)	2 (0-6)	4 (2-8)	6 (2.5-10.5)	4.5 (1.8-7.5)	2 (0-5)
No. of FFP transfusions (U)	4 (0-8)	5 (2-8)	8 (4-11.3)	3 (0-8)	3.5 (0.3-6)
ICU stay (days)	2 (1-4)	2 (1-4)	2.5 (1-6)	4 (2-5.5)	2 (2-9.3)
Hospital stay (days)	12 (8-18)	13 (8-20)	13 (7.5-23.8)	18 (9.4-29)	31.5 (13-46.3)
Peak AST first week (U/L)	1293 (594.5-2737)	1530 (716.8-3106)	2049.5 (1122.8-4999.8)	1391 (821-2196)	2064.5 (1239-6021.3)
Peak ALT first week (U/L)	922 (474.5-1743.5)	810 (406-1450)	991.5 (605.3-1701.5)	634 (290-1599)	1229.5 (789.3-2106.8)
INR day 1	1.6 (1.36-1.9)	1.7 (1.42-2.1)	1.8 (1.66-2.36)	1.61 (1.4-1.8)	2.13 (1.3-2.4)
Peak Creatinine first week (µmol/l)	106.1 (74-154.5)	126.8 (85.1-165.8)	185.64 (112.7-260.8)	190.1 (102.2-272.1)	134 (79-141.44)
Renal replacement therapy (%)	12%	6.15%	21.7%	9.1%	20%
Any complication (12 months) (%)	74.41%	75.9%	73.3%	68.2%	70%
Grade II complications (12 months) (%)	17.6%	19.5%	25%	13.6%	20%
Grade III complications (12 months) (%)	27.27%	32.8%	18.3%	45.5%	40%
Grade IV complications (12 months) (%)	15.4%	9.7%	23.3%	9.1%	10%
Primary non-function (%)	1.89%	1.5%	1.667%	0	10%
Bleeding (%)	5.65%	7.18%	8.3%	13.6%	10%
Hepatic Artery Thrombosis (%)	4.74%	1.54%	3.3%	0	0
Ischemic Cholangiopathy (%)	8.8%	6.82%	8.3%	4.55%	0
Anastomotic Strictures (%)	20.9%	20.67%	21.67%	13.6%	30%
Bile leak (%)	5.3%	7.8%	6.67%	4.55%	0
CCI ® until discharge (points)	8.7 (0-33.5)	20.9 (0-33.7)	20.9 (0-33.7)	20.9 (0-33.93)	10.45 (0-28.1)

Risk Factors	Benchmark cases (n=1012) ¶	MELD >20 – 25 (n=195)	MELD >25 – 30 (n=60)	MELD >30 – 35 (n=22)	MELD >35 (n=10)
CCI ® 3 months (points)	20.9 (0-39.5)	25.2 (0-40.3)	22.6 (0-42.4)	25.9 (0-40.73)	14.8 (0-33.38)
CCI ® 6 months (points)	26.2 (0-42)	26.2 (0-47.1)	29.6 (0-43.5)	33.7 (0-44.1)	23.6 (0-36.5)
CCI® 12 months (points)	29.6 (0-46.2)	33.7 (8.7-49.35)	29.6 (0-46.1)	39.5 (0-46.35)	27.9 (0-38.1)
Graft loss (12 month, %)	12.7%	11.79%	6.67%	4.55%	0
Retransplantation (12 months, %)	4.5%	2.05%	3.3%	0	0
Retransplantation overall (%)	8.93%	6.2%	8.3%	0	0
In Hospital Mortality (%)	3.26%	3.08%	1.667%	0	0
One-Year mortality (%)	8.39%	8.7%	1.667%	0	0
Follow up (graft survival, days)	1386 (646.5-2277.8)	1444 (766-2293.5)	1570.5 (645-2444.5)	1303.5 (568.5-1866.5)	1970 (1548.8-2503)
Follow up (patient survival, days)	1520 (822.75-2354.3)	1522 (842.5-2343)	1705 (950.8-2491.8)	1303.5 (568.5-1866.5)	1970 (1548.8-2503)

Values presented as median and IQR for continuous parameter and % for binary parameter; laboratory MELD > 35 points includes 2 DCD cases with a lab MELD of > 40 points, which were analysed together with the next lower group (MELD > 35 points), due to the otherwise too low case load in a separate group; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables), complication in 12 months = highest in 1 year of follow up; Based on the low numbers in high MELD groups statistical comparisons were not done. MELD; Model for End-Stage Liver Disease, RBC; Red Bloodcell Concentrate, FFP: Fresh Frozen Plasma, ICU ; Intensive Care Unit, AST; Aspartate Transaminase , ALT: Alanine Transaminase, INR; International Normalised Ratio, CCI; Comprehensive Complication Index.

Is the use of new machine perfusion concepts protective from complications in high-risk DCD donor cohorts?

Recipients of DCD livers with prolonged donor warm ischemia time performed significantly better when the graft was treated with dynamic preservation approaches. Particularly prolonged donor warm ischemia times increased the risk for IC and graft loss, which was reverted by all machine perfusion techniques. Importantly, a good proportion of IC did not lead to graft loss within the first year after liver transplantation. Similar findings were seen for HAT. In Supplementary Table 13 the detailed causes of graft loss during the first year of follow up are presented.

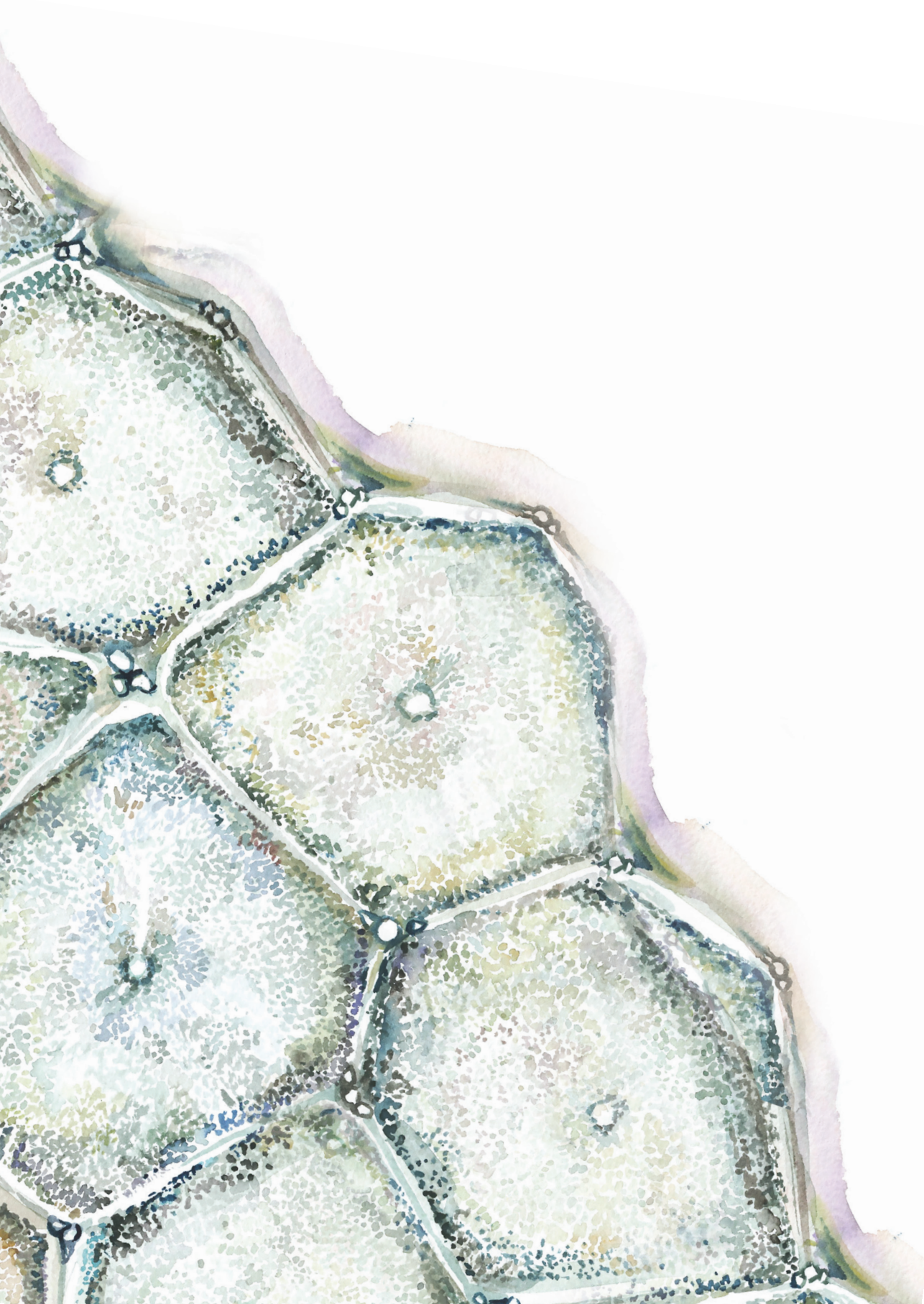
Supplementary Table 13: Graft loss comparing different risk groups and preservation techniques within last 5 years

IC was evident in more than 20% of recipients of DCD livers with a prolonged total and asystolic donor WIT with a graft loss of 10%. In contrast, either hypothermic machine perfusion alone or in combination with NRP were both protective from this DCD specific complication. No graft was lost in such cohorts despite the high donor risk.

Outcome parameter	Low Risk: Total donor WIT ≤ 30min & asystolic donor WIT ≤15 min		High Risk: Total donor WIT > 30 min & asystolic donor WIT > 15 min			p-value (Cold storage vs. cold storage + HOPE)	p-value (Cold Storage vs. NRP + cold storage + HOPE)
	Benchmark cases (n=1012) ¶	NRP + cold storage (n=49)	Cold storage (n=87)	Cold storage + HOPE (n=49)	NRP + cold storage + HOPE (n=63)		
Follow up (graft survival, days)	1386 (646.5-2277.8)	729 (429-1323)	892 (282-1491)	1160 (461-1922)	529 (281-785)	0.081	<0.001
Follow up (patient survival, days)	1520 (822.75-2354.3)	738 (453-1409)	1106 (531-1617)	1225 (526-1967)	541 (346-858)	0.242	<0.001
Ischemic Cholangiopathy (%)	8.8%	0%	22.06%	4.1% §	3.2% #	0.0071	0.0014
Graft loss (in hospital) (%)	5.8%	6.1%	11.49%	6.1%	7.9%	0.3761	0.5863
Graft loss (12 months) (%)	12.7%	10.2%	24.13%	12.2%	11.1%	0.1188	0.0558
Cause of graft loss (12 months)							
Primary non- function (%)	1.8%	0%	3.45%	2%	3.2%	1.0	1.0
Bleeding (%)	0.5%	2%	0 (0)	0 (0)	0%	1.0	1.0
Ischemic Cholangiopathy (%)	2.0%	0%	10.34%	0 (0) §	0% #	0.0262	0.0105
Hepatic Artery Thrombosis (%)	2.6%	2%	3.41 %	4.1%	6.3% *	1.0	0.4541
Sepsis (%)	1.7%	2%	1.15%	4.1%	1.6%	0.2944	1.0

Outcome parameter	Low Risk: Total donor WIT ≤ 30min & asystolic donor WIT ≤15 min		High Risk: Total donor WIT > 30 min & asystolic donor WIT > 15 min			p-value (Cold storage vs. cold storage + HOPE)	p-value (Cold Storage vs. NRP + cold storage + HOPE)
	Benchmark cases (n=1012) ¶	NRP + cold storage (n=49)	Cold storage (n=87)	Cold storage + HOPE (n=49)	NRP + cold storage + HOPE (n=63)		
Recipient death, cardiac (%)	0.6%	2%	2.29%	0%	0%	0.5356	0.5095
Recipient death, other cause (%)	0.5%	2%	1.15%	0%	0%	1.0	1.0
Cancer recurrence and new cancer (%)	0.8%	0%	1.15%	0%	0%	1.0	1.0
Recurrence underlying disease (%)	0.4%	0%	0%	0%	0%	1.0	1.0
Unknown and other	1.09%	0%	1.15%	2%	0%	1.0	1.0

Values presented as median and IQR for continuous parameter and number/proportion (%) for binary variables; ¶: Benchmark cohort cases; comparisons made with Mann-Whitney-U test (continuous variables) or Fisher exact test (binary variables); ::: IC lead to graft loss in 2 recipients with PSC as underlying disease on day 392 and 109 after LT in the cold storage high-risk group; §: 2x IC: treated with ERCP, no relisting or redo liver transplantation; #: 2x IC, treated with ERCP and PTC, no relisting or liver retransplantation *: 4/63 recipients underwent redo liver transplantation for HAT, 2x real HAT, 1x due to hepatic artery damage during =hepaticojejunostomy, 1x damage during embolization of a mycotic aneurysm; NRP: Normothermic regional perfusion; HOPE: Hypothermic Oxygenated Perfusion; DCD: Donation after circulatory death; SP: cohort from Spain; WIT: Warm ischemia time; IQR: Interquartile range; MELD: Model of end stage liver disease; RBC: Red blood cell; IC: Ischemic cholangiopathy; ICU: Intensive care unit; CCI: Comprehensive Complication Index; HAT: Hepatic artery thrombosis; Recipient death other ca



CHAPTER 5

Donor hepatectomy time influences ischemia-reperfusion injury of the biliary tree in donation after circulatory death liver transplantation

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Abstract

Background: Donor hepatectomy time is associated with graft survival after liver transplantation. The aim of this study was to identify the impact of donor hepatectomy time on biliary injury during donation after circulatory death liver transplantation.

Methods: First, bile duct biopsies of livers included in (pre)clinical machine perfusion research were analyzed. Secondly, of the same livers, bile samples were collected during normothermic machine perfusion. Lastly, a nationwide retrospective cohort study was performed including 273 adult patients undergoing donation after circulatory death liver transplantation between January 1, 2002 and January 1, 2017. Primary endpoint was development of non-anastomotic biliary strictures within 2 years of donation after circulatory death liver transplantation. Cox proportional-hazards regression analyses were used to assess the influence of hepatectomy time on the development of non-anastomotic biliary strictures.

Results: Livers with severe histological bile duct injury had a higher median hepatectomy time (p-value 0.03). During normothermic machine perfusion, livers with a hepatectomy time >50 minutes had lower biliary bicarbonate and bile pH levels. In the nationwide retrospective study, donor hepatectomy time was an independent risk factor for non-anastomotic biliary strictures after donation after circulatory death liver transplantation (Hazard Ratio 1.18 per 10 minutes increase, 95% Confidence Interval 1.06–1.30, p-value = .002).

Conclusions: Donor hepatectomy time negatively influences histological bile duct injury before normothermic machine perfusion and bile composition during normothermic machine perfusion. Additionally, hepatectomy time is a significant independent risk factor for the development of non-anastomotic biliary strictures after donation after circulatory death liver transplantation.

Introduction

The imbalance between the number of patients on the waiting list for liver transplantation (LT) and the number of available grafts from donation after brain death (DBD) donors has resulted in an increased use of livers from donation after circulatory death (DCD) donors. In 2018, 38% of all deceased donor LT in the Netherlands were performed with a DCD graft. (1)

LT from DCD donors can lead to inferior outcomes compared with LT with DBD grafts, especially with respect to graft survival (2-5), which is related to a higher chance of developing early allograft dysfunction and post-transplant cholangiopathy. (6-10) Among post-transplant cholangiopathies, non-anastomotic strictures (NAS), also known as ischemic type biliary lesions or ischemic cholangiopathy, is the most hazardous type, with a strong negative impact on graft survival. (11-13)

An important determinant of outcome after LT is ischemia reperfusion injury. Ischemia reperfusion injury occurs in both DBD and DCD-LT. However, DCD grafts suffer from an additional period of warm ischemia in the donor between withdrawal of life support and initiation of cold flush out, the so-called donor warm ischemia time (dWIT). Several studies have indicated that the length of the dWIT is a critical risk factor for negative outcome after DCD-LT. (2, 14, 15)

Unfortunately, the start of in situ cold flush out and cooling does not lead to adequate protection against ischemic injury, because the core temperature of the liver generally does not drop below 15 to 20°C during surgery. (16) At this temperature, organs are still metabolically active, resulting in rapid depletion of adenosine triphosphate and accumulation of metabolites during anaerobic metabolism. Liver core temperature first reaches a relatively safe range (<4°C) when organs are stored in a bag with cold preservation solution in a box with ice. Therefore, it is hypothesized that, apart from the dWIT, the duration of the hepatectomy time provides an additional risk factor for ischemic injury and could therefore impact outcome after LT.

A recent study published by Jochmans et al. and based on data from the Eurotransplant Registry supported this hypothesis. (17) In this study, donor hepatectomy time was an independent risk factor for patient mortality and graft loss. Moreover, DCD grafts appeared to be more susceptible to donor hepatectomy time than DBD grafts. More recently, Farid et al. assessed the influence of the donor hepatectomy time on the outcomes of DCD-LT in the United Kingdom, concluding that a hepatectomy time of more than 60 minutes was associated with a higher risk of primary non-function and graft failure. (18) Neither study, however, assessed the effect of donor hepatectomy time on the development of

post-transplant cholangiopathy after DCD-LT, neither did they evaluate whether hepatectomy time was different among procurement teams.

Several studies have shown a strong relation between bile duct injury (BDI) before implantation and the development of NAS after transplantation. (19, 20) If donor hepatectomy time influences the rates of NAS, this would be displayed in the severity of biliary injury before implantation. Additionally, bile composition during normothermic machine perfusion (NMP) of liver grafts can be studied to assess bile duct injury. (21, 22)

The aim of this study was to assess the impact of donor hepatectomy time in DCD donors on the development of biliary injury during DCD-LT. First, bile duct biopsies taken upon arrival in one of the 3 recipient centers were analyzed. Secondly, bile composition during NMP was studied. Last, the influence of hepatectomy time on the development of NAS after DCD-LT was studied in a nationwide retrospective database study.

Methods

Donation procedure and organ procurement

Until recently, all donor procedures/procurements in the Netherlands were performed by one of 5 regional procurement teams, each covering a certain region of the country. Each procurement team consists of a surgeon, surgical assistant, anesthesiologist, and 2 operation room assistants. In the Netherlands, withdrawal of life support in a patient eligible for DCD organ donation generally takes place at the intensive care unit. Premortem cannulation of the patient is not performed, and systemic heparinization is prohibited by Dutch law. When circulatory arrest has been determined, there is a mandatory 5 minutes “no-touch” period. After this “no-touch” period, the donor is transported to the operating theatre. A super-rapid sterno-laparotomy with pressurized, aortic-only perfusion is used as the standard procurement technique. Cold perfusion is currently executed with Belzer UW Cold Storage Solution (Bridge to Life, London, UK). Whether the liver is retrieved separately or en bloc with the pancreas is based on the preferences of the surgeon. On the back table, the liver is flushed via the portal vein with at least 500 ml cold preservation solution until clear perfusate is established. The common bile duct (CBD) and intrahepatic biliary tree are flushed with low pressure Belzer UW Cold Storage Solution. (23) As there are no clear Dutch guidelines on the sequence of organ procurement, the lungs in a DCD donor are usually procured before the abdominal organs. The implantation is usually executed with a caval sideclamp and veno-venous anastomosis, end-to-end arterial and portal anastomosis, and duct-to-duct biliary anastomosis. The standard reperfusion technique used is initial portal vein reperfusion.

Study design

This study consists of 3 parts. First, bile duct biopsies and bile composition of DCD livers were analyzed for a potential influence of hepatectomy time (parts A–B). Hereafter, to validate the findings, a nationwide retrospective database analysis was performed (part C).

Part A: Histological analysis of bile ducts

Of all DCD livers that underwent preclinical and clinical NMP in the University Medical Center Groningen between January 1, 2013 and January 1, 2019, bile duct biopsies before machine perfusion were collected. The only criterion required for inclusion was that the donor hepatectomy time was available. Biopsies were taken from the distal CBD before machine perfusion, fixed in 4% formalin, and subsequently embedded in paraffin. Slices of 4 μm were cut and stained with hematoxylin and eosin and subsequently examined using light microscopy. The BDI score was determined in a blinded fashion by 2 researchers, using a clinically relevant histological grading system. (20, 22) The BDI consisted of the combined scores for deep peribiliary gland injury, peribiliary vascular plexus injury, and stroma necrosis. The cutoff value used between low and high BDI was 4.75, as described previously. (22) Comparisons between groups were performed with the $\chi 2$ test or Fisher exact test where appropriate. Receiver operating characteristic curves were used to identify the most appropriate cutoff values.

Part B: Normothermic machine perfusion

All preclinical and clinical NMP procedures were performed with the Liver Assist device (Organ Assist, Groningen, the Netherlands). Protocols and outcomes are reported elsewhere. (22, 24, 25) To monitor biliary tree viability, bile was collected from an 8Fr biliary drain in the CBD. During NMP, bile samples were collected every 30 minutes under mineral oil to determine biliary pH, bicarbonate, and glucose, as these parameters are biomarkers of bile duct viability. (22) Bile composition was compared between the groups at different time points using the Mann-Whitney U test.

Part C: Retrospective nationwide study

In this nationwide retrospective cohort study, all adult LT performed with a DCD graft in the Netherlands between January 1, 2002 and January 1, 2017 were included. Exclusion criteria were multiorgan DCD transplantations, DCD retransplantations, transplantations involving machine perfusion, and procurement of DCD grafts by a foreign procurement team. Additionally, cases with missing information on hepatectomy times or donor agonal phase were excluded. Donor characteristics and information on the procurement procedure and the regional procurement

team were obtained via the Donor Data Application of Eurotransplant. Data of recipients and transplantation outcomes were obtained from the databases of the participating centers and were completed with data from the patients' electronic medical records.

Donor hepatectomy time was defined as the period between the start of cold flush in the donor and the storage of the liver in a bowl with cold preservation fluid and melting ice on the back-table. The dWIT was calculated as the time between withdrawal of life support and cold flush in the donor. Since in the normal situation the donor hepatectomy time is part of the cold ischemia time, the definition of the cold ischemia time has been altered to minimize the chance of confounding; cold ischemia time was defined as the period between the end of the donor hepatectomy and the removal of the liver from ice before implantation. Finally, recipient warm ischemia time was defined as the time between removal of the liver from ice until either portal or arterial reperfusion, whichever came first.

The endpoint of the retrospective study was the development of NAS within 2 years after transplantation. NAS was defined as donor bile duct strictures at any location but the anastomosis, in absence of hepatic artery thrombosis. To meet the endpoint, patients were required to have clinical symptoms of cholestasis (e.g., jaundice, itch, elevated total bilirubin) with subsequent imaging demonstrating bile duct strictures. If NAS developed after 2 years, it was considered to be related to recipient factors rather than donor factors. Univariable and multivariable Cox proportional-hazards regression models were used to evaluate independent risk factors for NAS. In both models, subjects that did not develop NAS within 2 years were censored at 2 years post transplantation. Patients who died or underwent retransplantation within the first 2 years after transplantation were censored at their date of death or date of retransplantation, respectively. Variables were included in the multivariable, backward stepwise, Cox model if univariable 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 donor hepatectomy time refer to an increase of 10 minutes in hepatectomy time. For the cold ischemia time and recipient warm ischemia time, the HR represent an increase of 1 hour and 1 minute, respectively.

In all 3 projects incorporated in this study, continuous variables were presented as median with both total range and interquartile range (IQR), whereas categorical variables were presented as number (percentage). All tests had a 2-sided design with a p-value below 0.05 considered significant. The analyses were performed using SPSS version 24 (IBM Corporation, Chicago, IL). This study was approved by the Medical Ethical Committee of the University Medical Center Groningen (METC.2017/504).

Results

Part A: Histological analysis of the bile duct

Of 40 consecutive NMP procedures between 2013 and 2019, 39 bile duct biopsies were collected. After exclusion of biopsies with an unknown donor hepatectomy time, 27 biopsies were included in the analysis. Livers with a high BDI score had a significantly longer median donor hepatectomy time compared with grafts with low BDI score (56 vs. 44 minutes, p-value = 0.03) (Figure 1A). Receiver operating characteristic-curve analysis showed a donor hepatectomy time of 50 minutes as the most suitable cutoff point. Of livers with hepatectomy time ≤50 minutes, 17% displayed high BDI vs. 64% in livers with a hepatectomy time >50 minutes (p-value = 0.01) (Figure 1B).

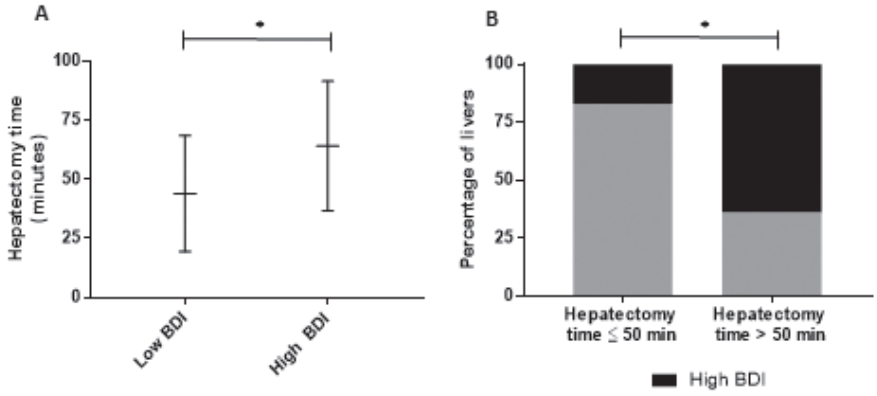

Figure 1: Hepatectomy time influences biliary injury before transplantation. (A) Liver grafts with a high BDI score had a longer median hepatectomy time compared with livers with a low BDI score (p-value = 0.027).
Data presented as median (IQR). (B) Livers grafts with a donor hepatectomy time >50 minutes have more severe BDI compared with livers with a hepatectomy time ≤50 minutes (p-value = 0.016). ∗Depicts a significant (p-value < 0.05) difference.

Part B: Normothermic machine perfusion

Of the 27 livers, livers with a hepatectomy time ≤ 50 minutes had more alkalotic bile during the first 4 hours of NMP. Subsequently, biliary bicarbonate levels were higher in livers with a hepatectomy time below 50 minutes. (Figure 2A & 2B). Glucose reabsorption, displayed by the glucose ratio between bile and perfusate, did not seem to be significantly influenced by hepatectomy time (Figure 2C).

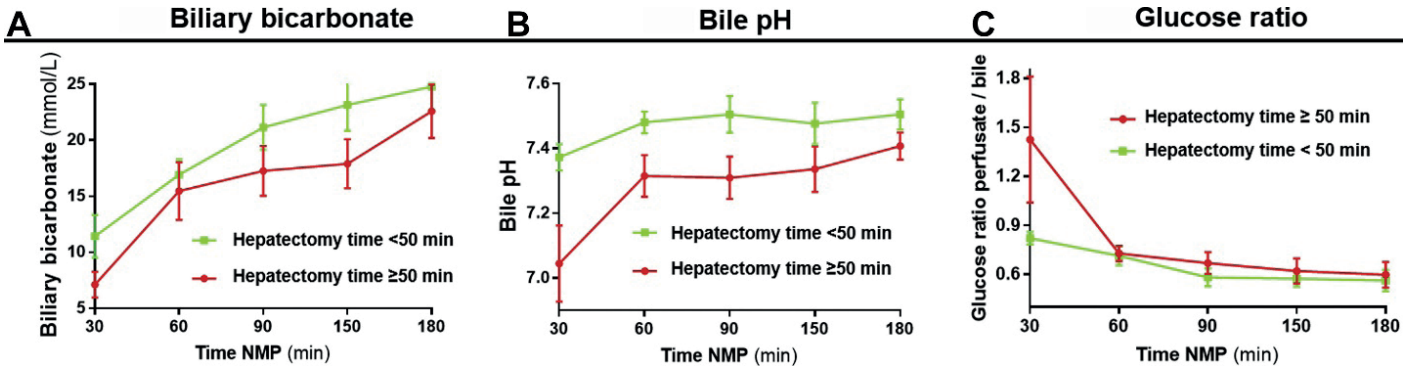

Figure 2: Hepatectomy time influences bile composition during NMP

(A) Hepatectomy time influenced biliary bicarbonate levels during NMP. (B) Bile pH was significantly lower in the group with hepatectomy time ≤50 minutes. (C) Hepatectomy time did not seem to significantly influence biliary glucose reabsorption.

Part C: Retrospective nationwide study

A total of 376 DCD-LTs were performed in the Netherlands between January 1, 2002 and January 1, 2017. One hundred and three cases met 1 or more of the exclusion criteria, resulting in a total of 273 included in this study (Figure 3). The median follow-up period of the complete cohort was 4.36 years (IQR 2.81–7.08, range 0–16.8 years).

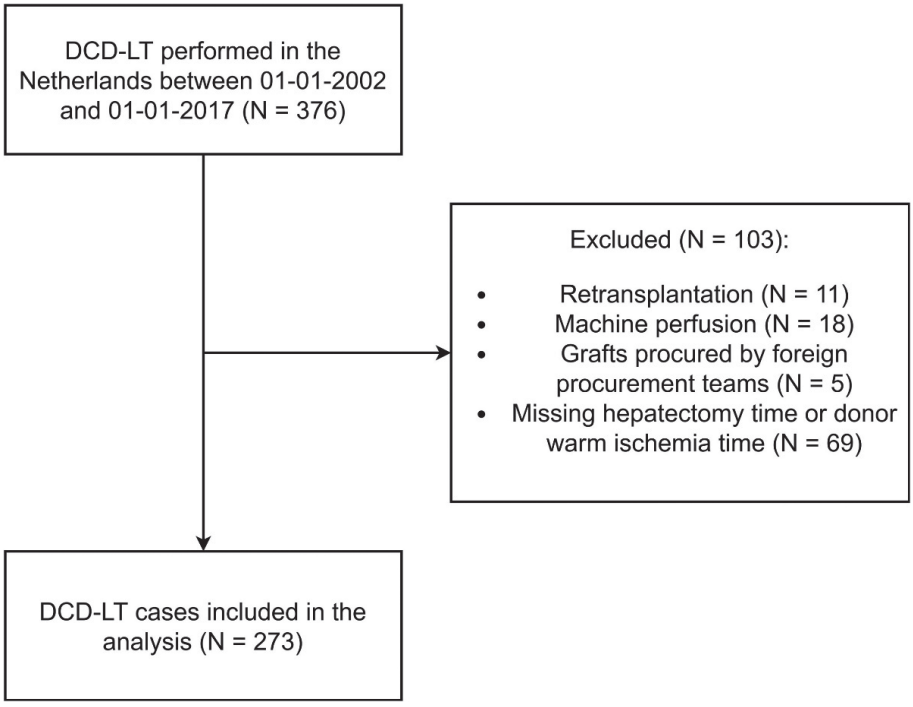

Figure 3: Flow chart of included subjects in the retrospective database study

Baseline characteristics are presented in Table 1. Median donor hepatectomy time for the entire cohort was 63 minutes (IQR 52.5–80.5, range 23–140 minutes). Lung procurement led to a significantly longer donor hepatectomy time of 69 minutes (IQR 59–80 minutes), when compared with a hepatectomy time of 61 minutes (IQR 49–81 minutes) in donors in which lungs were not procured (p-value = 0.02). The outcomes after DCD-LT in the complete cohort are shown in Table 1. Actuarial 1-, 3-, and 5-year graft survival rates were 75%, 64%, and 60%, and 87%, 79%, and 74% for patient survival, respectively.

Table 1: Donor and recipient demographics

Characteristic	Result (n=273)
Donor	
Age (years)	47.0 (35.5-54.0) Range 12-74
Gender	
Male	155 (56.8%)
Female	118 (43.2%)
Body mass index (kg/m²)	24.0 (22.0-26.0) Range 13-34
CVA as cause of death	
No	151 (55.3%)
Yes	122 (44.7%)
ALT peak (U/L)	43.0 (23.0-87.0) Range 6-7385
Last γGT (U/L)	34.0 (20.0-65.5) Range 4-747
Procurement	
Donor warm ischemia time (min)*	32.0 (26.0-38.0) Range 15-80
Donor hepatectomy time (min)†	63.0 (52.5-80.5) Range 23-140
Lung procurement	84.0 (30.8%)
Recipient	
Age (years)	57.0 (49.0-63.0) Range 22-70
Gender	
Male	197 (72.2%)
Female	76 (27.8%)
Body mass index (kg/m²)	25.7 (23.4-29.1) Range 17-46
HCC as indication for transplantation	
No	175 (64.1%)
Yes	98 (35.9%)
Laboratory MELD score	14.6 (10.0-21.0) Range 6-44
Transplantation	
Cold ischemia time (min)‡	359 (302-431) Range 131-743
Recipient warm ischemia time (min)§	34.0 (26.0-42.0) Range 17-144

Characteristic	Result (n=273)
Outcomes	
AST peak (u/L)	2115 (1165-4252) Range 129-20280
ALT peak (u/L)	1620 (771-2857) Range 162-10944
Intensive care unit stay (days)	2.0 (1.0-5.0) Range 0-185
Total hospital stay (days)	18.0 (13.0-27.0) Range 0-235
Primary non-function‖	8 (2.90%)
Non-anastomotic strictures	70 (25.6%)
Within two years post-transplant	66 (24.2%)
Hepatic artery thrombosis	14 (5.10%)
Survival	
Actuarial graft survival	
1 year	75%
3 year	64%
5 year	60%
Actuarial patient survival	
1 year	87%
3 year	79%
5 year	74%

Values are presented as median (interquartile range) or number [%]. *The time between withdrawal of life support and cold flush in the donor. †The period of time between the start of cold flush in the donor and the storage of the liver on ice on the back-table. ‡The time between the end of the donor hepatectomy and the removal of the liver from ice prior to implantation. §The time between removal of the liver from ice until either portal or arterial reperfusion. ||Patient death or retransplantation within 7 days of transplantation without clear cause. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVA , Cerebrovascular accident; γGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease.

Sixty-six patients (24.2%) were diagnosed with NAS within 2 years of LT. During the complete follow-up, 25 patients have undergone a retransplantation as a result of this complication. Baseline characteristics, stratified by recipient development of NAS, are provided in supplementary Table 1 . In a univariable Cox proportional-hazards regression model, donor hepatectomy time was an independent risk factor for the development of NAS (HR 1.14, 95% confidence interval [CI] 1.03–1.26, p-value 0.02). After adjusting for all covariates with a p-value below 0.20 in univariable analyses, donor hepatectomy time remained an independent risk factor for developing NAS within the first 2 years post LT (adjusted HR 1.18, 95% CI 1.06–1.30, p-value 0.02, Tables 2 and 3). Besides hepatectomy time, donor age and cold ischemia time were significant risk factors for NAS.

Table 2: Univariable Cox Proportional-Hazards regression model for developing NAS

	Hazard ratio	95% CI	p-value
Donor			
Age (years)	1.03	1.01-1.05	0.004
Gender			
Male	REF		
Female	1.14	0.70-1.86	0.59
Body mass index (kg/m²)	0.99	0.92-1.08	0.88
CVA as cause of death			
No	REF		
Yes	1.83	1.13-2.98	0.02
ALT peak (U/L)	1.00	1.00-1.00	0.09
Last γGT (U/L)	1.00	1.00-1.00	0.41
Procurement			
Donor warm ischemia time (minutes)*	1.03	1.01-1.05	0.01
Donor hepatectomy time (10-minutes)†	1.14	1.03-1.26	0.02
Lung procurement	0.60	0.34-1.06	0.08
Recipient			
Age (years)	0.994	0.97-1.02	0.60
Gender			
Male	REF		
Female	1.22	0.72-2.07	0.45
Body mass index (kg/m²)	1.02	0.97-1.07	0.55
HCC as indication for transplantation			
No	REF		
Yes	0.77	0.46-1.30	0.33
Laboratory MELD score	0.98	0.95-1.02	0.35
Transplantation			
Cold ischemia time (hours)‡	1.17	1.04-1.33	0.01
Recipient warm ischemia time (minutes)§	1.02	1.00-1.04	0.17

Univariable model. *The time between withdrawal of life support and cold flush in the donor. †The period of time between the start of cold flush in the donor and the storage of the liver on ice on the back-table. ‡The time between the end of the donor hepatectomy and the removal of the liver from ice prior to implantation. §The time between removal of the liver from ice until either portal or arterial reperfusion. Abbreviations: ALT, alanine aminotransferase; CVA, cerebrovascular accident; γGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Table 3: Multivariable Cox Proportional-Hazards regression model for NAS

	Hazard ratio	95% CI	p-value
Donor			
Age (years)	1.03	1.01-1.05	0.01
CVA as cause of death	-	-	0.29
No			
Yes			
ALT Peak	1.00	1.00-1.00	0.05
Procurement			
Donor warm ischemia time*	-	-	0.50
Donor hepatectomy time†	1.18	1.06-1.30	0.002
Lung procurement	0.47	0.26-0.84	0.01
Transplantation			
Cold ischemia time‡	1.22	1.08-1.38	0.001
Recipient warm ischemia time§	-	-	0.47

Multivariable model was conducted via backward stepwise approach. A dash (-) indicates that variable was removed from the model. *The time between withdrawal of life support and cold flush in the donor. †The period of time between the start of cold flush in the donor and the storage of the liver on ice on the back-table. ‡The time between the end of the donor hepatectomy and the removal of the liver from ice prior to implantation. §The time between removal of the liver from ice until either portal or arterial reperfusion. Abbreviations: ALT, alanine aminotransferase; γGT, gamma-glutamyltransferase.

Discussion

This is the first study that demonstrates the impact of donor hepatectomy time on the development of biliary injury during and after DCD liver transplantation. Hepatectomy time influences the severity of histological BDI before transplantation. Moreover, prolonged hepatectomy times negatively influences bile composition during NMP. Additionally, the retrospective study indicates that every 10-minute increase in donor hepatectomy time leads to an 18% increase in the risk of developing NAS.

Op den Dries et al. have shown that bile duct histology is highly predictive of NAS after liver transplantation. (20) In the current study, it is observed that prolonged hepatectomy times leads to an increased BDI score, depicting increased rates of deep peribiliary gland injury, peribiliary vascular plexus injury, and stroma necrosis. The results from this histology study demonstrate that the impact of hepatectomy time is already visible before graft reperfusion. In addition to histology, NMP can be used to assess biliary function. (21, 22) Similar results were observed during NMP; livers with prolonged hepatectomy time produced bile of inferior quality.

The results of the current study are roughly in line with those reported in the Eurotransplant registry study by Jochmans et al. and the United Kingdom-based study from Farid et al.: a prolonged donor hepatectomy time impairs the outcome of DCD-LT. However, neither of the studies were able to assess the influence of donor hepatectomy time on the development of biliary complications. Surprisingly, the median donor hepatectomy time in the Dutch cohort in the current study was substantially longer than that of the DCD-LT subgroup in the study of Jochmans et al. (63 vs. 50 minutes). (17) As within the Eurotransplant region, only the Netherlands, Belgium, and Austria perform DCD organ procurements; this implies that the donor hepatectomy time in the Netherlands is substantially longer compared with the other 2 countries. Moreover, the median hepatectomy time in our cohort was also considerably longer than in the United Kingdom as reported by Farid et al. (63 vs. 35 minutes). (18) As a result of this finding, the Dutch Committee on Independent Procurement Teams implemented several strategies to lower the hepatectomy time, such as raising awareness on the impact of the donor hepatectomy time and endorsing knowledge and skill exchange between the teams. Since 2018, this has resulted in a substantial decrease of the donor hepatectomy time in the Netherlands (mean of 42 minutes with a standard deviation of 12 minutes) without an increase in liver injuries, highlighting the importance of training in organ procurement and regular evaluation. (26)

The graft survival rates reported by Farid et al. are substantially higher than those in our cohort (1-, 3-, and 5-year graft survival of 86.5%, 80.9%, and 77.7% in the United Kingdom vs. 75%, 64%, and 60% in the Dutch cohort, Table 1). Since the patient survival rates have not been reported by Farid et al., it is not possible to evaluate whether the higher rate of graft loss in the Netherlands is the result of more patient deaths or of more retransplantations. However, it could possibly be explained by the difference in hepatectomy time between the 2 cohorts. Nevertheless, it would be valuable to thoroughly investigate this substantial difference in graft survival rates.

Surprisingly, procurement of the lungs seemed to have a protective effect on the development of NAS, despite the fact that lung procurement leads to a prolonged hepatectomy time. This finding is probably the result of the strict acceptance criteria for DCD lung donation handled by thoracic surgeons and lung physicians. Only lungs from optimal DCD donors are accepted, otherwise the lungs are not procured. Lung procurement is in that case a proxy for a more optimal donor.

Jochmans et al. stated that portal perfusion, next to standard in situ aortic cold flush, can accelerate liver cooling and might prevent the detrimental effect of prolonged hepatectomy time. (17) In a recent published study, Hameed et al. concluded that

in high-risk DBD donors, dual perfusion is superior. (27) Furthermore, Ghinolfi et al. concluded recently that dual perfusion has a protective effect on the development of ischemic type biliary lesions after LT with grafts from octogenarian donors. (28) However, a randomized controlled trial comparing aortic flush only and combined aortic and portal flush in DBD-LT, showed no difference in the incidence of post-transplant cholangiopathy. (29) Since DCD grafts could also be considered as high-risk grafts, it would be justifiable to evaluate the effect of dual perfusion versus aortic only perfusion in the DCD-LT population. Another method to potentially minimize the detrimental effect of both dWIT and hepatectomy time on the outcomes after LT is the use of normothermic regional perfusion. A recently published study by Hessheimer et al. showed that with the use of normothermic regional perfusion the rates of biliary complications and graft loss could be reduced substantially when compared with a super-rapid recovery. (30)

Recently, Kalisvaart et al. showed the importance of the agonal phase of the DCD donor and its influence on the outcomes after transplantation, considering an arterial oxygen saturation level below 80% as starting point for the functional donor warm ischemia time. (31) Unfortunately, in our cohort, data on blood pressures and saturation during the agonal phase were unavailable or improperly recorded. Therefore, we were forced to use another definition of the donor warm ischemia time. Since the agonal phase has proven to be of importance, we chose to use the period between withdrawal of life support and the initiation of cold flushing as the dWIT in this study rather than the time between cardiac arrest and cold perfusion. Additionally, as shown before, this study also underlines the importance of a short cold ischemia time for DCD grafts. (22, 32) Every hour of cold ischemia was associated with a 22% increased risk of NAS. Finally, donor age is once again shown to be an important risk factor for biliary complications.

An important strength of this study is the fact that histological analyses are combined with a study of bile composition during NMP and a nationwide retrospective database study. Another strength is that donor hepatectomy time is incorporated as a continuous variable into the multivariable model rather than as a dichotomous variable set around a certain cutoff for donor hepatectomy time. This latter would have led to a loss of valuable information. Another strong aspect of this study is the follow-up of all patients with detailed information on the development of biliary complications. One limitation of the database study is the retrospective design and relatively small cohorts. In addition, as part C was used to validate the findings in parts A and B, these cohorts consist of different patients. Moreover, in a substantial number of cases, hepatectomy time and/or dWIT was missing, leading to a high exclusion rate. Since we could not guarantee these variables to be missing at random, imputation of these variables was not desirable. We do not suspect that our results were confounded by this; however, bias cannot entirely be excluded.

In conclusion, donor hepatectomy time strongly influences biliary injury during and after DCD-LT. The donor hepatectomy time should be kept as short as possible, especially in the presence of other risk factors such as an older donor or prolonged cold ischemia time.

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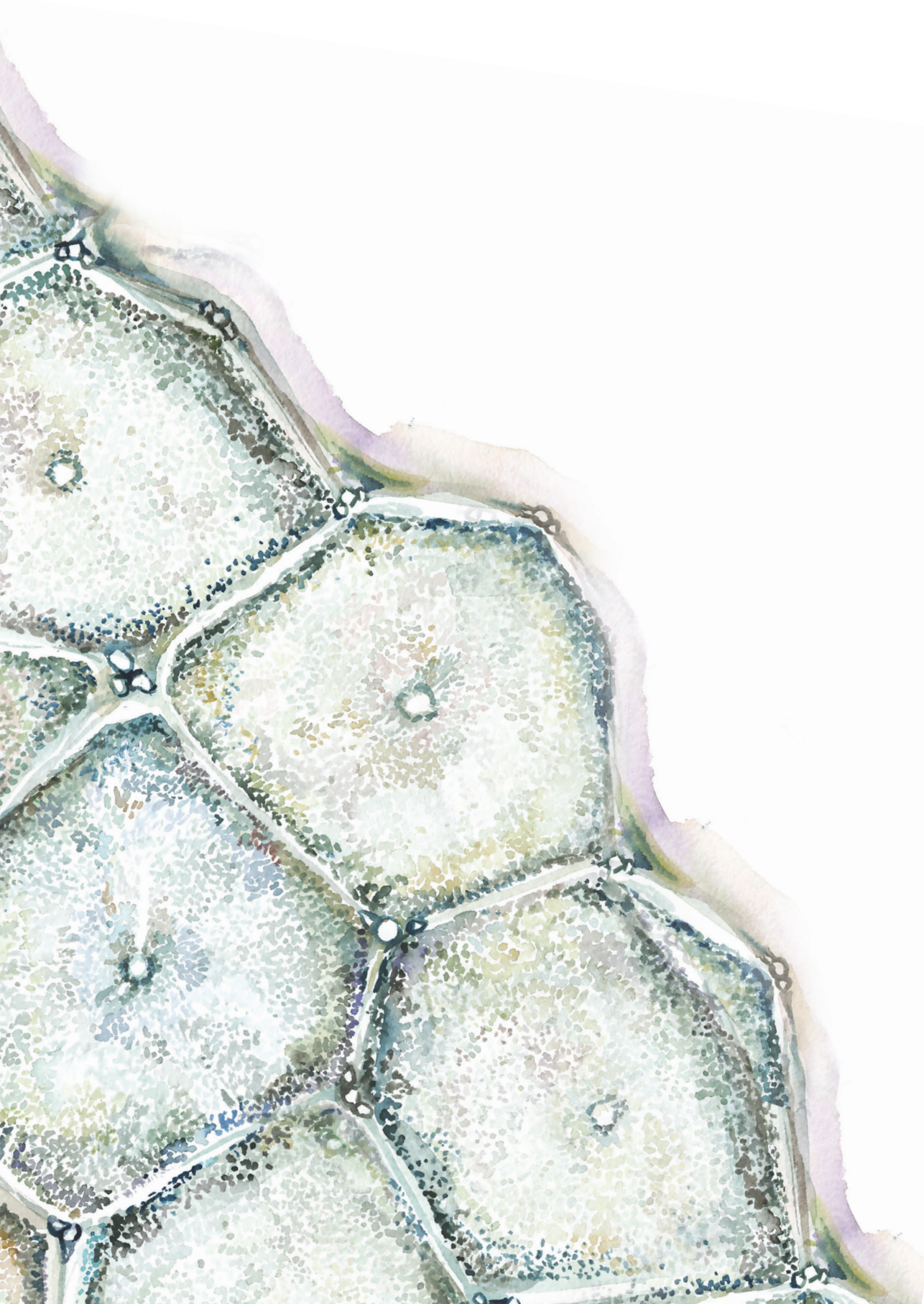
Appendix A: Supplementary Methods and Results

Supplementary Table 1: Donor and recipient demographics stratified by recipient NAS status

Characteristic	Patients with NAS (n = 66)	Patients without NAS (n = 207)	p-value
Donor			
Age (years)	50.0 (43.8-55.0)	46.0 (30.0-54.0)	0.02
Gender			0.67
Male	36 (54.5%)	119 (57.5%)	
Female	30 (45.5%)	88 (42.5%)	
Body mass index (kg/m²)	24.0 (22.0-26.0)	24.0 (22.0-26.0)	0.78
CVA as cause of death			0.03
No	29 (43.9%)	122 (58.9%)	
Yes	37 (56.1%)	85 (41.1%)	
ALT peak (U/L)	32.5 (22.8-56.3)	50.0 (24.0-101.0)	0.01
Last γGT (U/L)	27.0 (17.5-65.0)	35.0 (21.8-66.0)	0.18
Procurement			
Donor warm ischemia time (min)*	33.0 (29.0-39.3)	31.0 (25.0-38.0)	0.03
Donor hepatectomy time (min)†	69.5(56.0-88.3)	62.0 (52.0-77.0)	0.04
Lung procurement	15 (22.7%)	69 (33.3%)	0.10
Recipient			
Age (years)	54.7 (48.8-62.9)	57.0 (49.0-63.0)	0.59
Gender			
Male	46 (69.7%)	151 (72.9%)	0.61
Female	20 (30.3%)	56 (27.1%)	
Body mass index (kg/m²)	24.7 (23.4-29.5)	25.8 (23.5-29.0)	0.72
HCC as indication for transplantation			0.43
No	45 (68.2%)	130 (62.8%)	
Yes	21 (31.8%)	77 (37.2%)	
Laboratory MELD score	14.0 (9.0-20.5)	15.0 (10.0-21.2)	0.21
Transplantation			
Cold ischemia time (min)‡	384 (330-466)	352 (297-426)	0.004
Recipient warm ischemia time (min)§	36.0 (28.0-44.3)	33.0 (26.0-41.0)	0.10

Characteristic	Patients with NAS (n = 66)	Patients without NAS (n = 207)	p-value
Outcomes			
AST peak (u/L)	2231 (1418-4465)	2034 (1060-4291)	0.14
ALT peak (u/L)	1905 (1021-3042)	1489 (688-2586)	0.05
Intensive care unit stay (days)	2.0 (1.0-5.0)	2.0 (1.0-4.0)	0.75
Total hospital stay (days)	19.5 (13.0-32.3)	17.0 (13.0-26.0)	0.32
Primary non-function‖	0	8 (3.9%)	0.21
Hepatic artery thrombosis	2 (3.0%)	12 (5.8%)	0.53

Values are presented as median (interquartile range) or number [%]. *The time between withdrawal of life support and cold flush in the donor. †The period of time between the start of cold flush in the donor and the storage of the liver on ice on the back-table. ‡The time between the end of the donor hepatectomy and the removal of the liver from ice prior to implantation. §The time between removal of the liver from ice until either portal or arterial reperfusion. ‖Patient death or retransplantation within 7 days of transplantation without clear cause. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVA, Cerebrovascular accident; γGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease;



CHAPTER 6

The effect of arterialization time on outcomes of donation after circulatory death liver transplantation

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In preparation

Abstract

Background: Liver transplantation (LT) with grafts obtained after circulatory death (DCD) is associated with more early allograft dysfunction (EAD) than LT with grafts donated after brain death and are more prone to developing biliary complications. Since the biliary tree relies solely on arterial blood supply, it is hypothesized that initial arterial reperfusion can lead to fewer biliary complications. However, in the vast majority of LT, an initial portal vein reperfusion (IPR) approach is used. The aim of this study was to assess the influence of the additional time between portal and arterial reperfusion on outcomes after DCD-LT.

Methods: Data of all controlled DCD-LT with IPR performed in the Netherlands between 2001 and 1st of June 2018 were included. Primary endpoints were the incidence of EAD and non-anastomotic strictures (NAS) post-transplant. The influence of arterialization time on these endpoints was assessed with logistic regression and Cox Proportional-Hazards regression analyses.

Results: A total of 292 DCD-LT were included. Median arterialization time was 33 minutes (interquartile range 25-49). A prolonged arterialization time was not a significant risk factor for EAD or NAS. Both donor and recipient warm ischemia time were significant risk factors for EAD.

Conclusions: In DCD-LT, the time elapsed between portal and arterial reperfusion in DCD-LT is not a significant risk factor for developing EAD or NAS. A randomized controlled trial evaluating different reperfusion sequences would be highly beneficial.

Introduction

The ongoing critical donor organ shortage has led to greater use of grafts from donation after circulatory death (DCD) donors. In 2019, the proportion of DCD liver transplantations (LT) among all deceased donor LT in the complete Eurotransplant region, for the first time exceeded 10%. (1) When focusing solely on the three countries in the Eurotransplant region where DCD donation is allowed (Austria, Belgium and the Netherlands), this proportion is even 28%. (1)

The post-transplant outcomes of LT with DCD grafts (DCD-LT) have been found inferior to those of LT with grafts donated after brain death (DBD). (2, 3) An important explanation for this finding is the higher incidence of early allograft dysfunction (EAD) after DCD-LT. (4) Furthermore, patients receiving DCD liver grafts are more prone to develop biliary complications post-transplant. (5-7) The most incapacitating biliary complication for the recipient is the development of non-anastomotic strictures (NAS), also known as ischemic cholangiopathy or ischemic-type biliary lesions. (8, 9)

An important underlying mechanism in the development of NAS is ischemia reperfusion injury. During ischemia, depletion of adenosine triphosphate in cholangiocytes eventually leads to cell swelling and lysis. During the subsequent reperfusion, reactive oxygen species are formed which can activate an inflammatory cascade. (10) This results in apoptosis and necrosis of cholangiocytes and subsequent loss of the biliary epithelium. Cholangiocytes have proven to be more susceptible to ischemia reperfusion injury than are hepatocytes. (11) Besides ischemia reperfusion injury, ischemia can lead to irreversible damage of the peribiliary glands, thereby impairing their capacity to regenerate the biliary epithelium. (12)

The biliary tree relies mostly on arterial blood supply by both the hepatic artery and branches from the gastroduodenal artery. (13) Therefore, minimizing the biliary ischemia time by performing an initial artery reperfusion (IAR) technique during transplantation (i.e., reconstruction of the hepatic artery followed by reconstruction of the portal vein) could perhaps result in fewer biliary complications post-transplant. Especially in DCD grafts this could be beneficial. Nevertheless, most of the transplant centers worldwide currently use an initial portal vein reperfusion (IPR) approach. (14-16) The rationale for this approach is that anastomosis of the portal vein minimizing the anhepatic phase in the recipient. Furthermore, it has been shown that portal blood flow alone is sufficient for the liver to function adequately. (17)

Based on two recently published meta-analyses, there seems to be no difference in the occurrence of NAS between grafts revascularized through IPR or IAR. (18, 19) All included studies in these meta-analyses dealt with DBD liver transplantation, however, and the question remains whether this conclusion can be extrapolated to the DCD population. Furthermore, in a number of studies included in these meta-analyses the surgeon decided on the type of reperfusion technique, making it prone to bias. Unfortunately, there is no literature available on the incidence of EAD when using different reperfusion sequences.

In the absence of high-quality clinical evidence regarding the best reperfusion technique, it could be helpful to analyze whether the duration of additional arterial ischemia between reperfusion of the portal vein and reconstruction of the hepatic artery is of any influence on the development of EAD and NAS. In this national study we have tried to evaluate these effects among recipients of a DCD liver graft.

Methods

In this nationwide, retrospective cohort study, we included all adults who had received a DCD graft since the start of the DCD program in 2001 and the first of June, 2018. Multi-organ transplantation, retransplantation, transplantation with split livers and grafts recovered on machine perfusion were excluded. Furthermore, cases with an IAR approach as well as cases with an IPR approach with missing information on the time betwen portal and arterial reperfusion were excluded. The study has been approved by the medical ethics review board of the Erasmus MC University Medical Center Rotterdam (MEC-2019-0434).

Data collection and definitions

All three liver transplant centers in the Netherlands prospectively collect data on their LT program in a local database. We retrieved relevant data from these databases. Missing data were retrieved from individual medical records or from the Eurotransplant online application DonorData. Arterialization time was defined as the time between the reperfusion of the portal vein and the removal of the cross clamp of the hepatic artery. Donor warm ischemia time was defined as the time between the circulatory arrest in the donor and the start of the cold perfusion. The cold ischemia time was defined as the time between the start of cold perfusion in the donor and the liver being removed from ice. Recipient warm ischemia time was defined as the interval between removal of the liver from ice and portal reperfusion.

Primary and secondary outcomes

The primary endpoints of this study were the post-transplant incidences of EAD and NAS after DCD-LT. EAD was defined according to the Olthoff criteria. (20) NAS was defined as donor bile duct strictures located anywhere but the anastomosis, in the absence of a thrombosis of the hepatic artery, and having been demonstrated with imaging after clinical symptoms of cholestasis. Secondary endpoints were patient and graft survival.

Statistical analysis

Continuous and categorical variables are presented as median (interquartile range) and frequency (valid percentage), respectively. A univariable and a multivariable logistic regression model served to assess the influence of arterialization time on EAD. The post-transplant development of NAS was assessed with a univariable and a multivariable Cox-Proportional Hazards model. Arterialization time was included in both multivariable models, even if it didn't reach statistical significance in the univariable analysis. Covariates were included in the multivariable, backward stepwise, regression models if univariable regression yielded a p-value < 0.20. The threshold of 0.20 was chosen to lower the risk of overfitting of the model. The reported odds ratios (OR) and hazard ratios (HR) for arterialization time refer to an increase of one minute in arterialization time. For the cold ischemia time and recipient warm ischemia time, the OR and HR represent an increase of one hour and one minute, 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

Data of 292 DCD liver transplantations were included in this study. The median follow-up period of the complete cohort was 4.3 years (IQR 2.4-7.9). Baseline characteristics are presented in Table 1. Fifty-five percent of the donors was male; the median age of all donors was 47 years (IQR 37-53). A cerebrovascular accident was the main cause of death (41.8%), followed by trauma (24.7%). The median dWIT was 16 minutes (IQR 13-18).

The majority of the DCD-LT recipients were male (68.5%). A hepatocellular carcinoma was the most common primary indication for transplantation (34.9%). The median laboratory Model for End-Stage Liver Disease score at time of transplantation was 15 (IQR 10-20). The median cold ischemia time was 423 minutes (IQR 362-484); the median recipient warm ischemia time was 33 minutes (IQR 26 40). The median arterialization time was 33 minutes (IQR 25-49).

Table 1: Baseline characteristics

Characteristic	
Donor and procurement	
Age (years)	47.00 (37.00-53.00)
Gender	
Male	161 (55.1%)
Female	131 (44.9%)
Body mass index (kg/m²)	24.00 (22.00-26.00)
Cause of death	
CVA	122 (41.8%)
Trauma	72 (24.7%)
Anoxia	81 (27.7%)
Other	17 (5.8%)
Last AST (U/l)	44.00 (28.00-82.00)
Last ALT (U/l)	32.00 (20.00-61.00)
Last gGT (U/l)	32.00 (19.00-66.00)
Donor warm ischemia time (min) *	16.00 (13.00-18.00)
Donor risk index	2.10 (1.81-2.37)
Recipient	
Age (years)	56.00 (47.00-62.00)
Gender	
Male	200 (68.5%)
Female	92 (31.5%)
Body mass index (kg/m²)	25.92 (23.66-28.94)
Indication for LT	
HCC	102 (34.9%)
Cholestatic liver diseases (PBC/PSC)	54 (18.5%)
Alcoholic liver disease	37 (12.7%)
Viral hepatitis related cirrhosis	20 (6.8%)
NASH	18 (6.2%)
Cryptogenic liver cirrhosis	17 (5.8%)
Other	44 (15.1%)
Laboratory MELD score	15.00 (10.00-20.00)
Transplantation	
Use of portosystemic shunt	20 (7.0%)

Characteristic	
Type op biliary anastomosis	
Duct-to-duct	256 (89.8%)
Hepaticojejunostomy	29 (10.2%)
Cold ischemia time (min)	422.50 (362.25-483.75)
Recipient warm ischemia time (min)§	33.00 (26.00-40.00)
Arterialization time (min) ‡	33.00 (25.00-48.75)
Blood loss (ml)	3550.00 (2100.00-5500.00)
Post-operative	
Intensive care unit stay (days)	2.00 (1.00-5.00)
Total hospital stay (days)	18.00 (14.00-29.00)

Data are shown as median (IQR) and frequency (proportion). * : donor warm ischemia time is defined as the time between circulatory arrest and start of cold perfusion. §: recipient warm ischemia time is defined as time between liver being removed from ice and portal reperfusion. ‡: arterialization time is defined as the time between portal and arterial reperfusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVA, cerebrovascular accident; γGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

The actuarial one-, three- and five-year patient survival rates were 89%, 79% and 74%, respectively. The graft survival rates at one- three- and five-year follow up were 75%, 64% and 58%, respectively. Fifty-nine recipients required a retransplantation during the follow-up period, for which a biliary complication was the most common indication. In total, 134 recipients (45.9%) developed at least one biliary complication (i.e., anastomotic stricture, bile leakage and/or non-anastomotic strictures). Seventy-six recipients (26.0%) had been diagnosed with NAS, in most cases (58/76; 76.3%) during the first year post-transplant. The proportion of EAD post-transplant was 50.7%.

Table 2 depicts the outcomes of the logistic regression models for EAD. In both the univariable and multivariable regression model, arterialization time was not a significant risk factor for EAD. By contrast, both donor and recipient warm ischemia time were significant risk factors for EAD, with an odds ratio of 1.115 and 1.040, respectively. LT with DCD grafts from donors who had died from a cerebrovascular accident were associated with a significantly higher risk of EAD than grafts of donors who had died from other causes (OR 2.660, 95% confidence interval 1.386-5.105, p-value = 0.003). Interestingly, a graft from a female donor and a increasing age of the recipient were both protective factors for EAD.

Table 2: Logistic regression model for early allograft dysfunction

	Univariable			Multivariable		
	Odds ratio	**95% CI**	**p-value**	**Odds ratio**	**95% CI**	**p-value**
Donor and procurement						
Age (years)	1.023	1.002-1.046	0.035	-		0.157
Gender						
Male	REF					
Female	0.515	0.292-0.906	0.021	0.357	0.181-0.707	0.003
Body mass index (kg/m²)	1.047	0.966-1.135	0.259			
CVA as cause of death						
No	REF					
Yes	2.757	1.533-4.956	0.001	2.660	1.386-5.105	0.003
Last AST (U/l)	0.994	0.988-1.000	0.055	-		0.350
Last ALT (U/l)	0.995	0.990-1.001	0.084	-		0.271
Last γGT (U/L)	1.000	0.997-1.003	0.861			
Donor warm ischemia time (minutes)*	1.096	1.028-1.169	0.005	1.115	1.031-1.205	0.006
Recipient						
Age (years)	0.972	0.948-0.996	0.024	0.955	0.928-0.984	0.002
Gender						
Male	REF					
Female	1.206	0.665-2.189	0.537			
Body mass index (kg/m²)	1.034	0.971-1.101	0.294			
HCC as indication for transplantation						
No	REF					
Yes	0.737	0.414-1.313	0.301			
Laboratory MELD score	1.008	0.973-1.044	0.658			
Transplantation						
Cold ischemia time (hrs)	0.925	0.789-1.086	0.343			
Recipient warm ischemia time (min)§	1.025	0.999-1.052	0.056	1.040	1.007-1.073	0.016
Arterialization time (min) ‡	1.015	0.999-1.031	0.066	-		0.251
Type of biliary anastomosis						
Duct-to-duct	REF					
Hepaticojejustomy	1.375	0.528-3.580	0.514			

	Univariable			Multivariable		
	Odds ratio	**95% CI**	**p-value**	**Odds ratio**	**95% CI**	**p-value**
Use of portocaval shunt						
No	REF					
Yes	0.319	0.110-0.924	0.035	0.359	0.113-141	0.083

Multivariable model was conducted via backward stepwise approach. A dash (-) indicates that variable was removed from the model. * : donor warm ischemia time is defined as the time between circulatory arrest and start of cold perfusion. §: recipient warm ischemia time is defined as time between liver being removed from ice and portal reperfusion. ‡: arterialization time is defined as the time between portal and arterial reperfusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVA, cerebrovascular accident; γGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease;

In the univariable Cox Proportional-Hazards regression model for NAS-free survival, arterialization time had a borderline significance on the development of NAS post-transplant (HR 1.009, 95% CI 1.000-1.019, p-value = 0.053, table 3). In the multivariable model, however, arterialization loss its borderline significance and was even removed from the model. Donor age was a significant risk factor for developing NAS with a hazard ratio of 1.026. Furthermore, a high last level of alanine transaminase in the donor had a protective effect on the development of NAS (HR 0.992, 95% CI 0.985 – 0.999, p-value = 0.035).

Table 3: Cox proportional hazard model for NAS-free survival

	Univariable			Multivariable		
	Hazard ratio	**95% CI**	**p-value**	**Hazard ratio**	**95% CI**	**p-value**
Donor and procurement						
Age (years)	1.032	1.012-1.053	0.002	1.026	1.006-1.047	0.012
Gender						
Male	REF					
Female	0.858	0.543-1.355	0.511			
Body mass index (kg/m²)	0.968	0.907-1.033	0.327			
CVA as cause of death						
No	REF					
Yes	1.336	0.851-2.096	0.208			
Last AST (U/l)	0.993	0.987-1.000	0.035	-		0.877
Last ALT (U/l)	0.991	0.983-0.998	0.013	0.992	0.985-0.999	0.035
Last γGT (U/L)	1.001	0.999-1.003	0.532			
Donor warm ischemia time (minutes)*	1.039	0.992-1.088	0.109	-		0.103
Recipient						
Age (years)	0.995	0.976-1.014	0.594			
Gender						
Male	REF					
Female	1.034	0.634-1.687	0.893			
Body mass index (kg/m²)	1.013	0.963-1.066	0.610			
HCC as indication for transplantation						
No	REF					
Yes	0.771	0.472-1.259	0.299			
Laboratory MELD score	0.994	0.966-1.022	0.656			
Transplantation						
Cold ischemia time (hrs)	1.127	0.991-1.281	0.068	1.123	0.991-1.273	0.069
Recipient warm ischemia time (min)§	1.009	0.991-1.027	0.350			
Arterialization time (min) ‡	1.009	1.000-1.019	0.053	-		0.124

	Univariable			Multivariable		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
Type of biliary anastomosis						
Duct-to-duct	REF					
Hepaticojejustomy	1.062	0.509-2.214	0.873			
Use of portocaval shunt						
No	REF					
Yes	0.716	0.216-1.966	0.517			

Multivariable model was conducted via backward stepwise approach. A dash (-) indicates that variable was removed from the model. * : donor warm ischemia time is defined as the time between circulatory arrest and start of cold perfusion. §: recipient warm ischemia time is defined as time between liver being removed from ice and portal reperfusion. ‡: arterialization time is defined as the time between portal and arterial reperfusion. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CVA, cerebrovascular accident; γGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; MELD, model for end-stage liver disease;

Discussion

From the outcomes of this nationwide, retrospective cohort study, we may conclude that in DCD-LT with an initial portal reperfusion approach, the additional time between portal and arterial reperfusion neither is a risk factor for the occurrence of EAD post-transplant, nor influences the occurrence of NAS.

Our three-center study is rather similar to a recent single-center study by Gilbo and colleagues, who evaluated the impact of both donor hepatectomy time and implantation time on post-transplant outcomes. (21) Of 917 liver transplantation, the median arterialization time was 33 minutes (IQR 22- 44), which compares well with that in our cohort (33 minutes, IQR 25-49). In line with our study, the arterialization time was not a significant risk factor for the development of NAS. Still, the arterialization time had a significant effect on the incidence of EAD post-transplant. A possible explanation for the discrepancy in this respect between both studies is the inclusion of both DBD-LT and DCD-LT in the study of Gilbo and colleagues, whereas we had included only DCD-LT. A sub analysis on the 124 DCD-LT in the cohort studied by Gilbo and colleagues only considered the effect of the total implantation time on EAD and NAS, which consisted of the portal vein anastomosis time – which corresponds with the recipient warm ischemia time in our cohort – and the arterialization time. The total implantation time was a significant risk factor for EAD, but not for NAS. However, from the data published by Gilbo and colleagues it cannot be deduced whether this significant effect

of total implantation time on the incidence of EAD after DCD-LT is attributable to the time to portal revascularization, the additional time between portal and arterial revascularization, or a combination of both.

In the present study, both donor warm ischemia time and recipient warm ischemia time (i.e., time elapsed between liver being removed from ice and portal reperfusion), were significant risk factors for the development of EAD, in line with several published studies. (22-25) Two striking observations were donor gender (male donor) and donor cause of death being independent risk factors for the development of EAD. To our knowledge, this finding has not yet been reported in literature. However, in 2009, Singhal and colleagues found that grafts obtained from donors who suffered from a stroke had a worse graft survival when compared to other causes of donor death. The exact underlying mechanism remains unclear, but it has been proposed that elevation of intracranial pressure (i.e. during a cerebral bleed) leads to endothelial inflammation, negatively affecting the allograft. (26)

Our finding that arterialization time is not related to the development of NAS, is in line with results from several other studies. (21, 27, 28) In a multivariable analysis, Rammohan and colleagues found that arterialization time was not a significant risk factor for the development of biliary complications. (27) Cag and colleagues found no difference in arterialization time between recipients with and recipients without biliary complications post-transplant. (28) Furthermore, in both above-mentioned studies the cold ischemia time was of great importance for the development of biliary complications, and that it therefore should be kept as short as possible. We support this statement, grounded on our finding that CIT had a borderline significance in the multivariable model for NAS.

Higher age of the donor was a significant risk factor for developing NAS post-transplant in recipients in the current study. A previous study indeed suggests that a graft from an older donor is more susceptible to ischemia/reperfusion injury. (29) An interesting observation in our study is the protective effect of an increased last level of donor alanine transaminase on the development of NAS. We believe that this is not of clinical relevance, since 91.3% of the cohort had a last donor ALT level below 100 IU/L, which is classified as a mild elevation of transaminases. (30) Furthermore, information on the trend of transaminase levels in the donor prior to procurement was lacking.

A meta-analysis by Domagala and colleagues found no difference in patient and graft survival between LT with an IPR technique and LT with an IAR technique. (19) Moreover, another meta-analysis concluded that there is no significant difference in NAS between the two reperfusion techniques. (18) The results

of the present study underline these conclusions, implicating that once the portal vein has been revascularized, transplant surgeons can take their time in completing the arterial anastomosis. Still, this assumption needs cautious interpretation. First, a LT revascularized by using an IPR technique followed by a short arterialization time cannot simply be compared with a LT revascularized with an IAR technique. Furthermore, most of the studies included in both above-mentioned meta-analyses included only DBD liver grafts, whereas we had included only DCD-LT. A randomized controlled trial in which recipients of DCD livers are randomly assigned to either an initial portal reperfusion technique or an initial arterial reperfusion technique could provide the necessary evidence on what reperfusion technique is best for DCD liver grafts. A retrospective cohort study by our group, in which DCD-LT revascularized by IAR are compared with matched IPR cases is currently ongoing.

The strength of this study is that arterialization time was modeled as a continuous variable instead of a categorical variable with pre-specified cutoff points. The latter approach would have to a considerable loss of valuable information. Several limitations of the study need to be addressed. First, the retrospective design with its inherent shortcomings. For example, the length of time of arterial reperfusion or arterialization time was not always documented. These cases could unfortunately not be included in the analysis. Furthermore, we defined EAD using the Olthoff criteria, which have not been specifically developed for or adequately validated in the DCD-LT population. Therefore, a robust conclusion on the true effect of arterialization time on delayed graft function must be held back.

In conclusion, in DCD liver transplantations in which an initial portal reperfusion technique was used, the additional time between portal and arterial reperfusion seems to be unrelated to the development of early allograft dysfunction or non-anastomotic strictures. Further research comparing an initial portal reperfusion technique with an initial arterial reperfusion technique is highly recommended.

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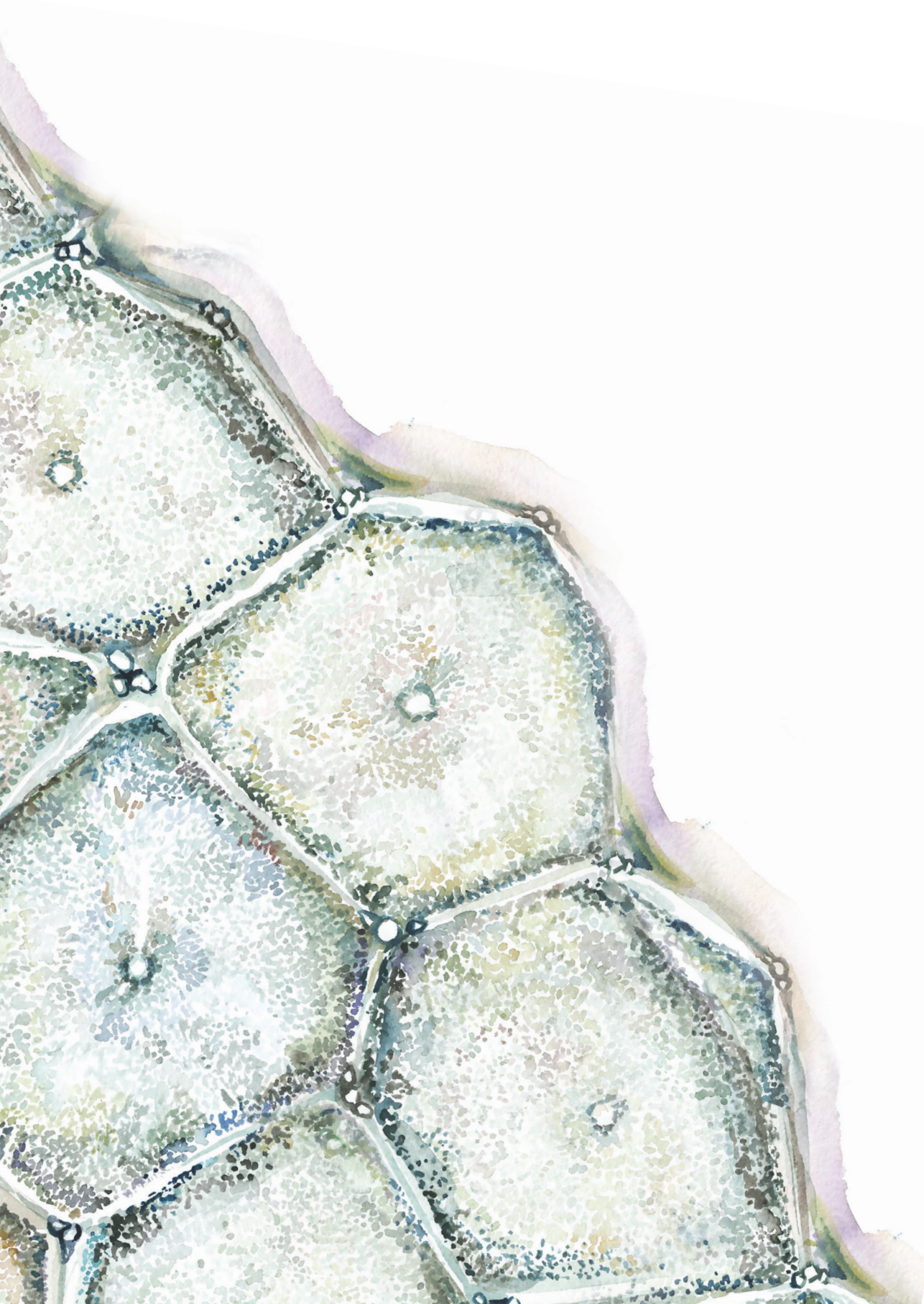

CHAPTER 7

Selected liver grafts from donation after circulatory death can be safely used for retransplantation - a multicenter retrospective study

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Abstract

Background: Due to the growing number of liver transplantations (LT), there is an increasing number of patients requiring retransplantation (reLT). Data on the use of grafts from extended criteria donors (ECD), especially donation after circulatory death (DCD), for reLT are lacking. We aimed to assess the outcome of patients undergoing reLT using a DCD graft in the Netherlands between 2001 and July 2018.

Methods: Propensity score matching was used to match each DCD-reLT with three DBD-reLT cases. Primary outcomes were patient and graft survival. Secondary outcome was the incidence of biliary complications, especially non-anastomotic strictures (NAS).

Results: 21 DCD-reLT were compared with 63 matched DBD-reLTs. Donors in the DCD-reLT group had a significantly lower BMI (22.4 vs. 24.7 kg/m^2, p-value = 0.02). Comparison of recipient demographics and ischemia times yielded no significant differences. Patient and graft survival rates were comparable between the two groups. However, the occurrence of non-anastomotic strictures after DCD-reLT was significantly higher (38.1% vs. 12.7%, p-value = 0.02).

Conclusions: ReLT with DCD grafts does not result in inferior patient and graft survival compared with DBD grafts in selected patients. Therefore, DCD liver grafts should not routinely be declined for patients awaiting reLT.

Introduction

Liver transplantation (LT) is a well-established treatment for patients suffering from end-stage liver disease. Due to the scarcity of available organs from deceased donors, the use of grafts from extended criteria donors (ECD) has increased substantially, of which grafts from donation after circulatory death (DCD) is a main parameter. (1) In 2018, a DCD graft was used in 38% and 9% of all deceased donor LT in the Netherlands and United States of America, respectively. (2, 3) In the United Kingdom, 26% of deceased donor LT were performed with DCD grafts (4).

Liver transplantation with DCD grafts (DCD-LT) is considered to be inferior compared to LT with grafts donated after brain death (DBD-LT), due to the increased risk of complications such as early allograft dysfunction (EAD) and biliary complications. (5-8) Among biliary complications, non-anastomotic strictures (NAS) are the most feared as they often require multiple interventions for biliary drainage, are largely irreversible and are known to have a negative impact on recipient and graft survival. (9) The incidence of NAS, also known as ischemic cholangiopathy (IC) or ischemic-type biliary lesions (ITBL), after DCD-LT varies between 3% and 39%. (6)

Since the use of grafts from marginal donors has increased, it is assumed that more recipients will develop post-transplant complications related to a suboptimal graft. Furthermore, due to improvements in surgical techniques, postoperative care and immunosuppressive regimes, the short-term survival after LT has improved significantly (10), resulting in a larger population surviving long enough to develop late graft failure. A retransplantation of the liver (reLT) is currently the only definitive treatment for allograft failure. However, it is well known that reLT is associated with inferior patient and graft survival compared with primary LT. (11, 12)

Despite DCD liver grafts being widely accepted, transplant physicians and surgeons tend to avoid the use of DCD grafts for reLT. However, since in some countries the availability of DBD grafts has decreased (13), the waiting time for an optimal, preferably DBD liver to become available for a reLT candidate could be too long with subsequent risk of deterioration of patient's condition, making him or her ineligible for reLT.

There is very little reported on the use of DCD grafts for patients requiring a reLT. Only one study has assessed the outcomes of ten patients undergoing reLT using DCD grafts. (14) The authors concluded that the use of DCD graft should be avoided if the recipient has a moderate to high Model for End-Stage Liver Disease

(MELD) score. Unfortunately, no comparison was made with reLT using DBD grafts. Since DCD-LT is common in the Netherlands, and reLT is not an official contraindication for the use of a DCD liver, we aimed to compare the outcomes of reLT with DCD grafts in the Netherlands with that of matched DBD cases.

Methods

In this multicenter retrospective study, all patients who underwent reLT using a controlled DCD liver graft (DCD-reLT) in the Netherlands from the beginning of the DCD-LT program in 2001 until July 1st 2018, were included. Pediatric LT (recipient < 18 years), reLT using a split graft, reLT in the setting of multi-organ transplantation and grafts preserved with machine perfusion were excluded. A pre-existent nationwide database on all liver retransplantations (reLT) performed between 1979 and July 2018 was used to match each DCD-reLT to three cases of reLT with DBD grafts (DBD-reLT). (15) For the matching, a propensity score matching approach with nearest-neighbor algorithm was used. The propensity scores were calculated using a logistic regression model with the following independent covariates: transplant center, number of consecutive reLT, year of reLT, donor and recipient age, last laboratory MELD score (Model of End-Stage Liver Disease) registered by Eurotransplant prior to transplantation, cold ischemia time (CIT), and interval between prior LT and ReLT. This latter matching criterion was chosen since an early reLT, is on the one hand technically less challenging than late reLT (easier hepatectomy with less adhesions), but on the other hand is performed in patients who may be sicker pre-reLT than patients undergoing a late reLT. (16, 17) DBD-reLT cases that met one of the previously mentioned exclusion criteria or had missing variables in one or more of the matching criteria were excluded prior to matching. Additional data on donor and organ procurement characteristics were obtained through the Eurotransplant Donor data database. Additional recipient data and data on follow-up were collected from prospective maintained databases and patients' electronic medical records. The study has been approved by the Institutional Review Board of the Erasmus MC University Medical Center Rotterdam (MEC-2019-0316).

In all DCD organ procurements in the Netherlands, withdrawal of life support takes place at the ICU or regular ward. After circulatory arrest, a mandatory no touch period of five minutes is carried out after which the donor is transported to the operating theatre. As described in the National protocol postmortem donor organ procurement, a super-rapid retrieval technique is used in DCD donors to minimize the donor warm ischemia time (dWIT). After cannulation of aorta and inferior vena cava, cold perfusion with University of Wisconsin (UW) solution is started. (18) Since premortem administration of heparin is prohibited by law, heparin is added to the perfusion solution. The standard method of implantation

is with a piggyback caval vein anastomosis, an end-to-end arterial and portal anastomosis, and a duct-to-duct biliary anastomosis.

The total dWIT was defined as time between withdrawal of life-supporting treatment and start of cold perfusion. The definition of asystolic dWIT was the time between circulatory arrest and cold perfusion. The CIT was defined as the period between the start of the cold perfusion in the donor and the removal of the liver from ice during the recipient procedure. The definition of recipient warm ischemia time (rWIT) used in this study is the interval between removal of the liver from ice and graft reperfusion (i.e., in the majority portal reperfusion).

The primary outcome measures of this study were patient and graft survival. Patient survival was defined as time between reLT and death, with or without functioning graft. Graft survival was calculated as time between the reLT and patient death (with or without functioning graft) or a successive retransplantation. Secondary outcomes were the incidence of three types of biliary complications: bile leakage, anastomotic strictures, and NAS. NAS was defined as any stricture of the bile duct except those localized near the biliary anastomosis and in absence of an hepatic artery thrombosis.

Continuous data were presented as median and interquartile range (IQR) and compared with the Mann–Whitney U test. Categorical variables were presented as number and percentages and compared with the Pearson chi-square test or the Fisher exact test where appropriate. Survival analyses was conducted using the Kaplan–Meier method, and comparisons were made with the log-rank test. All tests were two-sided with a p-value below 0.05 considered as significant. The propensity score matching was performed in RStudio, version 1.0.153 (RStudio Inc. Boston, MA, USA), using the MatchIt package. All other statistical analyses were performed using SPSS version 25 (IBM, Chicago, IL, USA).

Results

A total of 21 cases of DCD-reLT were included in this study. These cases were matched with 63 DBD-reLT cases. Donor and recipient demographics are given in Table 1. Compared with DBD-reLT donors, DCD-reLT donors had a significantly lower BMI (22.4 vs. 24.7 kg/m^2, p-value = 0.02). Furthermore, there was a trend toward significance regarding the donor cause of death (p-value = 0.06). The majority of the DBD donors had died from a cerebrovascular accident (CVA), whereas the cause of death among DCD donors was more equally distributed between trauma, CVA, and other causes. In DCD-reLT, the median asystolic dWIT was 15.0 minutes (12.0–18.0 minutes) whereas the total dWIT was 27.5 minutes (22.3–30.8 minutes).

The majority of the recipients was male, with a median age of 51.0 years (IQR, 46.0–56.5 years) in the DCD-reLT group and 56.0 years (IQR, 46.0–62.0 years) in the DBD-reLT group (p-value = 0.22). The most common indication for reLT was post-transplant cholangiopathy (43% in the DCD-reLT group, 44% in the DBD-reLT group), followed by vascular complications and recurrence of the primary disease.

Table 1: Donor and recipient demographics

		Total group n=84	DCD-reLT n=21	DBD-reLT n=63	P-value
Donor					
Gender					
	Male	42 (50.0)	10 (47.6)	32 (50.8)	0.80
	Female	42 (50.0)	11 (52.4)	31 (49.2)	
Age (years)		40.5 (24.0-51.5)	38.0 (19.5-45.0)	42.0 (25.0-53.0)	0.11
BMI (kg/m²)		23.5 (21.3-26.0)	22.4 (19.8-23.7)	24.7 (21.5-26.7)	0.02
Cause of death					
	CVA	43 (51.2)	7 (33.3)	36 (57.1)	0.06
	Trauma	26 (31.0)	7 (33.3)	19 (30.2)	
	Other	15 (17.9)	7 (33.3)	8 (12.7)	
Last γGT (U/L)		24 (17-52)	28 (18-34)	23 (17-53)	0.96
Last ALT (U/L)		32 (21-50)	23 (15-47)	36 (21-52)	0.10
Asystolic dWIT (min)†		n/a	15.0 (12.0-18.0)	n/a	n/a
Total dWIT (min)‡		n/a	27.5 (22.3-30.8) ∫	n/a	n/a
Recipient					
Gender					
	Male	54 (64.3)	12 (57.1)	42 (66.7)	0.43
	Female	30 (35.7)	9 (42.9)	21 (33.3)	
Age (years)		54.5 (46.0-61.8)	51.0 (46.0-56.5)	56.0 (46.0-62.0)	0.22
BMI (kg/m²)		24.3 (21.7-26.6)	22.7 (21.6-28.2)	24.3 (21.7-26.5)	0.77
Laboratory MELD score		20.0 (10.3-26.0)	19.0 (9.5-27.5)	20.0 (11.0-26.0)	0.70
Indication for reLT					
	PNF	7 (8.3)	3 (14.3)	4 (6.3)	0.41
	Vascular	23 (27.4)	3 (14.3)	20 (31.7)	
	Recurrence of disease	12 (14.3)	4 (19.0)	8 (12.7)	
	Other	5 (6.0)	2 (9.5)	3 (4.8)	

		Total group n=84	DCD-reLT n=21	DBD-reLT n=63	P-value
High urgency status		26 (31.0)	4 (19.0)	22 (34.9)	0.17
Number of reLT					
	1st reLT	72 (85.7)	18 (85.7)	54 (85.7)	>0.99
	≥ 2nd reLT	12 (14.3)	3 (14.3)	9 (14.3)	
Time between reLT and prior LT (days)		466 (13-2728)	1140 (166-3864)	368 (12-2685)	0.31
Graft type of prior LT					
	DBD graft	61 (72.6)	15 (71.4)	46 (73.0)	0.82
	DCD graft	22 (26.2)	6 (28.6)	16 (25.4)	
	Living	1 (1.2)	0	1 (1.6)	

Data are shown as median (IQR) and frequency (proportion). †: Asystolic dWIT is defined as the time between circulatory arrest and start of cold perfusion. ‡: Total dWIT is defined as time between withdrawal of life supporting treatment and cold perfusion. ∫: Proportion of missing data for this variable is 23.8%. ALT: Alanine transaminase, BMI: Body Mass Index, CVA: Cerebrovascular accident, DBD: Donation after Brain Death, DCD: Donation after Circulatory Death, dWIT: donor Warm Ischemia Time, γGT: Gamma-glutamyltransferase, LT: liver transplantation, MELD: Model for End stage Liver Disease, reLT: liver retransplantation

Table 2 shows operative data as well as data on the postoperative outcomes. Neither the CIT nor the rWIT differed significantly between the two groups. However, the peak ALT level in the first week post-reLT was significantly higher in the DCD-reLT group (1346 IU/l vs. 833 IU/l, p-value = 0.04). Patients were discharged from the hospital after a median of 25 days in the DCD-reLT group and 20 days in the DBD-reLT group (p-value = 0.15).

Table 2: Surgical and post-operative demographics

	Total group n=84	DCD-reLT n=21	DBD-reLT n=63	P-value
rWIT (minutes)	40 (32.8-46.3)	44.0 (35.0-48.0)	39.0 (31.5-43.0)○	0.07
CIT (minutes)	444 (377-524)	440 (355-518)	448 (389-527)	0.69
Blood loss (ml)†	3600 (2000-5900)	4819 (2675-8175)†	3200 (1767-5450)‡	0.09
Post-operative outcomes				
ICU stay (days)	2.0 (1.3-5.0)	2.0 (2.0-4.0)	2.0 (1.0-5.0)	0.90
Hospital stay (days)	21.0 (14.0-30.0)	25.0 (14.0-34.5)	19.5 (13.0-25.8) ∫	0.15
Peak ALT within 1st week	1011 (540-1626)	1346 (526-2518)	833 (526-1305)	0.04
Hepatic artery thrombosis	9 (10.7)	2 (9.5)	7 (11.1)	>0.99
Bile leak	9 (10.7)	2 (9.5)	7 (11.1)	>0.99
Anastomotic strictures	13 (15.5)	5 (23.8)	8 (12.7)	0.30
Non-anastomotic strictures	16 (19.0)	8 (38.1)	8 (12.7)	0.02
Death	24 (28.6)	5 (23.8)	19 (30.2)	0.58
Retransplantation	6 (7.1)	1 (4.8)	5 (7.9)	>0.99

Data are shown as median (IQR) and frequency (proportion). ○: Proportion of missing data for this variable is 3.2%. †: Proportion of missing data for this variable is 4.8%. ‡: Proportion of missing data for this variable is 15.9%. ∫: Proportion of missing data for this variable is 1.6%. ALT: Alanine transaminase, BAR: Balance of risk, CIT: Cold Ischemia Time, DBD: Donation after Brain Death, DCD: Donation after Circulatory Death, ICU: Intensive Care Unit, LT: liver transplantation, MELD: Model for End stage Liver Disease, reLT: liver retransplantation, rWIT: recipient Warm Ischemia Time

Survival rates

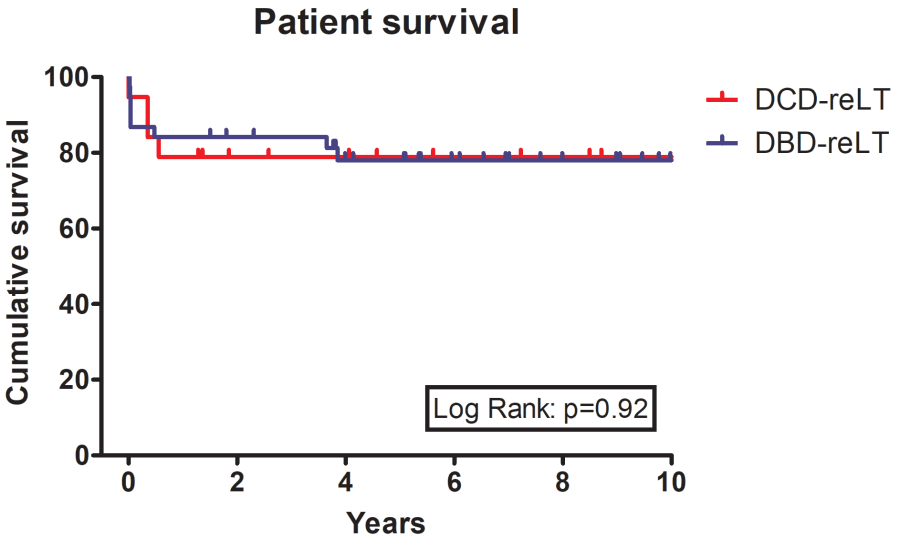
The median follow-up of the total cohort was 5.30 years (IQR, 1.49–8.73 years). The 30 days, 1-year, 5-year, and 10-year recipient survival in the DCD-reLT group was 95%, 81%, 81%, and 81%, respectively, compared with 90%, 82%, 72%, and 59% in the DBD-reLT group (p-value = 0.37, Figure 1). The causes of death of five recipients in the DCD-reLT group are listed in Table 3.

Table 3: Causes of death after DCD-reLT

Case	Graft type	Days between reLT and death	Cause of death
1.	DCD-reLT	1	Myocardial infarction in septic patient
2.	DCD-reLT	129	Multiple organ failure
3.	DCD-reLT	129	Recurrent giant cell hepatitis
4.	DCD-reLT	205	Pseudomonas infection in patient with recurrent HCV
5.	DCD-reLT	4941	Recurrent decompensated liver cirrhosis

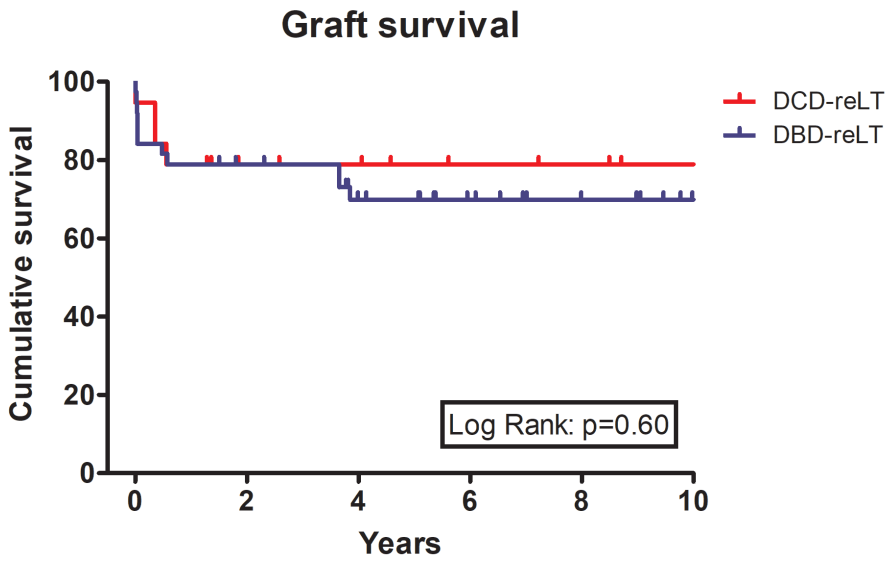
DCD: Donation after circulatory death, HCV: Hepatitis C virus infection, reLT: liver retransplantation

The 30 days, 1-year, 5-year, and 10-year graft survival was 95%, 81%, 81%, and 81% for the DCD-reLT group and 86%, 79%, 67%, and 53% in the DBD-reLT group (p-value = 0.20) (Figure 2). Six patients needed a subsequent retransplantation: three because of an early hepatic artery thrombosis (all in the DBD-reLT group), two due to ischemic-type biliary lesions (one in each group), and one patient in the DBD-reLT group due to recurrence of primary sclerosing cholangitis.



Number entering interval	0	2	4	6	8	10
DCD-reLT	19	12	11	8	7	5
DBD-reLT	38	30	23	17	11	6

Figure 1: Kaplan-Meier curve of patient survival after DCD-reLT and DBD-reLT
Patient survival is defined as death (with or without functioning graft).
DBD-reLT: liver retransplantation with graft from donation after brain death. DCD-reLT: liver retransplantation with graft from donation after circulatory death.



Number entering interval	0	2	4	6	8	10
DCD-reLT	19	12	11	8	7	5
DBD-reLT	38	28	21	15	10	5

Figure 2: Kaplan–Meier curve of graft survival after DCD-reLT and DBD-reLT
Graft survival is defined as death (with or without functioning graft) or consecutive retransplantation. DBD-reLT: liver retransplantation with graft from donation after brain death. DCD-reLT: liver retransplantation with graft from donation after circulatory death.

Biliary complications

In total, 10.7% of the recipients had a bile leakage. Furthermore, five recipients in the DCD-reLT group (23.8%) and eight in the DBD-reLT group (12.7%) developed an anastomotic stricture (p-value = 0.30). The proportion of recipients developing NAS was significantly higher in the DCD-reLT group (38.1% vs. 12.7%, p-value = 0.02). The majority of the NAS after DCD-reLT were of the focal type. The median time interval between reLT and diagnosis of NAS was 170 days (IQR 102–282 days).

Discussion

The relative shortage of available liver grafts has led to a more widespread use of DCD grafts. However, the outcomes after reLT with a DCD graft have rarely been reported in literature. This is the first study to analyze the outcomes after DCD-reLT and compare these with outcomes after matched DBD-reLT. Our results suggest that reLT with a DCD graft in selected patients does not result in inferior outcome when compared to matched DBD-reLTs.

The survival rates after DCD-reLT in this study are substantially higher than presented in the previous study on DCD-reLT performed by Perry et al. in 2011 (14). This could be due to the substantially lower MELD score in our population (median of 20.0 vs. a median of 27.0 reported by Perry et al.). Unfortunately, it is unclear whether in the study by Perry et al. the MELD score included (non) standard exception points. Since our median laboratory MELD score is that much lower, we are unable to refute or endorse the conclusion from Perry et al. that the use of DCD grafts should be avoided in high MELD recipients awaiting reLT. However, a recent published study by Taylor et al. concluded that accepting a DCD graft has a survival advantage over waiting for a DBD liver, especially in recipients with a high MELD score (19). As this study only included first-transplant recipients, it is doubtful whether the conclusions made by Taylor and colleagues can be extrapolated to the field of reLT. Based on our results, it is indicated that at least in recipients with low-to-moderate laboratory MELD score the use of a DCD graft is justifiable for reLT.

The significantly lower donor BMI in the DCD-reLT group is probably the result of strict selection by transplant physicians and surgeons. Since there seems to be some association between BMI and degree of steatosis, a known risk factor for poor outcome after LT (20, 21), transplant professionals may be reluctant to accept the liver from an overweight DCD donor for reLT. We believe that it is unlikely that the lower donor BMI of the DCD-group alone has resulted in the relatively high survival rates of this group, because median donor BMI of both groups was within the healthy weight category according to the WHO definition. (22)

When compared with DBD grafts, LT with DCD grafts is generally at higher risk of developing biliary complications post-transplant, especially NAS. A similar trend can be seen in the current study. Although the development of NAS post-transplant can have a substantial influence on the survival rates, we believe it should not discourage transplant professionals in using DCD grafts for the indication of reLT. Firstly, because the majority of the NAS cases reported after DCD-reLT in our study were of the focal type and could be treated conservatively by endoscopic therapy. Only two recipients required a new transplantation because of this complication.

Furthermore, the field of machine perfusion is evolving rapidly. Research has shown that with the use of machine perfusion, the incidence of biliary complications post-transplant can be reduced. (23-26) Currently, several international trials regarding machine perfusion are ongoing.

Surprisingly, the incidence of NAS after especially DBD-reLT in the current study is higher than expected. There could be several explanations for this. First, the high NAS incidence in the DBD-cohort could be the result of the matching. Furthermore, until recently, the donor hepatectomy time (i.e., the time between the start of cold perfusion in the donor and the liver being stored on ice) was relatively long in the Netherlands. Research has shown that a prolonged hepatectomy time is a risk factor for the development of NAS. (27, 28) Finally, the high incidences of NAS in this reLT cohort could also imply that a reLT, independent of graft type, has a higher risk of developing postoperative NAS. Unfortunately, literature on this topic is lacking.

With the renewed interest in the use of DCD grafts, we believe that the results of our study are very relevant for further practice in these centers. With careful selection, recipient and graft survival after DCD-reLT appear similar to the survival in DBD-reLT. Therefore, grafts for reLT should not be rejected based on the DCD status alone but a careful assessment of additional donor factors is needed for a case-by-case decision to use these grafts. Furthermore, making use of DCD donors for reLT may facilitate the current ethical debate regarding reLT. That is, if transplant surgeons and physicians will accept DCD grafts for retransplantation, more DBD grafts will remain available for recipients on the waiting list awaiting their first-transplant. At the same time, expansion of the donor pool with DCD donors will result in more expedited reLT for those in need. Finally, with the emerging technologies in the field of machine perfusion, it can be anticipated that the quality of DCD grafts can be improved, resulting in among other a decreased incidence of post-transplant cholangiopathy. (23, 29, 30)

One strength of this study is the comparison of outcome after DCD-reLT with a matched control group of DBD-reLT cases. This has made a proper comparison of the two groups possible, from which it can be concluded that survival after DCD-reLT is under certain circumstances similar to that after DBD-reLT. This study also has several limitations. Firstly, we had to define dWIT as time between withdrawal of life support and cold perfusion. We were unable to calculate the more important functional warm ischemia time in the donor since data on hemodynamic status during the agonal phase are lacking or improperly recorded. Furthermore, the study had a retrospective design, which is prone to bias and confounding. Finally, the sample size of this study is relatively small, which made detailed statistical analysis such as multivariate analysis impossible.

In conclusion, reLT with a DCD graft can yield similar patient and graft survival rates as reLT with donation after brain death. Therefore, DCD itself should not preclude the use of such donors in patients awaiting retransplantation. However, careful selection of the offered DCD livers probably remains mandatory, especially to minimalize the chance of developing NAS post-retransplant. Larger studies are needed to confirm our results.

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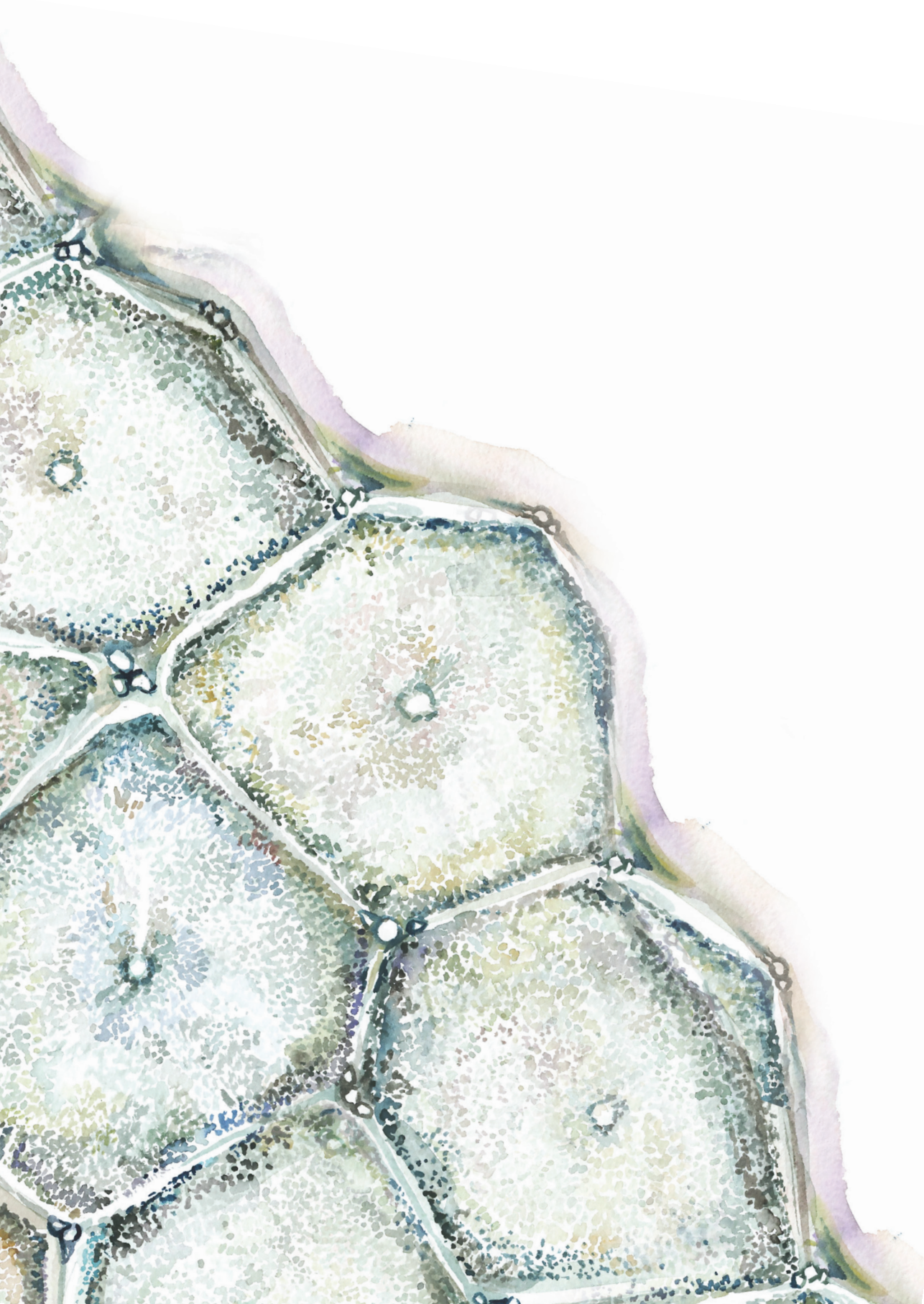

CHAPTER 8

Evaluation of Liver Graft Donation After Euthanasia

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Abstract

Background: The option of donating organs after euthanasia is not well known. Assessment of the results of organ transplantations with grafts donated after euthanasia is essential to justify the use of this type of organ donation.

Methods: All LTs with grafts donated after euthanasia (donation after circulatory death type V [DCD-V]), performed in Belgium and the Netherlands from the start of the donation after euthanasia program through July 1, 2018, were included in the analysis. A comparative cohort of patients who received grafts from donors with a circulatory arrest after withdrawal of life-supporting treatment (DCD-III) was also analyzed. Primary outcomes were recipient and graft survival rates at years 1, 3, and 5 after the LT. Secondary outcomes included postoperative complications within the first year after the LT.

Results: Among the cohort of 47 LTs with DCD-V grafts, 25 organ donors (53%) were women and the median (interquartile range [IQR]) age was 51 (44-59) years. Among the cohort of 542 LTs with DCD-III grafts, 335 organ donors (62%) were men and the median (IQR) age was 49 (37-57) years. Median (IQR) follow-up was 3.8 (2.1-6.3) years. In the DCD-V cohort, 30 recipients (64%) were men, and the median (IQR) age was 56 (48-64) years. Recipient survival in the DCD-V cohort was 87% at 1 year, 73% at 3 years, and 66% at 5 years after LT. Graft survival among recipients was 74% at 1 year, 61% at 3 years, and 57% at 5 years after LT. These survival rates did not differ statistically significantly from those in the DCD-III cohort. Incidence of postoperative complications did not differ between the groups. For example, the occurrence of early allograft dysfunction after the LT was found to be 13 (31%) in the DCD-V cohort and 219 (45%) in the DCD-III cohort. The occurrence of non-anastomotic biliary strictures after the LT was found to be 7 (15%) in the DCD-V cohort and 83 (15%) in the DCD-III cohort.

Conclusions: The findings of this cohort study suggest that LT with DCD-V grafts yield similar outcomes as LT with DCD-III grafts; therefore, grafts donated after euthanasia may be a justifiable option for increasing the organ donor pool. However, grafts from these donations should be considered high-risk grafts that require an optimal donor selection process and logistics.

Introduction

Few countries have accepted the possibility of euthanasia as an alternative to permanent, severe physical or mental illness. Currently, euthanasia is legalized under certain conditions in Belgium, Canada, Colombia, Luxembourg, and the Netherlands. (1) Euthanasia differs from physician-assisted suicide. During euthanasia, the physician administers medication to a patient to intentionally end their life, whereas in physician-assisted suicide, the patient self-administers the medication that has been prescribed by the physician.

Organ donation after euthanasia could help alleviate the current organ shortage. A retrospective study found that 10% of patients who underwent euthanasia in Belgium could have been a suitable organ donor. (2) Especially in patients for whom organ replacement therapy options are limited, including candidates for a liver transplantation, the use of organs donated after euthanasia could reduce waiting-list mortality. At present, organ donation after euthanasia is allowed in Belgium and the Netherlands and has been decriminalized in Canada. (3, 4) However, there is little awareness of the possibility to donate organs after euthanasia among both physicians and patients.

Although liver transplantation (LT) with grafts donated after euthanasia has been shown feasible in several countries, assessing the outcomes of LT with these grafts is essential to justify this type of organ donation to the general public. (5, 6) Recently, based on a single-center study, Gilbo et al. concluded that LT with grafts donated after euthanasia yielded similar survival rates as LT with grafts from donation after circulatory death (DCD) type III, defined as grafts from donors with a circulatory arrest after the withdrawal of life-supporting treatment. (7, 8) However, the study by Gilbo et al. had a small sample size and did not report information on postoperative complications, such as post-transplant cholangiopathy. (7)

As do grafts from DCD-III, organs donated after euthanasia undergo donor warm ischemia time (dWIT), which triggers the occurrence of post-transplant complications that could worsen long-term outcomes. (9, 10) As such, according to the modified Maastricht criteria, grafts donated after euthanasia are considered the fifth subtype of DCD (DCD-V). (8)

In general, the use of DCD grafts in LT has rapidly increased. Within the Eurotransplant region, the number of DCD liver grafts used in LT increased from 42 in 2010 to 160 in 2019. (11) When compared with LT with grafts from donation after brain death, however, LT with DCD grafts tends to yield a higher incidence of graft failure and biliary complications, of which non-anastomotic strictures are the most harmful. (9, 12, 13)

In this multicenter cohort study, the outcomes of LT with DCD-V grafts in Belgium and the Netherlands were examined. We aimed to assess these outcomes and to compare them with the results of the more commonly performed LT with DCD-III grafts.

Legal and practical aspects of euthanasia

Euthanasia was legalized in the Netherlands in 2001 and in Belgium in 2002. According to both the Dutch and Belgian law, patients who request euthanasia must be experiencing severe physical or mental distress with no chance for improvement and no reasonable alternative. (14, 15) Furthermore, a patient's appeal for euthanasia must be well considered and completely voluntarily. In addition to the physician handling the euthanasia request, an independent physician must reassess whether the request is justified. Euthanasia is performed by a physician who administers a drug that induces a coma (preferably, thiopental sodium; in the Netherlands, propofol is used as an alternative) followed by a nondepolarizing neuromuscular blocking agent (e.g., rocoronium bromide, atracurium besylate, or cistracurium besylate). (16, 17)

Legal and practical aspects of organ donation after euthanasia

In the Netherlands, the Erasmus MC University Medical Center and Maastricht University Medical Center developed a manual on organ donation after euthanasia, and the Dutch Transplant Society created a multidisciplinary national guideline for organ donation after euthanasia. (5, 18) In Belgium, a national guideline on DCD-V is nonexistent, but all transplant centers across the country have a local protocol for this type of organ donation. The most important ethical aspect of facilitating DCD-V is that the organ donation and euthanasia should be handled as 2 separate, strictly regulated processes. Neither the patients and their relatives nor the physicians should experience any form of social pressure or conflict of interest.

The process of DCD-V is initiated by a voluntary request from a patient whose euthanasia request has already been granted. After this request, a physician (often a general practitioner) contacts a transplant coordinator. The transplant coordinator evaluates the patient's medical record to ascertain whether the patient is a suitable organ donor. Often, additional screening investigations, such as blood tests and imaging, must be performed before a final decision can be made. The contraindications for DCD-V are similar to the contraindications for the other types of deceased donation. Despite some previous cases in which the coma-inducing drug was administered to the patient at home, today the complete euthanasia procedure is highly recommended to take place in the hospital. (19)

Donation and transplant procedure

After circulatory arrest has been declared by the physician who performed the euthanasia, the DCD-V procedure commences in a similar way as the DCD-III donation. To ascertain irreversible circulatory arrest, a 5-minute period of no touch is obligatory. In the Netherlands, transporting the donor to the operating theater during these 5 minutes is prohibited. In both Belgium and the Netherlands, a super-rapid sternolaparotomy is performed to procure donor organs. The implantation techniques are transplant center–specific but generally include the piggyback technique (or a variant of it) for the caval vein anastomosis, an end-to-end arterial and portal anastomosis, and a duct-to-duct biliary anastomosis.

Methods

Most transplant centers in the Netherlands and Belgium (n = 8) participated in this population-based cohort study.

Study population

All LT with DCD-V grafts performed in the Netherlands and Belgium from the start of the donation after euthanasia program (January 2012 for the Netherlands, and January 2005 for Belgium) through July 1, 2018, were included in this analysis. Liver grafts from DCD-V that were preserved with machine perfusion were excluded. We obtained LT data from prospectively collected databases maintained by many transplant centers. In case of missing data, we accessed individual medical records or the Donor Data application from Eurotransplant.

To compare the results of LT with DCD-V grafts with LT with DCD-III grafts (comparative cohort), we used a Dutch database that contains all adult LT with DCD-III performed between January 1, 2006, and January 1, 2017. Liver grafts recovered on machine perfusion and liver graft retransplantations were excluded from this database. This comparative cohort was extended to LT with DCD-III performed in the same period in 3 Belgian transplant centers (in Leuven, Antwerp, and Liège) that performed most of the LT with DCD-V.

Primary and secondary outcomes and definitions

The primary outcomes of this study were the recipient and graft survival rates at years 1, 3, and 5 after the LT. Patient loss was defined as death with or without a functioning graft, whereas graft loss was defined as either a recipient death or a retransplantation. Secondary outcomes were the occurrence of early allograft dysfunction, hepatic artery thrombosis, and non-anastomotic biliary strictures within the first year after the LT. As described, the dWIT can be divided into an agonal

phase and an asystolic phase. (20) In an LT with DCD-V graft, the agonal phase was defined as the time between administration of euthanatics (coma-inducing drug and non-depolarizing neuromuscular blocking agent) and circulatory arrest. In an LT with DCD-III graft, the agonal phase was defined as the period between withdrawal of life-supporting treatment and circulatory arrest. The definition of the asystolic phase was the same for both LT with DCD-III graft and LT with DCD-V graft: the time between circulatory arrest and start of cold perfusion.

The cold ischemia time was described as the period between the start of cold perfusion in the donor and the removal of the liver graft from ice before implantation. The recipient warm ischemia time was the period between the removal of the liver graft from ice and the portal or arterial reperfusion, whichever came first. Regarding the secondary outcome parameters, early allograft dysfunction was classified according to the Olthoff criteria and was diagnosed only in patients who were alive and did not undergo a retransplantation within week 1 after the LT. (21) Non-anastomotic biliary strictures were described as any stricture of the biliary tree other than those at the level of the anastomosis and in the absence of a hepatic artery thrombosis.

Statistical analysis

Continuous variables are presented as median (interquartile range [IQR]), whereas categorical variables are presented as frequency (valid percentage). To compare the 2 groups, we used either an unpaired $\chi 2$ test (categorical variables) or an unpaired Mann-Whitney test (continuous variables). Recipient and graft survival rates were calculated with the Kaplan-Meier method. A log-rank test was performed to assess the statistical differences in survival rates between the DCD-V and DCD-III cohorts.

All statistical analyses were performed in SPPS, version 25 (SPSS Inc). A 2-sided p-value < 0.05 was considered statistically significant. Data analysis was performed from September 2019 to December 2019.

Results

As of July 1, 2018, a total of 59 LT with DCD-V grafts had been performed in Belgium and in the Netherlands. Between January 1, 2012, and December 31, 2017, approximately 7% of all LT with DCD performed in both countries were with DCD-V grafts. In 12 cases, the liver graft underwent machine preservation, and these cases were excluded from further analysis. The final cohort comprised 47 LT with DCD-V grafts. The comparative cohort consisted of 542 LT with DCD-III grafts. The median (IQR) follow-up period of the complete cohort was 3.8 (2.1-6.3) years.

Donor, recipient and surgical characteristics

In the DCD-V cohort, 25 organ donors (53%) were women and 22 (47%) were men, with a median (IQR) age of 51 (44-59) years (Table 1). This composition was statistically significantly different from the DCD-III cohort, which comprised 335 men (62%) and 207 women (38%; p-value = 0.04), with a median (IQR) age of 49 (37-57) years. In the DCD-V cohort, a neurodegenerative disease (e.g., amyotrophic lateral sclerosis, multisystem atrophia, and Huntington disease) was the most common indication for euthanasia request (17 [36%]), followed by a psychiatric disorder (11 [23%]). Compared with donors in the DCD-III cohort, those in the DCD-V cohort had significantly lower levels of median (IQR) transaminase (aspartate aminotransferase: 26 [21-33] IU/L vs. 67 [36-140] IU/L; alanine aminotransferase: 25 [20-38] IU/L vs. 52 (25-115) IU/L; p-value < 0.001). (To convert aspartate aminotransferase and alanine aminotransferase to microkatals per liter, multiply by 0.0167.) The median (IQR) agonal dWIT was 7 (5-9) minutes, which was significantly shorter than that in the comparative cohort (14 [9-20] minutes) (p-value < 0.001). The median (IQR) asystolic dWIT was also significantly shorter in the DCD-V population (11 [8-14] vs. 12 [9-17] minutes; p-value = 0.03) (Table 1).

Table 1: Donor demographics

		DCD-V n = 47	DCD-III n = 542	P-value
Gender				
	Male	22 (47)	335 (62)	0.04
	Female	25 (53)	207 (38)	
Age (years)		51 (44-59)	49 (37-57)	0.17
BMI (kg/m²)		23 (20-26)	24 (22-26)	0.09
Indication for euthanasia				
	Neurodegenerative diseases	17 (36)	N/A	
	Psychiatric disorders	11 (23)	N/A	
	Multiple Sclerosis	8 (17)	N/A	
	Unbearable pain	3 (6)	N/A	
	Tetraplegia/quadriplegia	1 (2)	N/A	
	Locked in syndrome	2 (4)	N/A	
	Cerebrovascular accident	1 (2)	N/A	
	Other	3 (6)	N/A	
	Unknown	1 (2)	N/A	
Highest AST level (IU/L)		26 (21-33)	67 (36-140)	<0.001
Highest ALT level (IU/L)		25 (20-38)	52 (25-115)	<0.001
Agonal dWIT (minutes)[a]		7 (5-9)	14 (9-20) [c]	<0.001
Asystolic dWIT (minutes)[b]		11 (8-14)	12 (9-17)[d]	0.03

Data are shown as median (IQR) and frequency (valid percentages). Due to rounding, percentages may not add to 100%. a: Agonal dWIT is defined as the time between administration euthanatics (DCD-V) or withdrawal of life support (DCD-III) and cardiac arrest. b: Asystolic dWIT is defined as time between circulatory arrest and cold perfusion. c: Proportion of missing data for this variable is 14.4%. d: Proportion of missing data for this variable is 5.5%. ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, BMI: Body Mass Index, dWIT: donor warm ischemia time, DCD: donation after circulatory death.

In the DCD-V cohort, 30 recipients (64%) were men and 17 (36%) were women, with a median (IQR) age of 56 (48-64) years (Table 2). Median (IQR) recipient warm ischemia time was 39 (32-46) minutes and cold ischemia time was 356 (308-423) minutes. No statistically significant differences in recipient and surgical characteristics were observed between the DCD-V and DCD-III groups. For example, the median (IQR) body mass index (calculated as weight in kilograms divided by height in meters squared) for recipients was 25 (22-29) in the DCD-V cohort and 26 (23-29) in the DCD-III cohort (p-value = 0.12). Hepatocellular carcinoma was the most common indication for transplantation in both groups (13 [28%] vs. 177 [33%]; p-value = 0.10) (Table 2).

Table 2: Recipient and surgical demographics

		DCD-V n = 47	DCD-III n = 542	P-value
Gender				
	Male	30 (64)	401 (74)	0.13
	Female	17 (36)	141 (26)	
Gender mismatch				
	No mismatch	31 (66)	334 (62)	0.66
	Male donor – Female recipient	4 (9)	71 (13)	
	Female donor – Male recipient	12 (26)	137 (25)	
Age (years)		56 (48-64)	58 (51-64)	0.35
BMI (kg/m²)		25 (22-29)	26 (23-29) ∫	0.12
Indication for transplantation				
	Hepatocellular carcinoma	13 (28)	177 (33)	0.16
	Alcoholic liver cirrhosis	9 (19)	129 (24)	
	Cholestatic diseases (PBC/PSC)	6 (13)	56 (10)	
	Cirrhosis due to viral hepatitis	2 (4)	45 (8)	
	Cryptogenic cirrhosis	1 (2)	23 (4)	
	Acute liver failure	3 (6)	6 (1)	
	NASH	1 (2)	15 (3)	
	Other	12 (26)	81 (17)	
Laboratory MELD score		16 (11-23)	15 (10-20)[a]	0.19
Surgery				
rWIT (minutes)		39 (32-46)	39 (31-46)[b]	0.48
CIT (minutes)		356 (308-423)	373 (295-461)[b]	0.38

Data are shown as median (IQR) and frequency (valid percentages). Due to rounding, percentages may not add to 100%. ∫: Proportion of missing data for this variable is 20.7%. a: Proportion of missing data for this variable is 1.3%. b: Proportion of missing data for this variable is 0.2%. BMI: Body Mass Index, CIT: Cold Ischemia Time, DCD: Donation after Circulatory Death, MELD: Model for End stage Liver Disease, rWIT: recipient Warm Ischemia Time.

Postoperative course

The peak median (IQR) serum levels of both aspartate aminotransferase (895 [606-2047] IU/L vs. 1505 [837-3099] IU/L; P = .003) and alanine aminotransferase (674 [450-1223] IU/L vs. 1063 [544-2136] IU/L; P = .02) within week 1 after the LT were statistically significantly lower in the DCD-V cohort than in the DCD-III cohort (Table 3). However, no significant difference was found in the occurrence of early allograft dysfunction after the LT (13 [31%] vs. 219 [45%]; P = .09).

A total of 7 patients (15%) who underwent an LT with DCD-V graft had a diagnosis of non-anastomotic stricture of the biliary tree within the first year after the LT. This number was not statistically significant, compared with 83 patients (15%) in the comparative DCD-III cohort. Rates of primary non-function (2 [4%] vs. 9 [2%]) and hepatic artery thrombosis (3 [6%] vs. 23 [4%]) did not differ between the DCD-V and DCD-III cohorts (Table 3).

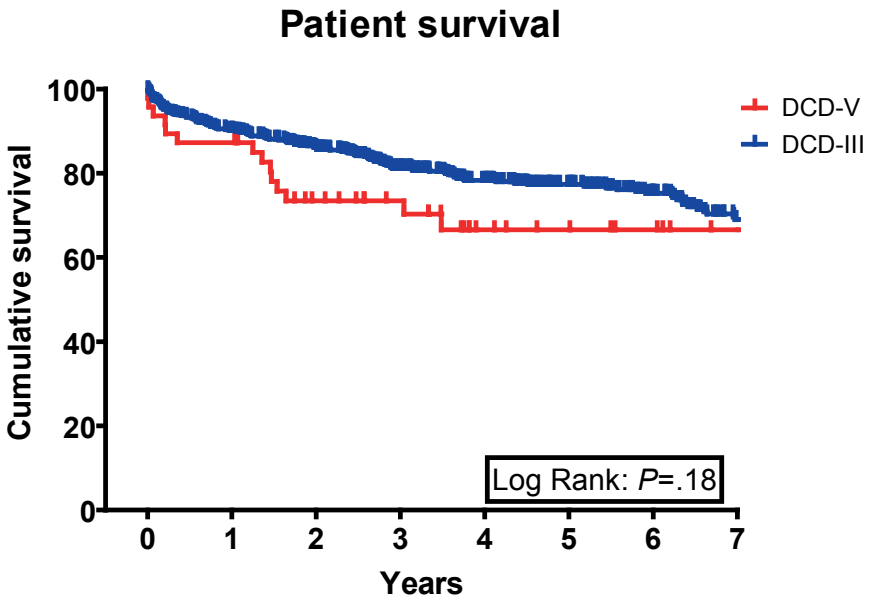
Table 3: Post-operative demographics and complications

	DCD-V n = 47	DCD-III n = 542	P-value
ICU stay (days)	3 (2-6)	3 (2-6)	0.82
Hospital stay (days)	17 (14-31)	18 (13-26)	0.73
AST peak first week (IU/L)[a]	895 (606-2047)[b]	1505 (837-3099)[c]	0.003
ALT peak first week (IU/L)[a]	674 (450-1223)[b]	1063 (544-2136)[c]	0.02
Bilirubin level day 7 (µmol/L)[a]	44(20-100)[b]	29 (16-72)[d]	0.16
Primary non-function	2 (4)	9 (2)	0.22
Early allograft dysfunction[a]	13 (31)[b]	219 (44.5)[c]	0.09
Hepatic artery thrombosis[e]	3 (6)	23 (4)	0.45
Non-anastomotic strictures[e]	7 (15)	83 (15)	0.94

Data are shown as median (IQR) and frequency (valid percentages). Due to rounding, percentages may not add to 100%. a: Patients who died or underwent retransplantation within seven days post-LT were excluded. b: Proportion of missing data for this variable is 10·6%. c: Proportion of missing data for this variable is 4·8%. d: Proportion of missing data for this variable is 9·2% e: Development of complication within the first year after transplantation. ALT: Alanine Aminotransferase , AST: Aspartate Aminotransferase, DCD: Donation after Circulatory Death, ICU: Intensive Care Unit, INR: International Normalized Ratio.

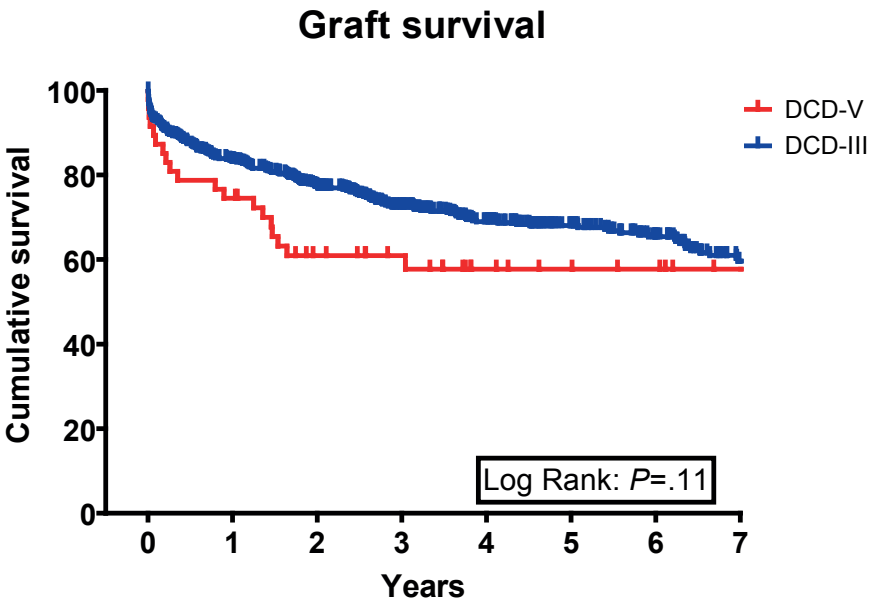
Recipient and graft survival

Recipient survival in the DCD-V cohort was 87% at 1 year, 73% at 3 years, and 66% at 5 years after LT. These rates did not differ significantly from the survival rates in the comparative cohort: 90% at 1 year, 81% at 3 years, and 77% at 5 years post-transplant (log-rank p-value = 0.18) (Figure 1). Graft survival among DCD-V recipients was 74% at 1 year, 61% at 3 years, and 57% at 5 years. In the DCD-III cohort, graft survival was 83% at 1 year, 72% at 3 years, and 68% at 5 years after LT (Figure 2). This difference in survival was not statistically significant (log-rank p-value = 0.11).



Number entering interval	0	1	2	3	4	5	6	7
DCD-V	47	41	29	23	13	10	7	3
DCD-III	542	480	427	340	274	206	159	104

Figure 1: Kaplan-Meier Curve of Recipient Survival From Liver Graft Donation After Circulatory Death Type V (DCD-V) vs. Type III (DCD-III)
DCD-III liver grafts were donated after a planned withdrawal of life-supporting treatments. DCD-V liver grafts were donated after euthanasia.



Number entering interval	0	1	2	3	4	5	6	7
DCD-V	47	35	24	19	11	8	6	2
DCV-III	542	441	383	304	236	174	135	88

Figure 2: Kaplan-Meier Curve of Graft Survival in Recipients of Liver Graft Donation After Circulatory Death Type V (DCD-V) vs. Type III (DCD-III)
DCD-III liver grafts were donated after a planned withdrawal of life-supporting treatments. DCD-V liver grafts were donated after euthanasia.

Discussion

To our knowledge, this study is the largest research thus far into the outcome of LT with grafts donated after euthanasia. The results show that LT with DCD-V liver grafts have recipient and graft survival rates that are similar to those of the more commonly performed LT with DCD-III grafts. Accordingly, DCD-V liver grafts can be used to enlarge the DCD donor pool by approximately 7%. However, because both the experience with this type of graft is limited and the results are not superior to those of LT with DCD-III, liver grafts donated after euthanasia should be considered extended-criteria grafts.

The results of the present study are not in line with our hypothesis that LT with DCD-V grafts have superior outcomes compared with LT with DCD-III grafts and that these outcomes may even be similar to outcomes of LT with grafts donated after brain death, which had a 5-year recipient survival rate of 80% and graft survival rate of 70%. (22)

This finding could be associated with a number of factors. First, patients who request euthanasia are often physically weakened. Because of their medical condition, patients can develop muscle atrophia, sarcopenia, and malnutrition. These conditions could have detrimental implications for the liver graft. Donors in the DCD-III cohort, especially those with trauma, often had a blank medical history. Second, the association between euthanatics and the DCD-V liver grafts is unclear. The non-depolarizing neuromuscular blocking agent is given in a relatively high dose and could therefore be hepatotoxic, especially given that this medication is eliminated mainly by the liver (through bile) and kidneys. (23) Furthermore, the postmortal effects of these medications as well as their effect during the first minutes of the cold flush of the graft is unknown. Further research into the effect of euthanatics on liver grafts is recommended. Meanwhile, the use of normothermic machine perfusion or normothermic regional perfusion to test the viability of DCD-V liver grafts may be helpful.

Optimal logistics is mandatory in the field of organ transplantation, especially when using high-risk grafts, which may describe DCD-V liver grafts. Therefore, a local allocation policy of DCD-V grafts, as used in the study by Gilbo et al., could facilitate optimal recipient selection. (7) Furthermore, the cold ischemia time can be kept as short as possible given that both organ procurement and transplantation are performed by a single team.

As we hypothesized, the agonal phase of the dWIT was significantly shorter among donors in the DCD-V cohort compared with donors in the DCD-III group. However, this shorter agonal phase did not seem to be associated with superior survival rates among recipients of DCD-V grafts compared with recipients in the DCD-III group. We were unable to calculate the functional dWIT in this study. Research has shown that an oxygen saturation of less than 80% should be considered as the start of the functional dWIT. (20) However, in LT with DCD-V grafts, the donor oxygen saturation and blood pressure levels are often not measured. In the few cases in which these parameters were measured, it was done noninvasively to minimize harm to the patient. This measurement cannot be compared with the typically invasive measurement method (ie, venous or arterial catheter) used in patients in the DCD-III cohort. Therefore, we chose the time of administration of euthanatics as the starting point of dWIT.

Significantly lower levels of alanine aminotransferase and aspartate aminotransferase were found in donors in the DCD-V cohort, which probably were associated with the lower post-transplant peak of aminotransferase levels. This finding may seem contradictory to our earlier statement that patients in the DCD-V cohort are physically weakened. However, donors in the DCD-III cohort, rather than those in the DCD-V group, are prone to having elevated transaminase levels associated with their traumatic or nontraumatic brain injury or cardiovascular event with possible resuscitation. (24-27) The absolute difference in transaminase levels between the two groups may be too small to have altered the outcome.

The DCD-V cohort comprised a substantially higher proportion of women. Although this finding was statistically nonsignificant in the current research, a higher risk of gender mismatch may be present among recipients of DCD-V liver grafts, especially woman-to-man transplantation. Research has shown that this type of gender mismatch is associated with lower survival rates. (28, 29)

When we compared the present study with the literature, we observed that recipient and graft survival rates at 3 years after LT with DCD-V grafts were substantially higher in the single-center analysis of Gilbo et al. than in this multicenter study. (7) This difference may be associated with both logistic and allocation policy differences between the Dutch and Belgian DCD cohorts.

Strengths and limitations

This study has some strengths. First, the study has a multicenter and international design, which enabled the inclusion of, to our knowledge, the largest population of donors and recipients of LT with DCD-V grafts reported in the literature. Second, we believe this study has the ability to create awareness about donation after euthanasia among the medical community and the general public.

According to the Dutch guideline, the conversation regarding the possibility of organ donation after euthanasia must be initiated by the patient and not by the physician. (18) The implementation of the new Donor Act in the Netherlands has revived the debate on whether this recommendation is ethical. (30, 31) On one hand, informing a patient about organ donation after euthanasia may put social pressure on the patient, which could potentially lead to a breach of trust. This conversation could be seen as a violation of a basic ethical principle in medical practice: primum non nocere (first, do no harm). On the other hand, withholding this information violates another important medical principle: patient autonomy. In both euthanasia and organ donation, the ability of patients to make their own choice using all available information is fundamental. Especially if the patient is registered as an organ donor, autonomy could be hampered if the physician does not inform the patient.

This study has some limitations. First, the sample size of the DCD-V group was relatively small. This limited size prevented us from performing more robust statistical analyses, such as regression analysis, to identify independent risk factors for inferior outcome of LT with DCD-V grafts. Second, even though many Dutch and Belgian transplant centers prospectively collect data on LT performed in their centers, the study design was retrospective and therefore prone to bias.

Conclusions

This cohort study found that LT with DCD-V liver grafts achieved results comparable to those in LT with DCD-III grafts. This finding suggests that DCD-V is a valuable source for increasing the organ donor pool. However, liver grafts from these types of organ donations should still be considered high-risk grafts that require an optimal donor selection process and favorable logistics.

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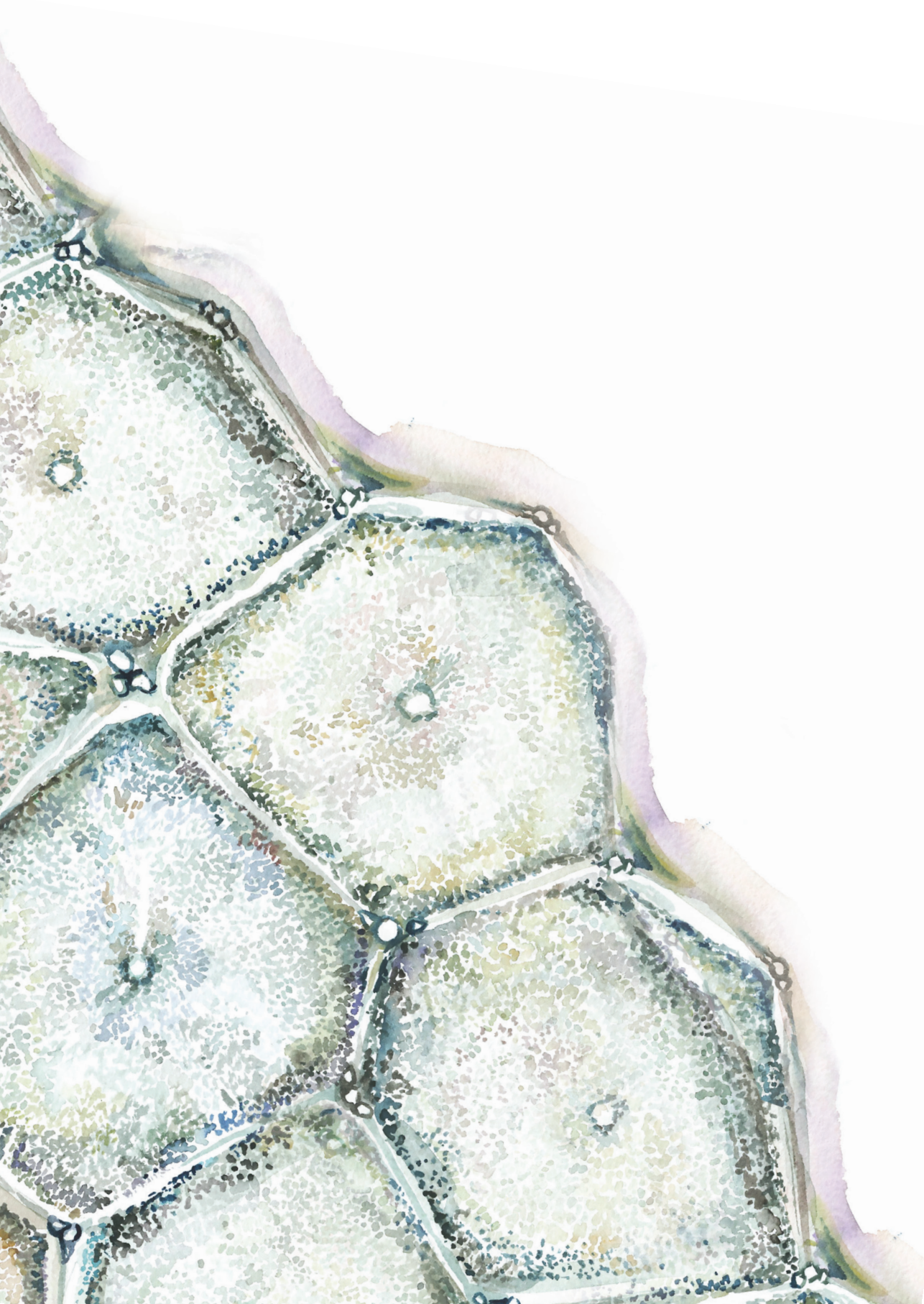

CHAPTER 9

General discussion and future perspectives

Over the years, several studies – including our study described in **chapter 3** – have addressed the lack of uniformity among transplant centers and countries regarding their policies about solid organ transplantation with grafts donated after circulatory death (DCD). (1-3) The common conclusion was that (inter)national consensus on certain aspects of DCD procurement and transplantation (e.g. a universal definition of donor warm ischemia time) would be highly beneficial in order to assure both donors and recipients a consistent standard of care . In 2019, the International Liver Transplant Society (ILTS) established a working group on DCD, Liver Preservation and Machine Perfusion. The 41 transplant experts participating in this working group were set to the task of critically and systematically reviewing the current literature on several topics related to DCD liver transplantation, including preservation and machine perfusion, and drafting propositions and recommendations on these topics. In January 2020, the working group's achievements were presented at an international consensus conference on DCD-LT, hosted by the ILTS. All 151 attendees were given the opportunity to comment on the statements and recommendations, and suggest emendations. After the consensus conference, the the working group finalized the statements and recommendations and published these as ILTS guidelines. (4-7) It is worth mentioning that the results of **chapters 7 and 8** have been incorporated in these guidelines. Furthermore, with the publication of our DCD-LT benchmarks study **(chapter 4)**, we have fulfilled the ILTS's recommendation to establish unique benchmarks for best achievable outcomes in DCD-LT. Table 1 provides a selection of the recommendations.

Table 1: Summary of ILTS guidelines on DCD-LT, adapted from (4-7)

Topic	Guidance
Donor risk factors	Routinely use DCD livers from donors aged ≤ 60 years, respecting other risk factors
	Routinely use DCD liver grafts from controlled DCD donors with a BMI of ≤ 25 kg/m^2
	In the absence of machine perfusion, avoid the use of DCD liver grafts with > 30% macrovesicular steatosis
DCD liver graft procurement	WLST may be preferentially performed in the operating room to minimize the fdWIT
	tdWIT should be specified as the time between withdrawal of treatment and cold flush
	The start of the fdWIT is defined as the timepoint where either the oxygen saturation < 80% or mean arterial pressure < 60mmHg
	fdWIT is of greater utility than tdWIT to assess the post-transplant risk of graft loss
	In case of a fdWIT of > 30 minutes, an increased risk for graft loss should be taken into account
	Donor hepatectomy time should be kept as short as possible (at most 60min from start of cold preservation).

Topic	Guidance
Adjuncts during DCD liver recovery	Fibrinolytic agents should be avoided in DCD donors, grafts, and recipients.
	Where it is legally permitted and in the absence of contraindications (e.g., intracranial hemorrhage), heparin should be given before WLST
	The cold preservation solution HTK should be avoided in DCD livers in cases where cold ischemia is estimated to be > 8 hours
Recipient risk factors	There is no specific recipient BMI cutoff for the use of DCD liver grafts
	Routinely use DCD livers in candidates with a laboratory MELD score of ≤ 25 points
	The use of DCD liver grafts for recipients with HCC, PSC, of PBC is not contraindicated
	Use livers from DCD donors for candidates with NASH selectively and in context of the overall medical risk
	Use DCD liver grafts selectively for recipients requiring a retransplantation and recipients listed for acute liver failure or with a high urgency status
Cold ischemia time	Implant controlled DCD donor livers ideally within 8 hours of cold ischemia
	Avoid the use of DCD liver grafts with a CIT of > 12 hours
	Use of the preservation solution HTK should be avoided in DCD liver grafts in which the expected CIT of > 8 hours
Early allograft dysfunction and complications	No recommendation can be made on the use of any specific model to define EAD because of the lack of validation studies in DCD-LT
	Future studies determining EAD should investigate the interactions between donor, recipient and perioperative factors in DCD-LT
	Unique benchmarks for best achievable outcomes in DCD-LT should be established
DCD after euthanasia	Category V DCD liver transplantation appears to offer results comparable to those of category III controlled DCD, and the use of livers arising through this process can be explored further.

BMI, body mass index; DCD, donation after circulatory death; dWIT, donor warm ischemia time; EAD, early allograft dysfunction; fdWIT, functional donor warm ischemia time; HCC, hepatocellular carcinoma; HTK, histidine-tryptophan-ketoglutarate; LT, liver transplantation; MELD, model for end-stage liver disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; tdWIT, total Donor Warm Ischemia Time; WLST, Withdrawal of Life Supporting Treatments;

An important aspect of DCD-LT that still lacks consensus is the definition of non-anastomotic strictures, also known as ischemic cholangiopathy (IC). In a systematic review from 2014 on biliary complications among recipients of a orthotropic liver graft, the incidence of IC after DCD-LT varied substantially across the individual studies. (8) The lowest incidence was 3%; the highest was 39%. (9) This large variability was partially the result of the variation in definitions of IC the individual transplant centers had formulated.

Therefore, it would be necessary for a new working group to review the current literature on biliary complications after DCD-LT and introduce a uniform definition of IC to be used by all transplant centers worldwide. A pivotal question the working group should address is whether the term 'ischemic cholangiopathy'

is tenable. In 2018, De Vries and colleagues proposed to replace this term with 'post-transplant cholangiopathy'. (10) Nevertheless, this suggestion is hardly reflected in the current literature.

Another topic the working group should address is whether different subtypes of IC must be distinguished. According to De Vries and colleagues, three subtypes of post-transplant cholangiopathy can be distinguished, which can occur separately or combined in a liver graft: (I) non-anastomotic biliary strictures of the extrahepatic or large intrahepatic bile ducts; (II) intraductal biliary cysts; and (III) bile duct necrosis with intrahepatic leakage and biloma formation. (10) More recently, Croome and colleagues have classified four distinct patters of IC; see Table 2. (11)

Table 2: Subtypes of ischemic cholangiopathy, adapted from (11)

Pattern of IC	Explanation
Diffuse necrosis	Diffuse narrowing of the intrahepatic bile ducts with irregularities and filling defects
Bilateral multifocal/ multifocal progressive	Mild to moderate stenosis of the second-order and peripheral bile ducts, progressively worsening over time
Confluence dominant	Strictures and casts confined to the biliary confluens, with relative preservation of the second-order and peripheral bile ducts
Minor form	Mild radiologic abnormalities, never developing to more extensive strictures

IC, ischemic cholangiopathy;

Croome and colleagues found that each pattern was associated with a specific clinical course. For example, the occurrence of pruritus was significantly higher among DCD liver grafts recipients who developed diffuse necrosis or multifocal progressive IC, when compared with patients with the confluence dominant or minor form of IC. Furthermore, all patients with diffuse necrosis required biliary stenting, whereas only 11% of patients with a minor form of IC required stenting. Besides the differences in clinical course among the four patterns of IC, graft survival differed significantly between the four groups, being the lowest in the diffuse necrosis and multifocal progressive groups. (11)

Other important topics that should be addressed in developing a uniform definition of IC are: (I) the diagnostic modalities needed to diagnose IC; (II) whether or not to include biliary irregularities that do not require endoscopic or surgical interventions; and (III) whether or not to include irregularities/strictures of the biliary tree in the absence of clinical symptoms (i.e. pruritus, jaundice, cholangitis and elevated liver enzymes).

Quality control and continuous learning

Since the field of liver transplantation is rapidly evolving, as can be concluded from the more than ten thousand articles published in the last five years that use the Medical Subject Heading (MESH) liver transplantation, transplant centers would do well to regularly appraise their performance. Our study on benchmarks in DCD-LT **(chapter 4)** provides an important tool for evaluating local DCD-LT programs. First, transplant surgeons and physicians can easily calculate the proportion of DCD-LT performed in their center that fulfill the benchmark criteria and are therefore considered as the 'ideal' DCD-LT cases with relatively the lowest donor and recipient risk. This proportion can be used as covariate when comparing the outcomes of DCD-LT between different transplant centers, enabling a fairer comparison of different DCD-LT programs. Furthermore, if a center's proportion of benchmark cases differs substantially from that of other centers, this may be a reason to evaluate this center's policy on accepting or declining liver grafts. Second, transplant centers can assess whether their outcomes exceed the benchmark thresholds which we have proposed to serve as reference values. If this is the case, efforts should be directed at finding a possible explanation as well as implementing interventions to improve the outcomes.

As shown in the previous paragraph, scientific research can help pinpointing possible shortcomings in performance. In **chapter 5**, we found that the median donor hepatectomy time in the Netherlands was significantly longer than that in other countries of the Eurotransplant region and the United Kingdom. (12, 13) In response, the Dutch Transplant Society implemented several strategies to shorten the donor hepatectomy time, such as renewed education on organ procurement and exchanging knowledge between procurement teams. (10) These strategies have proven to be successful, seeing that the median donor hepatectomy time in the Netherland has decreased with more than twenty minutes over the past years.

Organ donation after euthanasia starting at home

The study presented in **chapter 8** dealt with the outcomes of LT with grafts donated after euthanasia (DCD type V). We recommended that the complete euthanasia procedure should be performed in the hospital, although case-reports have been published in which the euthanasia procedure was started at the donor's home. (14) Shortly after the publication of our study, Sonneveld and Mulder published a letter to the editor in which they stated that our recommendation was unsubstantiated and potentially damaging to the concept of organ donation after euthanasia starting at home (ODEH). (15)

We never intended to depict ODEH as inferior to organ donation after a complete euthanasia procedure in the hospital. As the (inter)national experience with ODEH is scarce – e.g., until June 2020 only three cases are known in the Netherlands, – we think that developing a guideline on ODEH is called for. In the meantime, organ donation after euthanasia performed completely at the hospital should be advocated.

Since organ donation after euthanasia and ODEH are delicate matters, developing a guideline on ODEH should not be the work of physicians only, but a joint project including medical ethicists, jurists and members of patient associations. Important issues to address are possible periprocedural complications and how to act on these. For example, is it allowed to administer inotropic agents during transport of a patient whose blood pressure drops below the level required for adequate organ perfusion? Or, in the case of a cardiac arrest before administration of the non-depolarizing neuromuscular blocking agent, should we refrain from resuscitation? A recently published case report by Tajaâte and colleagues perfectly describes – in our opinion – a case of ODEH in which all periprocedural risks were anticipated on. (16) Moreover, all risks of complications had been discussed with the patient and his family. We think this case report can serve as the foundation for the development of a guideline on ODEH.

Future perspectives

The results from the studies presented in this thesis lead to new research questions. One important aspect of DCD-LT that deserves more thorough study is whether the reperfusion sequence affects the outcome of DCD-LT. A randomized controlled trial in which adult recipients of a primary DCD liver graft will be randomized between (A) initial portal vein reperfusion, (B) initial hepatic artery reperfusion, or (C) simultaneous reperfusion, is highly recommended to answer this question. All grafts included in such a trial should have been preserved using static cold storage, to minimize the risk of bias by including grafts that have been preserved by different types of machine perfusion. The primary endpoint of this study could be the development of ischemic cholangiopathy. Since early dysfunction of the liver graft is associated with inferior outcomes after LT, their determinants (post-transplant levels of transaminases, bilirubin and creatinine) could be secondary outcome measures. (17, 18) In a subsequent study these primary and secondary outcomes measures can be assessed in DCD liver grafts that have been preserved using machine perfusion.

In several studies, we determined peak alanine transaminase (ALT) and aspartate transaminase (AST) levels in the first week after DCD-LT, as well as the bilirubin level on the seventh day post-transplant. It is important to have these values since they are incorporated in the most commonly used definition of early

allograft dysfunction, the Olthoff criteria. (18) However, the Olthoff criteria have not been specifically evaluated for the DCD-LT population. (19-22) Therefore, it could be of great interest to validate the existing models for EAD among the 2219 cases of DCD-LT that have been included in the DCD-LT Benchmark study **(chapter 4)**. Besides, the raw data of that study can be used to develop a DCD-LT-specific model for early allograft dysfunction. Of note, an adapted definition of early graft dysfunction is probably necessary for livers that have been exposed to machine perfusion, since post-operative peak levels of AST and ALT have proven to be significantly lower after machine perfusion when compared to static cold storage. (23) These lower levels are likely to be the result of dilution, because in machine perfusion a substantially higher amount of perfusate is used (washout effect). Moreover, when oxygenated machine perfusion is used, the accumulated transaminases in the graft will be released in the perfusate instead of being released in the recipient immediately post-transplant. (24)

The study presented in **chapter 7** demonstrated that DCD grafts can be used for the purpose of retransplantation. Of course, we are fully aware of the limited sample size of the study and that the findings should be interpreted cautiously. Nevertheless, in many transplant centers patients listed for retransplantation are still not considered eligible for receiving a DCD graft. The Universitätsspital Zürich is currently finalizing a manuscript of a benchmark study on retransplantation. including 1063 cases from 21 transplant centers. We encourage the principal investigators of this study to create two separate sets of benchmark cutoffs: one for retransplantations with grafts donated after brain death and one for retransplantations with grafts donated after circulatory death. If the benchmark values do not differ significantly between the two groups, this finding could perhaps convince transplant physicians and surgeons that DCD grafts are acceptable for the purpose of retransplantation.

In **chapter 8**, we hypothesized that the outcomes of DCD-LT with grafts donated after euthanasia (e.g., type V DCD-LT) would be better than the outcomes of type III DCD-LT. The analysis revealed, however, similar outcomes. A possible explanation is toxicity of the euthanatics for the liver. Therefore, we will start an in-vitro project in which primary hepatocytes are exposed to several different dosages of thiopental, propofol or rocuronium in order to create a dose-response curve. In parallel, blood will be collected from DCD-V donors, and serum levels of the coma-inducing drug (either thiopental or propofol) and rocuronium will be measured. With this information we can assess if there is any hepatotoxicity of euthanatics. If this indeed seems to be the case, further research should focus on the development of a tool to assess whether a liver graft of a specific DCD-V donor has been exposed to toxic concentrations of euthanatics – for example by measuring the concentrations of euthanatics in machine perfusate.

Based on the promising results of studies investigating machine perfusion in liver grafts, the question is not *if*, but *when* machine perfusion will become the gold standard, especially for grafts from extended criteria donors, such as DCD liver grafts. However, of all relevant studies published over the last decade, only three were randomized controlled trials, which are known to have the highest level of evidence in evidence-based medicine. (25-30) Therefore, more high-quality research is necessary before machine perfusion can be safely implemented as standard of care. Seven experts in the field of machine perfusion have recently developed twelve recommendations for future clinical trials on liver machine perfusion preservation, as listed in Table 3. (24)

Table 3: Recommendations for future clinical trials regarding machine perfusion In liver transplantation, adapted from (24)

	Recommendations
1.	Use of standardized nomenclature
2.	Registration of study in public trial registries and publication of study protocol in peer-reviewed journal
3.	A randomized controlled trial or meta-analysis of existing trials studies are study designs of choice
4.	Randomization time should depend on primary endpoint of trial (by last at organ offer when assessing organ utilization rate, at final organ acceptance when assessing post-transplant outcomes)
5.	Develop multicenter consortia trials rather than single center trials
6.	Implementation of an international registry of all machine perfusion cases in liver transplantation
7.	Use clinical data as primary outcomes instead of surrogate laboratory endpoints
8.	Use static cold preservation as control cohort first before comparing different machine perfusion techniques
9.	Redefinition of early allograft dysfunction
10.	Intention-to-treat analysis
11.	Collection of biospecimen is recommended (e.g. bile, biopsies of liver and bile duct)
12.	Contingency plan (i.e. back-up allocation of graft)

With the growing interest in personalized medicine, it would be highly beneficial to develop a prediction model especially for the DCD-LT population, in which not only donor and recipient characteristics are incorporated, but also procurement-related factors such as donor warm ischemia time. This model should be presented as a risk calculator that can be accessed by anyone, free of charge. Rather than a categorical outcome parameter (e.g., low risk, intermediate risk and high risk), the result of the risk calculator should be presented as the probability of event-free survival. The event of interest can be either graft loss, death of any cause or ischemic cholangiopathy. If all variables incorporated in the risk calculator are

available before transplantation, such a calculator enables transplant physicians and surgeons to find a perfect match between donor and recipient.

Conclusion

As a result of the growing imbalance between the demand for liver grafts and the availability of liver grafts, the use of grafts donated after circulatory death has grown substantially since the beginning of this century. New surgical techniques, machine perfusion and new insights on for example the pathophysiology of early allograft dysfunction and biliary complications, have led to a new era in which DCD-LT can become as successful as LT with DBD grafts.

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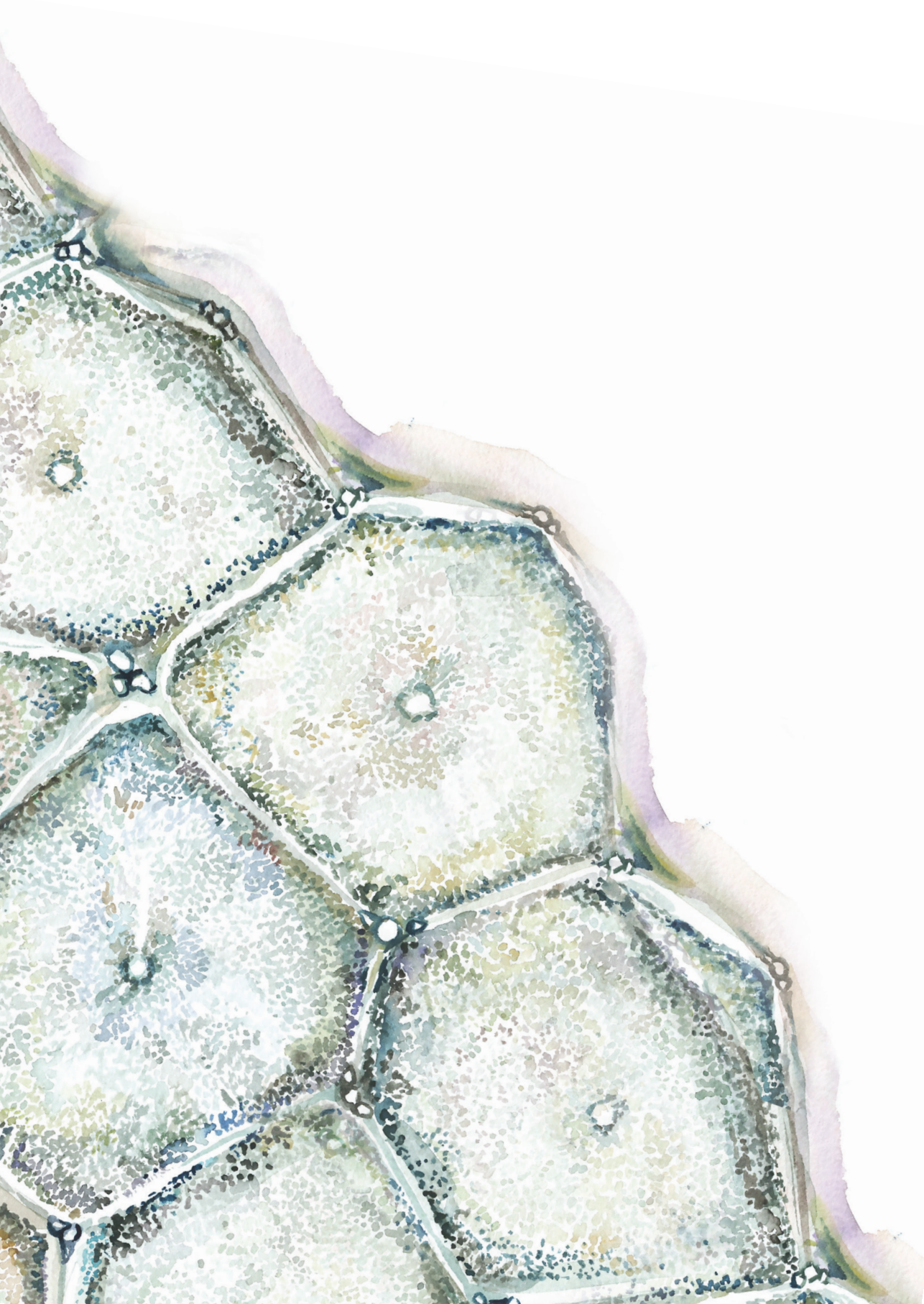

CHAPTER 10

Summary

The studies performed during the course of my PhD-trajectory aimed to give actual insight in the state of liver transplantation with grafts donated after circulatory death (DCD-LT) in the Netherlands as well as to find new ways to use this type of liver graft to its full potential.

Chapter 2 gives an insight into 20 years of DCD-LT in the Netherlands. Over the span of two decades, 600 DCD-LT have been performed in the Netherlands. While substantial changes in characteristics of both the donor and recipient population have occurred, patient and graft survival of DCD-LT have remained stable. Nowadays, approximately one third of all liver transplantations performed in the Netherlands make use of a DCD graft.

Chapter 3 reports the results of an internet-based survey we conducted among 119 transplant surgeons in four European countries – Belgium, the Netherlands, Spain and the United Kingdom – on their practices regarding DCD-LT. It appeared there were major differences in practices between and even within countries. For example, the majority of the respondents from Spain and the United Kingdom defined the starting point of the donor warm ischemia time as the moment when the saturation/blood pressure drops below a certain threshold, whereas over half of the Dutch respondents take the moment of cardiac arrest as starting point. Furthermore, a high rate of respondents had violated national or center-specific DCD-LT protocols on topics such as the donor and recipient upper age limits. These survey results led us to conclude that the development and implementation of an international consensus guideline would be highly beneficial to ensure a consistent standard of patient care and to legitimately compare the outcomes of DCD-LT between and within countries.

To identify the best possible outcomes after DCD-LT, we made use of the concept of benchmarking **(chapter 4)**. To this aim, we selected 1012 'low-risk' DCD-LT from a cohort of over 2000 controlled DCD-LT performed in 17 transplant centers in Europe and North America. The outcomes of these benchmark cases served to set cut-off target values for the most relevant parameters. In the benchmark cohort, the one-year survival was 91.6%, with a retransplantation rate of 4.5% within the first year. Despite these relatively good outcome, a relatively high proportion of recipients developed at least one severe complication (i.e. ≥ Grade III complication conform the Clavien Dindo classification) within the first year post-transplant, with a benchmark cut-off of ≤ 66%. Other benchmark cut-offs were ≤ 16 days for hospital stay post-transplant, ≤ 16.8% for ischemic cholangiopathy, and ≤ 38.9 points for the comprehensive complication index at one-year post-transplant. These benchmark cut-offs can serve as comparators in future research as well as references for individual recipients or specific patient groups. In a second part of the study, we compared the outcomes of 'higher–risk' groups (i.e., grafts with a

prolonged donor warm ischemia time, recipients with a higher MELD score, and retransplantations) with those of the benchmark cohort. These comparisons revealed an shortened graft survival among the high-risk group, with outcomes that exceeded the benchmark cut-off values. Lastly, we evaluated the outcomes of DCD-LT with grafts retrieved using machine perfusion. Although most of these grafts had a prolonged donor warm ischemia time – and were therefore considered high-risk, the outcomes were comparable to those of the benchmark group, thus proving the protective value of machine perfusion.

The study presented in **chapter 5** dealt with the effect of the donor hepatectomy time (i.e., the time elapsed between the start of cold perfusion in the donor and the liver being stored on ice) on the development of biliary injury during and after DCD-LT. This nationwide study consisted of three parts. First, we assessed the bile duct injury score (BDI) in bile duct biopsies collected from DCD liver grafts that had been included in a trial on normothermic machine perfusion. All biopsies had been collected prior to the start of machine perfusion. Grafts with a high bile duct injury score (≥ 4.75) had been exposed to a significantly longer donor hepatectomy time when compared with grafts with a low BDI score (p-value = 0.027). Second, we assessed the bile composition in samples collected at standard time points during normothermic machine perfusion. The bile produced by DCD grafts with a prolonged donor hepatectomy time (cut-off 50 minutes based on the receiver operating characteristic curve) was of inferior quality with a higher pH level and a higher concentration of bicarbonate. Third, we related the donor hepatectomy time to the development of non-anastomotic strictures among 237 recipients of a DCD graft. A Cox proportional hazards regression model revealed that with every ten minutes increase in donor hepatectomy time, the risk of the development of non-anastomotic strictures increased by 18%. Thus, we concluded that the donor hepatectomy time should be kept as short as possible.

In **chapter 6**, the focus was shifted from the effect of donor hepatectomy time on the outcomes of DCD-LT towards the influence of the arterialization time (i.e. the time between portal reperfusion and the arterial anastomosis being completed). Based on the findings from this nationwide study on DCD-LT with an initial portal reperfusion sequence, we concluded that arterialization time was not an independent risk factor for the development of early allograft dysfunction and/or non-anastomotic strictures. This conclusion might suggest that the time window in which a transplant surgeon must complete the arterial anastomosis is not too strict. Still, more research on this issue is highly recommended, preferably with a randomized controlled trial in which recipients of a DCD-LT are randomly assigned to one of three different reperfusion sequences (initial portal reperfusion, initial arterial reperfusion and simultaneous reperfusion).

Chapter 7 describes a multicenter, retrospective cohort study on outcomes of 21 retransplantations using DCD grafts (DCD-reLT) compared with outcomes of retransplantation using DBD grafts (DBD-reLT). Using the propensity score matching approach, each DCD-reLT was matched with three DBD-reLT. The propensity score model included the following covariates: year of reLT, transplant center, number of consecutive reLT, interval between prior LT and reLT, both donor and recipient age, MELD score and cold ischemia time. Both patient and graft survival were comparable between DCD-reLT and DBD-reLT. The occurrence of non-anastomotic strictures was higher in the DCD-reLT group (38.1% versus 12.7%, p-value = 0.02). Fortunately, most of these strictures had only required conservatively endoscopic interventions; nor more than two patients had required a new liver graft. With the ongoing development of machine perfusion, we may expect that the incidence of non-anastomotic strictures will decline in the future. Based on the results of this study we have concluded that DCD liver grafts can be safely used for the purpose of retransplantation.

Chapter 8 presents the results of the largest study thus far on the outcomes of liver transplantation with grafts donated after euthanasia (i.e., DCD type V liver transplantation according to the modified Maastricht classification). In this multicenter, retrospective cohort study, 47 DCD-V liver transplantations performed in Belgium and the Netherlands were compared with 542 liver transplantations with a regularly controlled DCD graft (i.e. DCD type III). Donors in the DCD-V cohort had significantly lower levels of alanine aminotransferase and aspartate aminotransferase (25 versus 52 U/l and 26 versus 67 U/l, respectively; both p-values < 0.001). As expected, the donor warm ischemia time was significantly shorter in potential donors in the DCD-V group, whose lives are actively ended. Both patient and graft survival were similar between the two groups as well as the incidence of post-operative complications such as early allograft dysfunction (31% versus 45%; p-value = 0.09) or non-anastomotic strictures (15% in both groups; p-value = 0.94). Hence, liver grafts donated after euthanasia are a justifiable option to increase the donor pool, but should be treated as high-risk grafts requiring adequate recipient selection.

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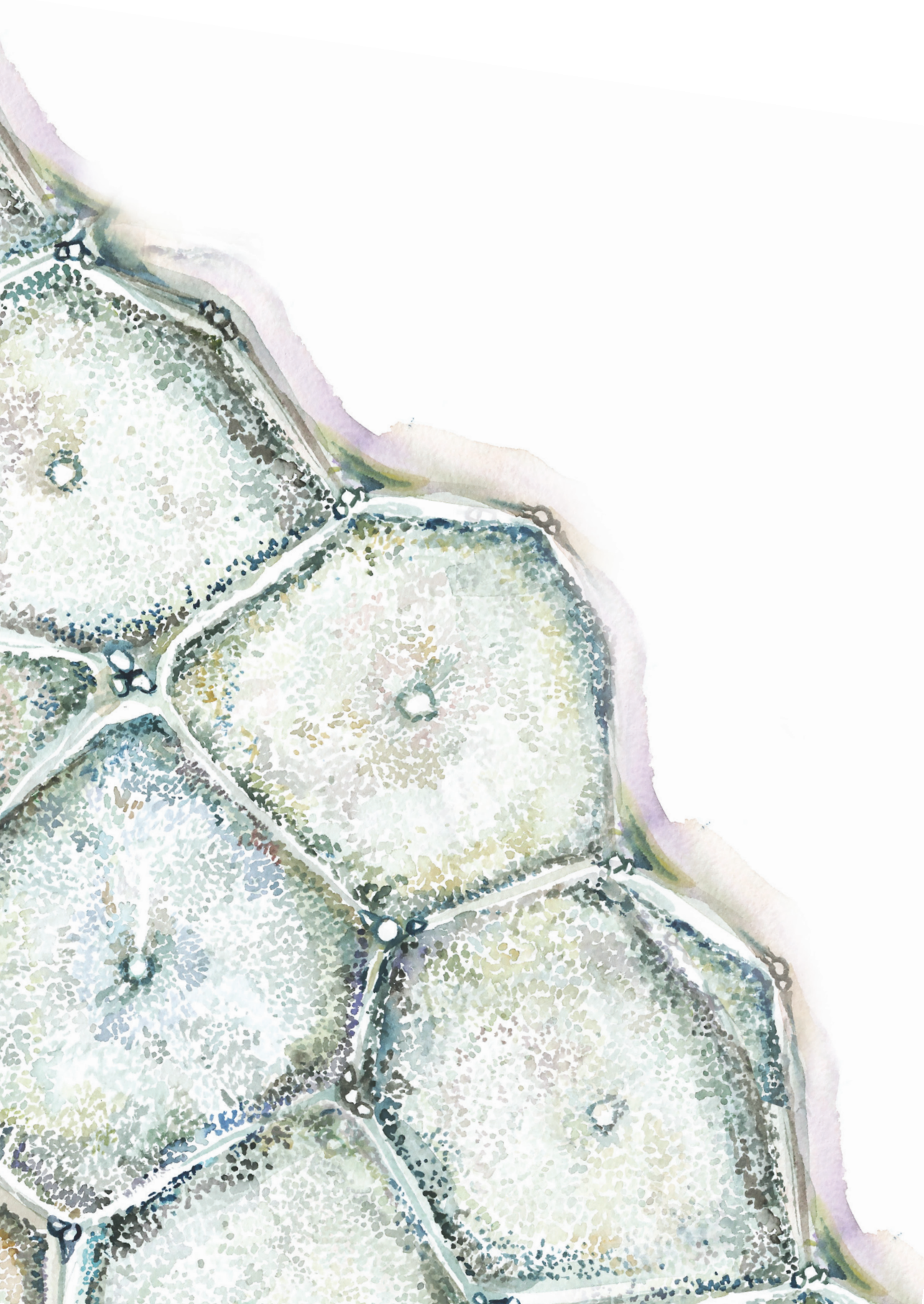


CHAPTER 11

Nederlandse samenvatting

Dit proefschrift tracht de huidige stand van zaken rondom levertransplantatie met organen gedoneerd na circulatiestilstand (DCD levertransplantatie) helder te belichten. Tevens is gezocht naar nieuwe manieren om dit type donorlevers optimaal te benutten, omdat er nog altijd schaarste heerst in het aantal beschikbare donororganen.

Hoofdstuk 2 van het proefschrift biedt een globaal overzicht van 20 jaar DCD levertransplantatie in Nederland. Tussen de implementatie van DCD levertransplantatie in Nederland in 2001 en de zomer van 2020 is een totaal van 600 DCD levertransplantaties verricht. Tegenwoordig wordt bij een derde van de levertransplantaties in Nederland gebruik gemaakt van een DCD lever. De afgelopen twee decennia hebben substantiële veranderingen plaatsgevonden in zowel donor- als ontvangerpopulatie. Het overlevingspercentage is hierbij echter stabiel gebleven.

In **hoofdstuk 3** beschrijven wij de resultaten van een online enquête over DCD levertransplantatie verspreid onder 119 transplantatiechirurgen uit vier Europese landen (België, Nederland, Spanje en het Verenigd Koninkrijk). De resultaten van deze enquête tonen aan dat er grote verschillen bestaan in de werkwijze rondom DCD levertransplantatie tussen de vier verschillende landen, en ook dat er binnen een land verschillen bestaan. Een voorbeeld dat deze internationale variëteit kenmerkt, is de definitie van de warme ischemietijd in de donor (dWIT). Het merendeel van de respondenten uit Spanje en het Verenigd Koninkrijk definieert de start van de dWIT als het moment waarop de bloeddruk of saturatie van de donor onder een vooraf afgesproken waarde daalt. Echter, de meeste Nederlandse respondenten definieert de circulatiestilstand in de donor als startpunt voor de dWIT. Bij de enquête kwam ook naar voren dat een substantieel deel van de respondenten heeft afgeweken van nationale of centrumspecifieke protocollen aangaande DCD levertransplantatie. Hierbij kan gedacht worden aan het overschrijden van de leeftijdsgrens voor DCD donoren. Op basis van de uitkomsten van de enquête hebben wij geconcludeerd dat internationale consensus wenselijk is om patiënten gelijkwaardige zorg te kunnen bieden en om in de toekomst de resultaten van DCD levertransplantatie tussen verschillende landen en/of transplantatiecentra legitiem met elkaar te kunnen vergelijken.

Met behulp van benchmarking hebben wij in **hoofdstuk 4** getracht om de best mogelijke resultaten van DCD levertransplantatie te identificeren. Dit onderzoek bestaat uit drie delen. In het eerste deel zijn 1012 “laag-risico” DCD levertransplantaties geselecteerd uit een cohort van ruim 2000 DCD levertransplantaties die zijn verricht in zeventien transplantatiecentra in Europa en Noord-Amerika. Op basis van de resultaten van de benchmark cases hebben

wij specifieke streefwaarden berekend voor de belangrijkste uitkomstmaten na DCD levertransplantatie. De één-jaarsoverleving na DCD levertransplantatie bedraagt 91.6% in het benchmark cohort met een percentage retransplantaties van 4.5% binnen het eerste jaar. Ondanks deze relatief goede uitkomsten is het percentage ontvangers van een DCD lever dat in het eerste jaar na transplantatie een ernstige complicatie ontwikkelt (Graad III of hoger volgens de Clavien Dindo classificatie) vrij hoog met een berekende streefwaarde van ≤ 66%. Andere belangrijke streefwaarden zijn een opnameduur van ≤ 16 dagen na transplantatie, een ≤ 16.8% incidentie van ischemische cholangiopathie en een maximale CCI (comprehensive complication index) score van 38.9 punten, berekend één jaar na transplantatie. De berekende streefwaarden kunnen fungeren als een ijkpunt voor toekomstig onderzoek alsmede als referentie voor individuele (groepen) ontvangers van DCD levers. In het tweede deel van de benchmarkstudie zijn de uitkomsten van "hoog-risico" DCD levertransplantaties vergeleken met de uitkomsten van het benchmark cohort. Tot de "hoog-risico" groep behoren DCD levertransplantaties met een langere dWIT, ontvangers met een hogere MELD-score (Model for End Stage Liver Disease) en retransplantaties. Uit dit deel van de studie kwam naar voren dat de uitkomsten van de "hoog-risico" groep de in het eerste deel van de studie berekende streefwaarden overschreden. Het derde en laatste deel van de studie stond in het teken van DCD levertransplantatie met levers die gepreserveerd zijn door middel van machineperfusie. Ondanks het feit dat het leeuwendeel van deze levers werd blootgesteld aan een langere dWIT en dus voldeed aan de definitie van onze "hoog-risico" groep, bleek transplantatie met deze levers tot vergelijkbare uitkomsten te leiden als die van het benchmark cohort. Hieruit kan worden geconcludeerd dat machineperfusie een positief effect heeft op de kwaliteit van het te transplanteren orgaan.

In **hoofdstuk 5** van dit proefschrift hebben wij het effect van de hepatectomietijd in de donor - de tijd tussen de start van de koude perfusie in de donor en het moment dat de lever uit het lichaam van de donor is verwijderd en op ijs is geplaatst - op de ontwikkeling van biliaire complicaties na DCD levertransplantatie geëvalueerd. Dit betreft een nationale studie die bestaat uit drie delen. In het eerste deel van de studie hebben wij galwegbiopten van DCD levers beoordeeld. Deze biopten zijn verzameld in het kader van een andere studie naar de effecten van normotherme machineperfusie en zijn afgenomen voordat de lever werd aangesloten op de machine. Het bleek dat galwegbiopten met een hogere mate van galwegschade, gedefinieerd als het hebben van een bile duct injury (BDI) score van 4.75 of hoger, waren blootgesteld aan een significant langere donor hepatectomietijd in vergelijking met galwegbiopten met een lage BDI score (p-waarde 0.027). Voor het tweede deel van de studie is het effect van de donor hepatectomietijd op de samenstelling van gal bestudeerd. Hiervoor is gebruik gemaakt van galmonsters die op verschillende momenten tijdens normotherme

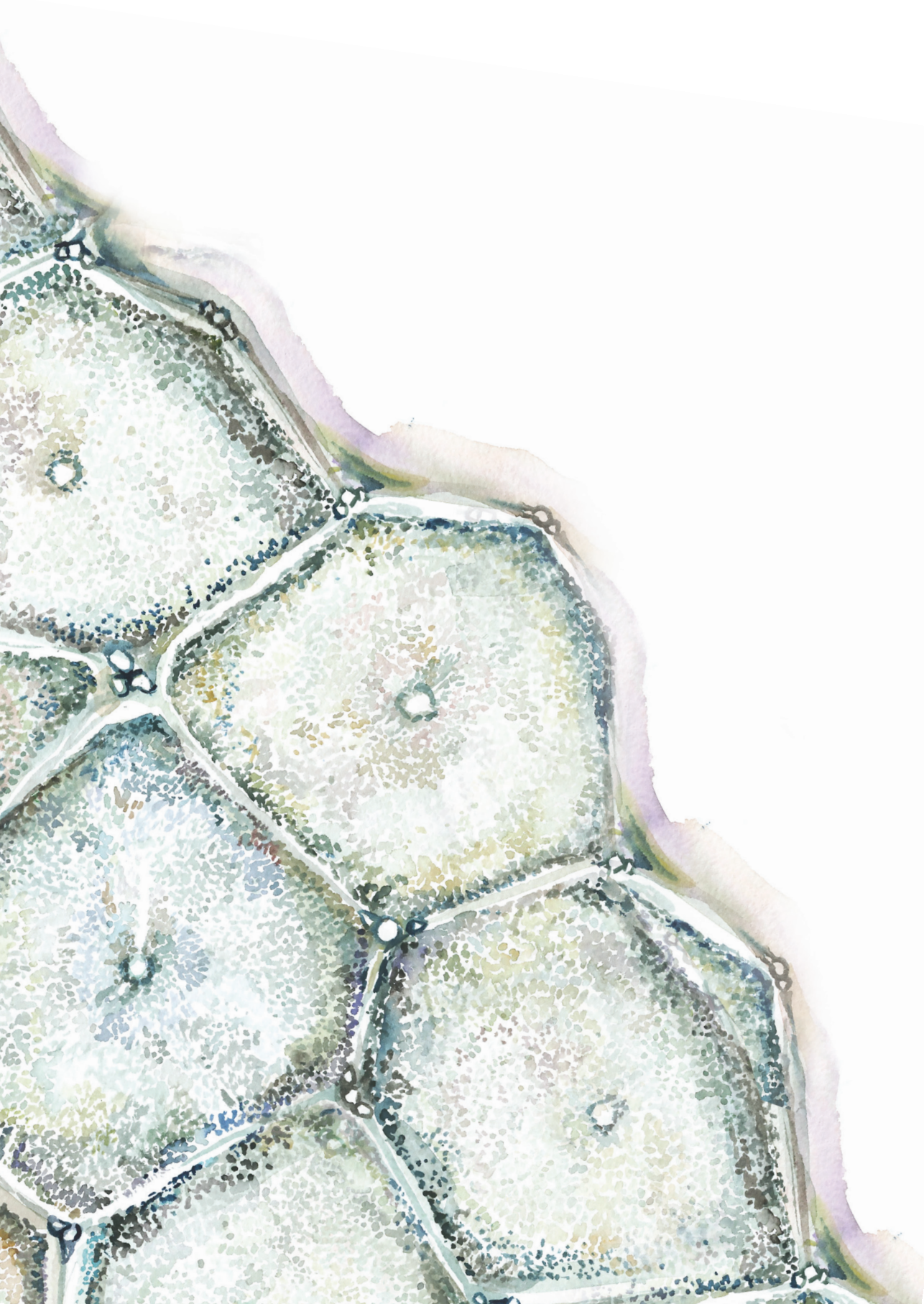
machineperfusie zijn afgenomen. De galmonsters van DCD levers die waren blootgesteld aan een donor hepatectomietijd van 50 minuten of langer, bleken van slechtere kwaliteit (hogere pH en hogere concentratie van bicarbonaat) dan de galmonsters van DCD levers met een kortere donor-hepactectomietijd. De afkapwaarde van 50 minuten was gekozen op basis van de receiver operating characteristic curve. In het derde en laatste deel van de studie hebben wij het effect van de duur van de donor hepatectomietijd op het ontwikkelen van niet-anastomotische stricturen onderzocht in een cohort van 237 ontvangers van een DCD lever. Op basis van de resultaten van een Cox-regressie model hebben wij geconcludeerd dat donor hepatectomietijd een onafhankelijke risicofactor is voor het ontwikkelen van niet-anastomotische stricturen; met iedere tien minuten toename van donor hepatectomietijd neemt het risico op het ontwikkelen van niet-anastomotische stricturen met 18% toe. Op basis van resultaten van de drie onderdelen tezamen, hebben wij geconcludeerd dat het noodzakelijk is de donor hepatectomietijd zo kort mogelijk te houden.

In **hoofdstuk 6** wordt aandacht geschonken aan het effect van de arterialisatietijd - de tijd tussen reperfusie van de vena porta en reperfusie van de arteria hepatica in de ontvanger - op de resultaten van DCD levertransplantatie. In deze nationale, retrospectieve studie, bestaande uit 292 DCD levertransplantaties, werd door middel van een Cox-regressie model aangetoond dat de arterialisatietijd geen risicofactor is voor het hebben van vroege transplantaatdysfunctie (early allograft dysfunction) en niet-anastomotische stricturen. Ondanks het feit dat de resultaten van deze studie suggereren dat een transplantatiechirurg geen tijdslimiet heeft voor het creëren van de arteriële anastomose, is nader onderzoek strikt noodzakelijk. Bij voorkeur door middel van het ontwikkelen van een randomized controlled trial waarin de verschillende reperfusie technieken (initiële portale reperfusie, initiële arteriële reperfusie en simultane reperfusie) met elkaar worden vergeleken in een cohort van DCD levertransplantaties.

In **hoofdstuk 7** rapporteren wij de resultaten van een nationale cohortstudie waarin de uitkomsten van retransplantatie met een DCD lever zijn vergeleken met die van retransplantatie met een lever afkomstig van een hersendode donor (DBD). Tussen oktober 2001 en juli 2018 hebben in Nederland 21 retransplantaties plaatsgevonden waarbij gebruik is gemaakt van een DCD lever. Elk van deze retransplantaties is door middel van de statistische techniek *propensity score matching* gematcht met drie retransplantaties met een DBD lever. Hiervoor werden de volgende matchingvariabelen gekozen: het jaar waarin de retransplantatie plaatsvond, het transplantatiecentrum waar de retransplantatie plaatsvond, het aantal levertransplantaties dat de patiënt heeft ondergaan, het interval tussen de retransplantatie en de voorgaande transplantatie, de leeftijd van zowel de donor als de ontvanger, de MELD score en de duur van de koude

ischemietijd. De DCD- en DBD-groep verschillen wat betreft donorkarakteristieken alleen in body mass index (22.4 versus 24.7 kg/m2, p-waarde = 0.02). Er zijn geen verschillen waargenomen in ontvangerkarakteristieken tussen beide groepen, noch in ischemietijden. Zowel de patiëntoverleving als de overleving van het transplantaat waren vergelijkbaar tussen de DCD en DBD groep. In de DCD groep waren er significant meer gevallen van niet-anastomotische stricturen (38.1% versus 12.7%, p-waarde = 0.02). Het merendeel van deze gevallen kon echter relatief eenvoudig behandeld worden met een endoscopische ingreep. Slechts twee patiënten dienden nogmaals een retransplantatie te ondergaan vanwege niet-anastomotische stricturen. Op basis van de resultaten van deze studie is geconcludeerd dat DCD levers veilig kunnen worden gebruikt in patiënten die op de wachtlijst staan voor een retransplantatie. Hierbij wordt opgemerkt dat zorgvuldige donor- en ontvangerselectie essentieel blijft.

In **hoofdstuk 8**, het laatste hoofdstuk van dit proefschrift, presenteren wij de resultaten van de tot op heden grootste studie naar de uitkomsten van levertransplantaties met organen gedoneerd na euthanasie (DCD type V volgens de aangepaste Maastricht classificatie). In deze studie zijn 47 levertransplantaties, waarbij levers afkomstig zijn van donoren die hun organen hadden afgestaan na euthanasie (DCD-V groep), vergeleken met 542 “reguliere” DCD levertransplantaties (DCD-III groep). Donoren in de DCD-V groep hadden een significant lagere serumspiegel van alanine aminotransferase en aspartaat aminotransferase (respectievelijk 25 versus 52 U/l en 26 versus 67 U/l, beide met een p-waarde < 0.001). Zoals verwacht was de dWIT in de DCD-V groep significant korter dan in de DCD-III groep, omdat in het geval van euthanasie medicatie wordt toegediend om het leven actief te beëindigen. Dit is in alle andere gevallen van orgaandonatie vanzelfsprekend niet het geval. Zowel patiëntoverleving als de overleving van het transplantaat verschilden niet significant tussen de DCD-V en DCD-III groep. Ook de incidentie van post-operatieve complicaties zoals vroege transplantaatdysfunctie en niet-anastomotische stricturen verschilden niet tussen beide groepen. Derhalve kan worden geconcludeerd dat het gebruik van levers afkomstig van donoren na euthanasie, waardevolle kansen biedt om het aantal beschikbare donororganen te vergroten. Hierbij wordt de kanttekening gemaakt dat levers afkomstig van dit type donoren dient te worden gezien als een “hoog-risico orgaan” waarbij goede ontvangerselectie essentieel is.



APPENDICES

A

Abbreviations
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Abbreviations

ACLF	Acute on chronic liver failure
ALF	Acute liver failure
ALD	Alcoholic liver disease
ALT	Alanine transferase
AS	Anastomotic strictures
AST	Aspartate transferase
BE	Belgium
BDI	Bile duct injury
BMI	Body mass index
CBD	Common bile duct
CCA	Cholangiocarcinoma
CCI	Comprehensive complication index
CIT	Cold ischemia time
CVA	Cerebrovascular accident
DBD	Donation after brain death
DC	Hospital discharge
DCD	Donation after circulatory death
DWIT	Donor warm ischemia time
ECD	Extended criteria donor
ES	Spain
FWIT	Functional donor warm ischemia time
GGT	Gamma-glutamyltransferase
HAT	Hepatic artery thrombosis
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HOPE	Hypothermic oxygenated perfusion
HPS	Hepato-pulmonal syndrome
HR	Hazard ratio
HTK	Histidine-tryptophan-ketoglutarate
IAR	Initial arterial reperfusion
IC	Ischemic cholangiopathy
ICU	Intensive care unit
IGL-1	Institute George Lopez-1
ILTS	International liver transplant society
IPR	Initial portal reperfusion
IQR	Interquartile range
ITBL	Ischemic-type biliary lesions
LT	Liver transplantation

MAP	Mean arterial pressure
MELD	Model for end-stage liver disease
NAS	Non-anastomotic strictures
NASH	Non-alcoholic steato-hepatitis
NL	The Netherlands
NMP	Normothermic machine perfusion
NRP	Normothermic regional perfusion
ODEH	Organ donation after euthanasia starting at home
PBC	Primary biliary cirrhosis
PNF	Primary non-function
PSC	Primary sclerosing cholangitis
PVT	Portal vein thrombosis
RBC	Red blood cell concentrate
RELT	Liver retransplantation
RRT	Renal replacement therapy
RWIT	Recipient warm ischemia time
SBP	Systolisch blood pressure
SBP	Spontaneous bacterial peritonitis
SETH	Sociedad Española de transplante hepatico
SIG	Speciel interest group
SUPPL	Supplementary
TDWIT	Total donor warm ischemia time
UK	United Kingdom
UW	University of Wisconsin
WHO	World health organization
WLST	Withdrawal of life-supporting treatment

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PhD Portfolio

Name PhD student: Marjolein van Reeven
PhD period: 2017-2022
Promotor: Professor J.N.M. IJzermans
Erasmus MC department: Surgery
Research school: Molecular Medicine

PhD training	Year	Workload (ECTS)
Courses		
BROK® (Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers)	2017	1.5
Scientific Integrity, Erasmus MC	2017	0.3
Open Clinica, Erasmus MC	2017	0.1
Basic Introduction Course on SPSS, Molecular Medicine	2017	1.0
Survival Analysis Course, Molecular Medicine	2018	0.5
Masterclass Privacy Analytics, Erasmus University Rotterdam	2018	0.5
The Photoshop and Illustrator CC 2019 Workshop, Molecular Medicine	2019	0.3
Biomedical English Writing Course, Molecular Medicine	2020	2.0
Scientific presentations		
International Congress of the International Liver Transplantation Society , Prague	2017	2.0
European Society of Transplantation Congress, Barcelona	2017	3.0
International Congress of the International Liver Transplantation Society, Lisbon	2018	3.0
International Congress of the International Liver Transplantation Society, Toronto	2019	1.0
Bootcongres Nederlandse Transplantatie Vereniging, Amsterdam	2019	3.0
European Society of Transplantation Congress, Copenhagen	2019	3.0
Eurotransplant Annual Meeting, Leiden	2019	2.0

Attendance at (inter)national conferences and seminars		
Chirurgendagen, Nederlandse Vereniging van Heelkunde	2017	1.0
Bootcongres Nederlandse Transplantatie Vereniging, Rotterdam	2018	1.0
Erasmus Liver Day	2017	1.0
Erasmus Liver Day	2018	1.0
ILTS Consensus Conference	2019	1.0
Teaching		
Coaching bachelor students medicine	2017-2020	2.0
Supervision of thesis from research master student	2017-2018	2.0
Supervision of thesis from master student medicine	2019-2020	1.0
Supervision of systematic review from bachelor students medicine (3x)	2018-2019	1.5
Total		**34.7**

Dankwoord

Circle Of Life

From the day we arrive on the planet
And blinking, step into the sun
There's more to see than can ever be seen
More to do than can ever be done

Some say, "Eat or be eaten"
Some say, "Live and let live"
But all are agreed
As they join the stampede
You should never take more than you give

In the circle of life
It's the wheel of fortune
It's the leap of faith
It's the band of hope
Till we find our place
On the path unwinding
In the circle, the circle of life

Some of us fall by the wayside
And some of us soar to the stars
And some of us sail through our troubles
And some have to live with the scars

There's far too much to take in here
More to find than can ever be found
But the sun rolling high through the sapphire sky
Keeps great and small on the endless round

In the circle of life
It's the wheel of fortune
It's the leap of faith
It's the band of hope
Till we find our place
On the path unwinding
In the circle, the circle of life

It's the wheel of fortune
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On the path unwinding
In the circle, the circle of life

Dit proefschrift was nooit tot stand gekomen zonder de hulp en steun van collega's, vrienden en familie. Ik wil jullie daar allen heel hartelijk voor bedanken. In het bijzonder wil ik graag de volgende mensen bedanken:

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About the author

Marjolein van Reeven was born on the 22nd of July 1992 in Capelle aan den IJssel. In 2010, she graduated from secondary school with honors (R.K. Scholengemeenschap Emmauscollege, Rotterdam). Subsequently, she studied medicine at the Erasmus University Rotterdam. During her clinical rotations, she did an elective in Gastroenterology (Erasmus University Medical Center Rotterdam) and Pediatric Oncology (Sophia Children's Hospital Rotterdam), followed by a senior rotation at the department of Internal Medicine at the Ikazia Hospital Rotterdam. The first steps of this thesis were made during her clinical rotation at the department of Surgery of the Erasmus University Medical Center Rotterdam. After obtaining her medical degree in December 2016, she started her PhD trajectory under supervision of prof. dr. J.N.M. IJzermans (Department of Surgery, division of Hepatopancreatobiliary and Transplant Surgery), ultimately leading to this thesis. During her PhD period, she completed a master's degree in Clinical Epidemiology at the Netherlands Institute for Health Sciences, Rotterdam. In January 2022, she has started her residency training in Internal Medicine at the Reinier de Graaf Hospital Delft (supervisor dr. H. Boom).