Marit Kalisvaart

Risk assessment in liver transplantation

The impact of donor organ quality on surgical and renal outcomes

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RISK ASSESSMENT IN LIVER TRANSPLANTATION

The impact of donor organ quality on surgical and renal outcomes

RISICOANALYSE IN LEVERTRANSPLANTATIE

De invloed van donor orgaankwaliteit op chirurgische en renale uitkomsten

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Marit Kalisvaart
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PROMOTIECOMMISSIE

Promotor

Prof. dr. J.N.M. IJzermans

Overige leden

Prof. dr. H.J. Metselaar
Prof. dr. R. Zietse
Prof. P. Muiesan

Copromotor

Dr. J. de Jonge

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Chapter 1

General introduction &
outline of this thesis

ABBREVIATIONS

AKI	Acute kidney injury
ALT	Alanine aminotransferase
AST	Aspartate transaminase
AUC	Area under the curve
BAR	Balance of risk
BMI	Body mass index
CCI	Comprehensive Complication Index
CKD	Chronic kidney disease
DBD	Donation after brain death
DCD	Donation after circulatory death
DRI	Donor risk index
DWIT	Donor warm ischemia time
DM	Diabetes mellitus
EAD	Early allograft dysfunction
ECD	Extended criteria donor
ESLD	End-stage liver disease
ESRD	End-stage renal disease
FFP	Fresh frozen plasma
HAS	Hepatic artery stenosis
HAT	Hepatic artery thrombosis
HCC	Hepatocellular carcinoma
HR	Hazard ratio
HRS	Hepatorenal syndrome
ICU	Intensive care unit
IDDM	Insulin dependent diabetes mellitus
INR	International normalized ratio
IQR	Interquartile range
IRI	Ischemia/reperfusion injury
ITBL	Ischemic-type biliary lesions
KCH	King's college hospital
KDIGO	Kidney Disease Improving Global Outcomes
MAP	Mean arterial pressure
MELD	Model for end-stage liver disease
MMF	Mycophenolate mophetil
MI	Multiple imputation
MVN	Multivariate normal regression
NHS	National Health Service
NHSBT	NHS Blood & Transplant
PNF	Primary non function
RBC	Red blood cells
RRT	Renal replacement therapy
SBP	Systolic blood pressure
SD	Standard deviation
UK	United Kingdom
UNOS	United Network for Organ Sharing
US	United States
UW	University of Wisconsin
WoT	Withdrawal of Treatment
WIT	Warm ischemia time

HISTORY OF LIVER TRANSPLANTATION

The first successful human liver transplantation was performed in 1967 by Thomas Starzl and colleagues (1). This life-saving treatment has evolved tremendously over the years and has become the golden standard for patients with end-stage liver disease (ESLD) and/or hepatocellular carcinoma (HCC). From the beginning, most liver transplantations in the Western world have been performed with grafts from deceased donors (2). Unfortunately, there has always been a significant disbalance between the number of patients in need of a liver transplant and the availability of donor organs, increasing the length of the waiting list and resulting in patients dying before an organ becomes available (3).

Expanding the donor pool

World-wide, most grafts retrieved from deceased donors come from heart beating or 'donation after brain death' (DBD) donors. The worsening organ shortage has led to several strategies to expand the donor pool. In the nineties marginal or 'extended criteria donor' (ECD) grafts were introduced, including grafts from older donors and steatotic grafts (4). Quickly thereafter, grafts from non-heart beating or 'donation after circulatory death' (DCD) donors were assumed to have a large potential to further increase the donor supply. The initial results with DCD grafts were promising and these organs have been a regular source of organs since the early years of this century in several countries, including the United States (US) and United Kingdom (UK), and across the European mainland (5–7). However, DCD donation involves an extra period of warm ischemia before the organs can be retrieved, potentially impairing organ quality. Consequently, the use of DCD grafts for liver transplantation has been associated with an increased incidence of postoperative complications, such as primary non function (PNF), acute kidney injury (AKI) and ischemic-type biliary lesions (ITBL) (8–10). These DCD-specific complications lead to inferior graft and patient survival rates, compared to when DBD grafts are used (11,12). However, more recent studies report satisfactory patient and graft outcomes in case of strict donor and recipient selection criteria (13,14).

The liver transplantation recipient

Liver transplantation should be suggested for every patient suffering from ESLD, cirrhosis with HCC or acute liver failure in whom the liver transplant would extend life expectancy or improve the quality of life, as advised by the 'Clinical practice guidelines for liver transplantation' from the *European Association Study of the Liver* (15). The disbalance in the equilibrium between available donors and patients in need of

a liver transplant is an accumulative burden for the waiting list. To limit the waitlist mortality, many countries introduced a 'sickest first' policy for the allocation of liver grafts, following the Model for End-stage Liver Disease (MELD) score (16,17). The MELD-model is based on the dysfunction of the bilirubin metabolism, coagulopathy and renal failure in end stage liver disease, using the serum bilirubin, international normalized ratio (INR) and serum creatinine levels to predict 90-day mortality. Consequently, less patients died while awaiting a liver transplant, but the sickest patients were selected for transplant, including patients with severe renal failure (18).

Balance of Risk

The increased use of DCD and other marginal grafts and prioritization of the sickest patients on the liver transplantation waiting list is a potential "disastrous" combination. Therefore, it is pivotal to identify the specific marginal aspect of each graft and comprehend its grade of marginality. With this in mind, each graft can be allocated to the appropriate recipient. For example, a patient with a HCC that might quickly cross the Milan-criteria and would be excluded from liver transplantation could be evaluated for transplantation with a DCD graft, as it is likely that such a marginal graft is more quickly available than a standard graft. The additional risk of PNF, biliary and renal complications should be considered, but the DCD transplant can be the only option to cure the patient from his malignant disease (19–21). In contrast, a patient who is critically ill with a high MELD-score and admitted on ICU due to decompensated ESLD could be too sick to receive a marginal graft. As complications are more likely to occur with marginal grafts, a very sick recipient may not have the reserve to recover from this event. Therefore, DCD grafts and other marginal grafts are in general not considered for patients with high MELD-scores in countries, such as the UK and the Netherlands (22). Dutkowski and colleagues presented a new score system to evaluate the balance of risk (BAR-score) to detect and avoid unfavourable combinations of donor and recipient factors and this score has also proven to be useful in the acceptance for liver grafts in patients with higher MELD-scores (>30 points) (23,24).

The kidneys at risk in liver transplantation

The 'renal' risk for liver transplantation recipients increased significantly over the last years due to (I) the pre-transplant renal impairment and (II) the increased use of marginal grafts. These grafts are more susceptible for hepatic ischemia/reperfusion injury (IRI) and this not only impacts on the initial function of the liver graft, but also has a negative effects on other organs, including the kidney (25). This phenomenon, caused by the release of tissue debris, pro-inflammatory cytokines and chemokines

after a period of organ ischemia, leads to a systemic inflammatory response similar as seen in sepsis and multi-organ failure (26–29). This response is considered to play an important role in the pathogenesis renal dysfunction after liver transplantation (30–32). Previous studies have shown that not only DCD grafts, but also marginal DBD grafts, are associated with an increased risk of post-transplant AKI (10,33). There is a wide variety in the severity of AKI in the early postoperative phase and not all patients require renal replacement therapy (RRT). Also, most patients recover quickly from this initial hit, but some have a slow recovery or will remain dialysis-dependent. The multifactorial origin of AKI and its deleterious effects on various short- and long-term outcomes make it essential to identify the patients at risk for AKI after liver transplantation.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis is to evaluate emerging risk factors for potential postoperative complications with the recent developments in liver transplantation practice. The focus lies on two important pillars: the evolving use of DCD grafts over the last two decades and the implications of graft quality on renal issues in liver transplantation. The two intertwine as the strategy to use DCD grafts to overcome the donor organ shortage is associated with specific complications, such as AKI.

The use of DCD grafts in liver transplantation is discussed in **Part I** of this thesis. **Chapter 2** serves as a synopsis of the introduction and development of DCD grafts in liver transplantation. This chapter includes the current knowledge about the challenges with the use of this type of grafts and strategies that have been developed worldwide to improve the outcomes of these grafts. In **Chapter 3** we present a comparison study about the short and long-term outcomes after liver transplantation of DCD and DBD grafts using the novel Comprehensive Complication Index. **Chapter 4** follows as a more in-depth analysis of the impact of the donor warm ischemia time (WIT) in DCD grafts with a focus on the course of hemodynamic parameters after the donor withdrawal of treatment (WoT). A new risk score to define futility in DCD liver transplantation is presented in **Chapter 5**. The UK DCD Risk Score stratifies the risk for graft loss using three risk groups to improve the decision making process of matching DCD grafts and recipients.

In **Part II** the risks factors for renal complications after liver transplantation are assessed. An overview of AKI and renal dysfunction on the long-term is given in

Chapter 6. It summarizes previous studies about AKI and chronic kidney disease (CKD) and discusses the multifactorial origin of kidney problems in liver transplantation candidates and recipients. Graft quality and IRI are an upcoming issue and the postreperfusion syndrome is the first presentation of severe IRI after reperfusion of the new graft. Therefore, we have evaluated the relation between the postreperfusion syndrome and post-transplant AKI in **Chapter 7**. The severity of IRI is also determined by the length of ischemic periods during preservation of the liver graft. In **Chapter 8** the impact of all the consecutive warm ischemia times of DCD grafts on the development of AKI is discussed. We present a new prediction model for postoperative AKI in **Chapter 9.** The AKI Prediction Score comprehends the graft, recipient and surgical risk factors into one model to identify the patients at risk for severe AKI directly after liver transplantation. **Chapter 10** focusses on the relation between the use of marginal grafts and long-term renal dysfunction. The results of this thesis are summarised and discussed with recommendations for future research in **Chapter 11**.

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DCD
CCI
QEHB

PART I

PREDICTING OUTCOME IN DCD LIVER TRANSPLANTATION

Chapter 2

The benefits & challenges of DCD liver transplantation: review of the literature

THE INTRODUCTION OF DCD GRAFTS

DCD (or at that time so-called non-heart beating donation) has been explored since the start of human organ transplantation. In fact, the first human heart, lung, kidney and liver transplants were performed with DCD grafts (1–4). However, since the Harvard consensus report to determine brain death in 1968, liver transplantation with organs retrieved from brain death donor became the golden standard (5). Due to the rapid developing organ shortage, donation after circulatory death was revived in the Western world during the early 1980s, with a pioneering role for the Netherlands using DCD kidneys for transplantation (6). Due to the complexity of the surgical procedure, the immediate life-saving concept and potential lethal complications, it took a bit more time for transplant surgeons to engage in DCD liver transplantation, but DCD grafts are part of the liver transplantation practice since the early 2000s in North America and many European countries.

Classification of DCD donation

Starzl and colleagues reported in 1995 their first series of thirteen DCD grafts used for transplantation (7). They used two types of grafts:

§ Uncontrolled DCD grafts: a patient that is (in the process of being) pronounced brain death, with a sudden, unexpected cardiac arrest. Thereafter, cardiopulmonary resuscitation CPR is started and the patient is transported to theatre for the organ donation procedure.

§ Controlled DCD grafts: a patient has irreversible neurological damage, but either do not fulfil the brain death criteria or the family requests withdrawal of life support before organ donation. In these cases, the life support is withdrawn and the organ procurement procedure starts after a certain period (variable between countries) after circulatory arrest.

As expected, the actual transplantation rate of livers from potential donors was higher in the controlled group (86% vs. 60%). The results of the uncontrolled DCD grafts were disappointing, with half of the patients requiring immediate retransplantation due to PNF and inadequate portal flow and two other patients lost their grafts later due to other causes. All the grafts after controlled DCD donation functioned well (n=6) and after two years three patients were alive with functioning grafts. In the following years, the option of DCD grafts was explored in other countries. The widely used 'Maastricht Classification' (**Table 1**) for DCD donation were introduced by Kootstra and colleagues in 1995, stratifying the potential DCD donors into four (and later five) groups (8–10). This classification is more specific about the location and unexpected

Table 1 – Modified Maastricht classification for donors after circulatory death
Adapted from Thuong *et al*, Transplant International, 2016 (10).

Category I	*Found dead out of hospital*
Uncontrolled	IA. Cardiocirculatory death outside hospital with no witness. Totally uncontrolled.
	IB. Cardiocirculatory death outside hospital with rapid resuscitation attempt.
Category II	*Unsuccessful resuscitation*
Uncontrolled	IIA. Unexpected cardiocirculatory death in ICU
	IIB. Unexpected cardiocirculatory death in hospital with rapid resuscitation
Category III	*Awaiting cardiac arrest*
Controlled	IIIA. Expected cardiocirculatory death in ICU
	IIIB. Expected cardiocirculatory death in OR (withdrawal phase >30 min)
	IIIC. Expected cardiocirculatory death in OR (withdrawal phase <30 min)
Category IV	*Cardiac arrest while brain death*
Uncontrolled	IVA. Unexpected cardiocirculatory arrest in a brain dead donor (in ICU)
Controlled	IVB. Expected cardiocirculatory arrest in a brain dead donor (in OR or ICU)
Category V	Euthanasia
Controlled	VA. Medically assisted cardiocirculatory death in ICU or ward.
Uncontrolled	VB. Medically assisted cardiocirculatory death in OR.

aspect of the DCD donation. The most common used categories are II (uncontrolled) and III (controlled). Liver transplantation with type II DCD donors has been common practice Spain and has later been explored in Italy and France (11–13). Due to the unpredictable warm ischemia times in type II DCD liver grafts, the outcomes of liver transplantation with these grafts are capricious with higher rates of PNF and biliary complications, with initial one-year graft survival rates of only 50% (14,15). The Spanish law allows for premortal cannulation of the donors and therefore, the clinicians quickly engaged in the use of perfusion techniques with cardiopulmonary bypass of the donor, to limit the warm ischemia times and to optimize organ quality. Although initially only a small percentage of the potential grafts were used for transplantation, the results of this normothermic regional perfusion with type II grafts were improving and therefore it is now common practice in this country (16,17).

Type III DCD organ donation

All the following chapters in this thesis will concern liver transplantation with *type III controlled DCD grafts*. Type III DCD organs have been the preferred option from the beginning in the US, Canada, and several European countries, including the Netherlands, Belgium and the UK (18). Regularly, a type III DCD donor is admitted on ICU and there is a controlled WoT. This can either be either in ICU, anaesthetic room or

operation theatre. After circulatory arrest, there is an obligatory waiting time before death can be declared. This time is 5 minutes by law in the Netherlands, but varies between 5 and 20 minutes in Europe (10). Because the general practice for WoT in the Netherlands is on ICU, there is an additional transport time of the donor to the operation theatre, which extends the period of donor warm ischemia time (DWIT) (19). The aim of the organ procurement in DCD donation is to safely retrieve the organs as quickly as possible. The principle of the super-rapid technique consists of coordinated surgical steps including a rapid thoraco-laparotomy, cannulation of the abdominal aorta for cold perfusion, venous exsanguination and cross-clamping of the thoracic aorta and followed by separate resection of all organs (20). An overview of organ donation by graft type in the Netherlands during the last years is displayed in **Figure 1**, showing a steady increase of controlled DCD donation from the year 2000 (21). After the start of the DCD program in 1999, due to a part of the donors that do not proceed to donation and a number of liver grafts discarded before implantation, the number of liver transplants performed with DCD grafts grew at a slower pace (**Figure 2**), with 45 DCD transplants in 2014.

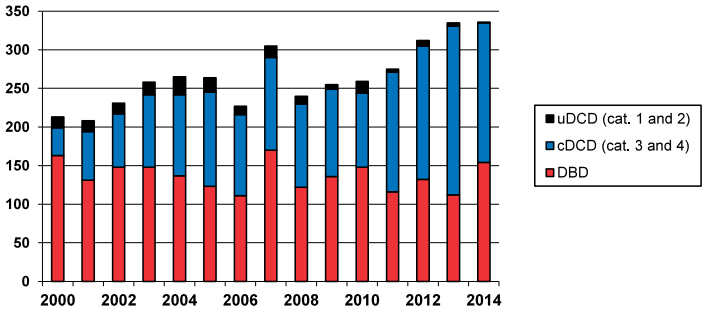

Figure 1 – Number of deceased organ donors in the Netherlands
From Leiden *et al*, Netherlands Journal of Medicine, 2016 (21).

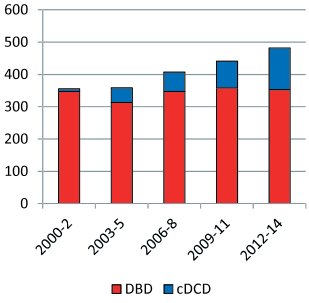

Figure 2 – Liver transplantation by graft type in the Netherlands since 2000
From Leiden et al, Netherlands Journal of Medicine, 2016 (21).

Donor warm ischemia time

The obligatory DWIT in DCD donation is responsible for the additional period of warm ischemia and aggravation of hepatic IRI (22). **Figure 3** shows an overview of the separate period between the WoT until the start of cold perfusion. At this moment, there is no consensus about the actual definition of DWIT and various definitions are used (10). Overall, DWIT can be divided into two periods: the agonal phase (from WoT until circulatory arrest) and the asystolic phase (from circulatory arrest until the start of cold perfusion). For the graft to be considered for liver transplantation, the length of the full period of DWIT cannot extend one hour in most countries, including the UK and the Netherlands (23,24). On average, the time for a potential DCD donor to die is 36 minutes (25). However, DWIT is a dynamic period and some donors have a much longer period of hypoxia or hypotension during the agonal phase, with a potential major impact on the severity of hepatic IRI. The impact of DWIT on postoperative outcomes after DCD liver transplantation has been studied before, but different definitions of DWIT were used and consequently, the length of an acceptable DWIT in these studies ranges from 10 to 35 minutes (26–30). A common way to assess DWIT is with the functional DWIT. This period is a combination of agonal phase starting with the drop in saturation (SpO2 <80%) or blood pressure (MAP or SBP <50 mm Hg), until the start of cold perfusion. In many countries, the cut-off for discarding a DCD liver is 30 minutes of functional DWIT.

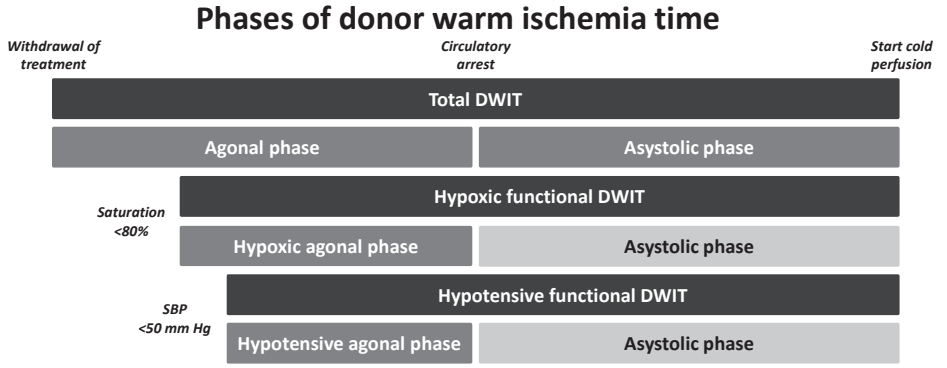

Figure 3 – Overview of the separate periods of donor warm ischemia time

The potential of expanding the donor pool

Even after more than 20 years of liver transplantation with DCD grafts, there are no exact numbers on what the real contribution of DCD grafts is to the patients waiting for a liver. Transplantation with DCD grafts comes with a certain risk of PNF or other complications and some patients will even die before a second organ becomes avail-

able. Therefore, the actual benefit and perspective for the patient on the waiting list is more complicated to estimate. In an early nationwide comparative study from US (2006) of DCD and DBD grafts, an increased relative risk (RR 1.85) for graft failure was reported with the use of DCD grafts (31). However, a following study in 2014 with US *United Network for Organ Sharing* (UNOS) data confirmed the inferior graft survival rates with DCD grafts, but the patient survival on the long-term was comparable with DBD grafts, implicating these patients are relisted an retransplanted timely (32).

Survival outcomes in comparison with DBD grafts

Since the start of DCD liver transplantation, multiple groups from all over the world have reported their experience with these grafts, some of them also comparing the results with DBD grafts. The first report from the University of Wisconsin with 19 DCD grafts in 1999, already reported an increased incidence of PNF leading to more graft loss in this group, but comparable patient survival with recipients of DBD grafts (33). In the following years, more centres reported their experience with mixed results (34,35). National data from US arrived in 2003, summarizing the centre experiences, with increased retransplantation rates and inferior survival with the use of DCD grafts (36). The first results from Europe (King's College, UK) were promising, with satisfactory results with grafts of selected DCD donors (37). Results of the Dutch DCD program (2010) were similar, with satisfactory survival rates, despite a higher risk for biliary strictures (38). Importantly, duration of DWIT and cold ischemia time were both identified as risk factors in DCD liver transplantation and should be minimized. Additional donor risk factors for DCD graft failure that are identified are older donors and high donor weight (graft steatosis). Older recipients, those with high MELD-scores (>35) and life-support prior to transplantation are also at increased risk for graft failure (28).

With the additional risk of DCD grafts in mind, many have adapted the strategy to allocate these grafts to recipients with lower MELD-scores, such as patients with HCC at risk for exceeding Milan-criteria (39,40). This leads to differences between DCD and DBD liver transplantation in donor, graft and recipient risk factors, compromising the analysis of outcome. Therefore, several groups have published their data using propensity score matching, a method to equalize study groups in non-randomised studies, by matching the cases of the intervention group (DCD) to certain cases from the control group (DBD) by preselected characteristics (41). The studies from the early years in DCD liver transplantation and those performed without matching demonstrate mixed positive and negative results. Interestingly, the general sentiment of the five studies using propensity score matching display significant inferior results

with DCD grafts: PNF and ITBL were more frequently observed in recipients of DCD grafts that were matched to a DBD counterpart, leading to inferior graft and overall survival rates (30,42–45). The overall negative results of these studies with matched cases shows the 'real' additional risk of DCD donation in comparison to brain-death donors. It also suggest that balancing the risk between donor and recipient in a combined DCD & DBD donor pool is essential to optimize the results of the population on the liver transplant waiting list.

The trends from studies published in the last three years is that the known complications of using DCD grafts are still a serious issue and these patients have an increased risk for graft loss (29,44). However, due to the early identification of patients with serious complications, they can be relisted earlier and receive optimal bridging treatment towards retransplantation, leading to comparable patient survival on the long-term (29). The Birmingham group from the UK has used more marginal DCD grafts over the last years, with comparable survival rates with DBD grafts, by selecting the right patient to receive a DCD graft (44). More recently, they have shown that DCD grafts from older donors are not associated with poorer outcomes, provided that other risk factors are eliminated (46). Historically, transplant surgeons in the US have been more cautious with the use of DCD grafts, displayed by the low rate of DCD liver transplantation nationally (only 6% of total deceased donor transplants in 2016 (47). However, recently voices arise in the US to approach DCD grafts with a different strategy to limit the duration of cold ischemia time and only select patients with relatively low MELD-scores (48).

Specific postoperative complications

Primary non-function

Due to the additional warm ischemic period during DCD donation, there is a significant depletion of intracellular energy reserves leading to more severe hepatic IRI (49). Prolonged duration of DWIT has been linked to non-viability of DCD grafts in pigs and the first studies in humans already showed higher incidence of PNF (7,33,36,50). Incidence rates up to 12% of have been reported over the years, but this was not always significantly more compared to DBD grafts (30,35,36,51,52). Furthermore, some have described their DCD experience without any cases of PNF (53–55). Additional to a long DWIT, donor age, graft steatosis and prolonged cold ischemia are thought to increase the risk for PNF (56).

Ischemic-type biliary lesions

Biliary complications are considered the Achilles heel of DCD liver transplantation. Especially strictures not related to the biliary anastomosis created during the transplant, the so-called non-anastomotic strictures, ischemic cholangiopathy or ischemic-type biliary lesions (ITBL) are more frequently observed when DCD grafts are used. ITBL are defined as symptomatic strictures of the intrahepatic or hilar bile ducts after liver transplantation, in the presence of a patent hepatic artery (57). Every graft prepared for liver transplantation experiences a certain period of warm and cold preservation, leading to significant injury to the luminal biliary epithelium, but this does not necessarily lead to ITBL. Although the exact pathogenesis of ITBL remains unknown, recent literature proposes that injury to specific components of the bile duct, i.e. the peribiliary glands and vascular plexus during preservation is associated with develop-

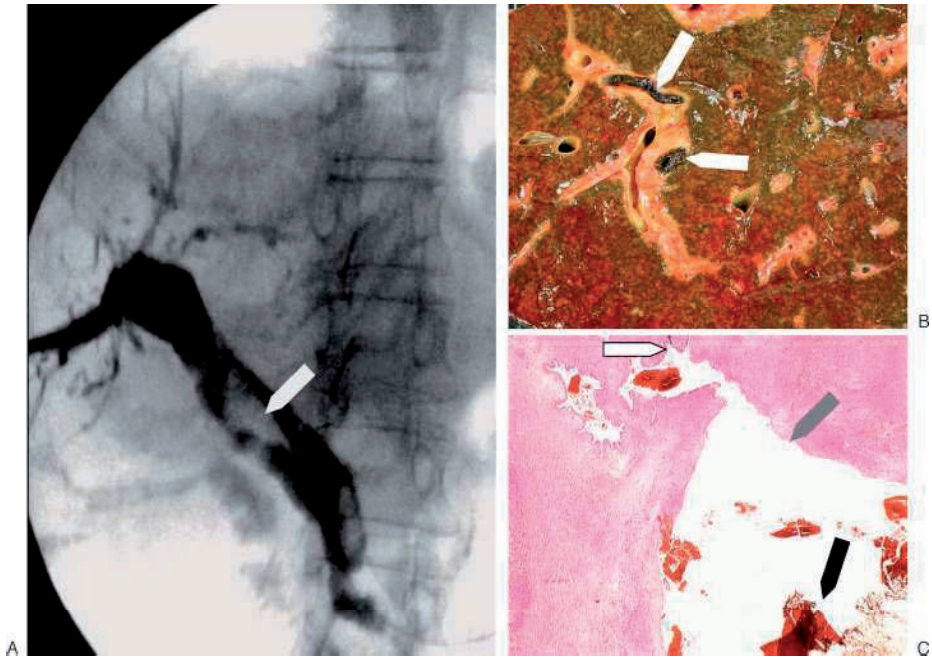

Figure 4 – Macroscopic, radiological and histological appearance of ITBL after liver transplantation. From Deltenre *et al*, Seminars in liver disease, 2008 (62).
Biliary casts. (A) Typical appearance on endoscopic retrograde cholangiopancreatography in a patient with ischemic cholangiopathy following liver transplantation. Filling defects (white arrow) can be seen in dilated bile ducts, with mildly irregular margins. (B) Gross appearance at sectioning in an excised liver. Solid brown material can be seen within large bile ducts (white arrows). (C) Microscopic appearance of a large bile duct. Biliary epithelium is lacking in some areas (gray arrow) and is reserved in other areas (white arrow). Solid, bile-stained material is observed within the lumen (black arrow). (Courtesy of Dr. Annie Sibert, Service de Radiologie, and Dr. Vale´ rie Paradis, Service d'Anatomie et de Cytologie pathologiques, Hôpital Beaujon, Clichy, France.)

ment of ITBL (58,59). This suggests that adequate preservation of these structures is essential for a timely regeneration of the biliary epithelium. Additional damage of bile salts after transplantation and subsequent influx of immune cells leading to inflammation and fibrosis can result in ITBL (60). The extra period of warm ischemia during DWIT potentially leads to the additional injury of the vital structures of the biliary tree, increasing the risk for development of ITBL with DCD grafts. Patients can develop the strictures within months after liver transplantation (**Figure 4**), regularly requiring endoscopic and surgical interventions, but often retransplantation cannot be avoided (61).

In the largest meta-analysis (2011) about ITBL after DCD liver transplantation comprehending 11 studies by Jay et al, ITBL was observed in 16% of the recipients receiving a DCD graft and they had an 10.8x increased odds of developing ITBL, compared to DBD recipients (63). As a result, DCD recipients also had a 2.6-fold increased odds of retransplantation. A more recent meta-analysis from 2014 showed similar results with ITBL occurring in 16% and 3% after liver transplantation with DCD and DBD grafts, respectively (64). ITBL has a multifactorial origin and a wide variety of ITBL-rates have been observed between single-centre experiences and ranges from 3% to 38% (40,42,44,64–71). An evaluation of the IDOL-consortium involving 10 centres across the US, ITBL was present in 12%, ranging from 6% to 26% in the individual centres (72). Identified risk factors for ITBL are prolonged duration of warm and cold ischemia and specifically the length of donor warm ischemia. There is no consensus about the impact of donor age and development of ITBL and these grafts should not be avoided for age per se (45,46,66,73,74).

Acute kidney injury

AKI is a common issue after liver transplantation and is the result of donor, surgical and recipient risk factors. The kidney is a known organ to suffer from hepatic IRI, due to the release of pro-inflammatory cytokines and reactive oxygen species, acting like a systemic inflammatory response syndrome (75). With the evolving use of marginal grafts, including DCD grafts, graft quality takes more part in the development of post-transplant AKI (76). The Birmingham group was the first to describe an increased frequency of AKI with the use of DCD grafts and was observed in 53% of the recipients, compared to 32% of the DBD recipients (**Figure 5**) (77). These findings were later confirmed by others (54). The increased incidence of AKI did not lead to more CKD in the Birmingham cohort. However, an indirect relationship between DCD liver transplantation and end-stage renal disease on the long-term was observed in a nationwide-US registry study (78).

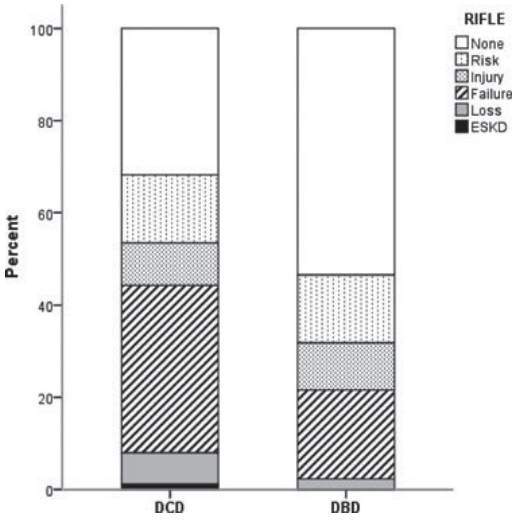

Figure 5 – Comparison of acute kidney injury after liver transplantation using DCD or DBD grafts.
From Leithead et al, American Journal of Transplantation, 2012 (77).
Stacked bar graph demonstrating the proportion of DCD liver transplant recipients and DBD recipients who developed acute renal dysfunction during the immediate postoperative period.

Cost-Effectiveness & Quality of Life

The use of DCD grafts is also associated with higher costs after transplantation, due to the development of complications requiring multiple interventions, hospital admissions and even retransplantation, such as ITBL (79). This has been shown in several cost-effectiveness studies from US and the Netherlands (80,81). Due to the long-term problems with intensive and multidisciplinary treatments, ITBL has a significant impact on the quality of life of the patients as well (82). Recipients of DCD grafts have a significant better quality of life on the long-term, when compared to recipients who remained waiting on the waiting list for a DBD liver transplant (83).

Although DCD liver transplantation is not without risk, it has expanded the donor pool significantly over the last years. Avoiding the DCD-specific complications and preventing the need for retransplantation are the most important issues to solve in the near future to improve outcomes and quality of life and reduce costs. Machine perfusion techniques have the potential to improve outcomes of marginal grafts, such as DCD grafts and assessment of the risk factors for graft failure in DCD liver transplantation is pivotal to identify these grafts that have the most benefit of these new preservation techniques (84).

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Chapter

Comparison of postoperative outcomes between DCD and DBD liver transplantation using the Comprehensive Complication Index

Marit Kalisvaart
Jubi de Haan
Wojciech Polak
Herold Metselaar
Bas Wijnhoven
Jan IJzermans
Jeroen de Jonge

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SUMMARY

Liver transplantation is complex surgery and the increasing use of marginal grafts is pressurizing current postoperative outcomes. DCD grafts in particular are associated with PNF and ITBL with subsequent impaired graft survival rates. The aim of this study was to test the total burden of complications in the early postoperative period after liver transplantation between DCD and DBD grafts with the novel Comprehensive Complication Index (CCI). We performed a retrospective single-centre study of all liver transplantations performed since the start of DCD program (2001-2015). CCI (at hospital discharge and six months) was the result of ALL complications weighted by their Clavien-Dindo grade. A multiple logistic regression model was used to identify factors associated with a complex postoperative course (CCI at six months >60). In total, 441 cases were included: 115 DCD and 326 DBD grafts. Median in-hospital CCI was comparable for both groups (DCD 38.2; DBD 36.7; p=0.429). Six-months post-operative median CCI was significantly higher for DCD grafts (53.4 vs 47.2; p=0.041). Also, more DCD recipients underwent retransplantation for ITBL in this period (4% vs 1%; p=0.031). Logistic regression identified recipient BMI (p=0.046), recipient WIT (OR 1.032; 95%CI 1.008-1.056; p=0.008), and DCD graft (OR 3.913; 95%CI 1.200-12.767; p=0.024) as risk factors for a CCI >60. In conclusion, this analysis shows a comparable complication rate during the index hospital stay for DCD and DBD LT, but the CCI increases significantly for DCD recipients in six months after transplantation. Reduction of biliary complications, especially ITBL, is needed to improve the outcomes for DCD grafts.

INTRODUCTION

DCD grafts are increasingly used to overcome the organ shortage in liver transplantation (1,2). The use of these marginal grafts is associated with several complications, such as PNF, AKI and ITBL, resulting in inferior graft and recipient survival (3–6). However, recent studies show that with careful selection of DCD grafts and recipients the survival rates can reach a level comparable DBD liver transplantation (7–10). Furthermore, DCD grafts have shown to be a useful source of organs for patients waiting for a transplant with an HCC. Their time on the waitlist can be shortened before they cross Milan criteria and they are more likely to tolerate an extended criteria organ, due to their relatively low biological MELD-score (11–13).

The complexity of the surgical procedure, the severe disease of the patient and the marginal graft puts patients receiving a DCD graft more at risk for postoperative complications. However, the diversity of complications makes it difficult to compare postoperative outcomes and solid endpoints, like early postoperative mortality, have become rare due to successful retransplantation. The Comprehensive Complication Index (CCI) is a novel tool to combine all complications into one number that comprehends the entire burden of postoperative morbidity (14). It has shown its efficacy in gastro-intestinal surgery to serve as a primary outcome measure and compare treatment groups (15,16). In the early postoperative period after liver transplantation vascular complications, infections, acute rejection, EAD and kidney injury are the most common problems, while chronic rejection, recurrence of liver disease and biliary complications develop several months after transplant (17). These later (biliary) complications require often interventional therapy as well, such as endoscopic treatment, surgery or even retransplantation (18).

The aim of this study was to compare all postoperative morbidity with the novel CCI between DCD and DBD grafts up to six months after liver transplantation.

METHODS

This retrospective study was performed with approval of the Erasmus MC Institutional Review Board (MEC-2014-670). All consecutive patients who underwent deceased donor liver transplantation with age ≥18 years in our center from the start of the DCD program were included (10/2001 - 08/2015). Exclusion criteria were retransplantation, split-liver transplantation, combined liver kidney transplantation and transplant for acute hepatic failure.

Table 1 - donor, recipient and surgical characteristics of DCD and DBD liver transplantation

Donor	**DBD (n=326)**	**DCD (n=115)**	**Total (n=441)**	**P-value**
Age (years)	54 (44-63)	47 (38-56)	51 (43-61)	<0.001
Male gender (%)	164 (50)	64 (56)	228 (52)	0.324
Body mass index	24.5 (22.7-26.9)	22.7 (21.6-25.4)	24.2 (22.6-26.6)	0.017
Cause of death (%)				
Trauma	57 (18)	29 (25)	86 (20)	
Anoxia	12 (4)	24 (21)	36 (8)	
Cerebrovascular accident	251 (77)	57 (50)	308 (70)	
Other	6 (2)	5 (4)	11 (3)	<0.001
Location (%)				
Local	26 (8)	15 (13)	41 (9)	
National	244 (75)	97 (84)	341 (77)	
International	56 (17)	3 (3)	59 (13)	<0.001
Donor risk index				
Official	2.0 (0.4)	2.5 (0.5)	2.1 (0.5)	<0.001
Excluding DCD status	2.0 (0.4)	1.7 (0.3)	1.9 (0.4)	<0.001
Donor warm ischemia time (min)				
Asystolic (n=112)	.	16.8 (4.8)	.	.
Ischemic agonal phase (n=88)	.	13.0 (7.0)	.	.
Recipient				
Age (years)	53 (43-60)	55 (48-62)	53 (44-60)	0.016
Male gender (%)	213 (65)	79 (69)	292 (66)	0.513
Body mass index	25.2 (22.7-28.4)	25.3 (23.2-29.1)	25.2 (22.9-28.6)	0.655
Type of liver disease (%)				
Hepatitis B	30 (9)	9 (8)	39 (9)	
Hepatitis C	44 (14)	23 (20)	67 (15)	
Biliary cirrhosis	111 (34)	27 (24)	138 (31)	
Alcohol related cirrhosis	55 (17)	22 (19)	77 (18)	
Other	86 (26)	34 (30)	120 (27)	0.188
Hepatocellular carcinoma (%)	75 (23)	36 (31)	111 (25)	0.078
Child Pugh score	8 (6-10)	8 (7-10)	8 (6-10)	0.572
MELD score	14 (9-20)	15 (9-20)	14 (9-20)	0.409
Sodium (mmol/L)	138 (135-140)	138 (134-140)	138 (135-140)	0.323
Medical history(%)				
Diabetes Mellitus	70 (22)	29 (25)	99 (22)	0.408
Coronary artery disease	11 (3)	3 (3)	14 (3)	0.687
Hypertension	17 (5)	6 (5)	23 (5)	0.999
Surgical				
Operation time (hours)	7.5 (6.7-8.7)	7.6 (6.9-8.4)	7.5 (6.8-8.7)	0.551
Cold ischemia time (hours)	6.9 (5.6-8.3)	6.5 (5.8-7.4)	6.7 (5.7-8.0)	0.338
Recipient warm ischemia time (min)	28 (24-34)	28 (24-35)	28 (24-35)	0.850
Blood loss (L)	3.8 (2.4-6.5)	4.5 (3.2-6.8)	4.0 (2.5-6.5)	0.067
RBC transfusion (units)	3 (1-6)	3 (1-6)	4 (3-7)	0.734
FFP transfusion (units)	4 (0-8)	4 (0-8)	3 (1-6)	0.687
Platelet transfusion (units)	1 (0-2)	1 (0-2)	4 (0-8)	0.188
Peak postoperative serum AST (U/L)	856 (555-1699)	2657 (1311-4905)	1061 (630-244)	<0.001

AST, aspartate transaminase; DBD, donation after brain death; DCD, donation after circulatory death; FFP, fresh frozen plasma; MELD, model for end stage liver disease; RBC, red blood cells.
Continuous variables are presented in median and interquartile range.

All collected donor and recipient characteristics are displayed in **Table 1**. This data was used to calculate the donor risk index (DRI), MELD and Child-Pugh score as well (19,20). Graft allocation in the Netherlands is based on the MELD-principle. WoT of DCD donors is on the ICU and there is an obligatory 5-minute waiting time after circulatory arrest. The super-rapid retrieval technique is used to minimize the asystolic phase of DWIT (21). Exclusion criteria for DCD donors included age >60 years, body mass index (BMI) >28, aspartate transaminase (AST) >120 U/L and serum sodium > 160 mmol/L. The vascular and biliary flush is performed with University-of-Wisconsin preservation fluid. Prior to 2013 histidine-tryptophan-ketoglutarate was used as well. Standard surgical technique included piggyback cavo-caval anastomosis with only incidental use of a portocaval shunt. Operation time, cold ischemia time, and recipient WIT, intraoperative blood loss with subsequent transfusion requirements were all recorded. The peak serum AST level in the first 72 hours was used as a surrogate marker for hepatic IRI, a known marker for EAD (22). The standard immunosuppression regime changed during the study period. Until 2012 the regimen consisted of tacrolimus from day 0 and prednisolone (for 3 months). In 2012 mycophenolate mophetil (MMF) (from day 0) and basiliximab (day 0 and 4) were added and introduction of tacrolimus was postponed for kidney protection until day 5.

Duration of hospital and ICU admission, recipient and graft survival were all documented. The CCI was calculated using the original algorithm with all complications that occurred during hospital admission and up to six months after transplantation using the Clavien-Dindo classification (14,23). The range of the CCI is 0-100, with 100 equaling death. In case of retransplantation, a grade 4A complication was scored and thereafter follow up was terminated to prevent progression of CCI points for the first graft that were actually a complication from the re-transplant graft. ITBL was defined as postoperative symptomatic strictures and associated dilatation of the intrahepatic or hilar bile duct(s). These symptoms were (1) confirmed by endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography or percutaneous transhepatic cholangiography, (2) in the presence of a patent hepatic artery confirmed by ultrasound and (3) required biliary endoscopic or percutaneous intervention or retransplantation.

Statistical analysis

Data were analyzed with IBM SPSS Statistics V24 (IBM Corporation, Armonk, NY, USA). The student's t-test was used to compare normally distributed continuous variables. Nonparametric continuous variables were compared using the Mann-Whitney-U test. To compare categorical variables the Chi-square test or Fisher's exact test were used.

P-values <0.05 were considered statistically significant. A multiple logistic regression model with all relevant donor, recipient and surgical characteristics was used to identify factors associated with a complicated course after liver transplantation (CCI >60 at six months after transplant). Long-term survival rates were estimated using Kaplan-Meier methods. The median follow up period was 6.9 years.

RESULTS

During the study period 634 liver transplants were performed at our center: 120 DCD and 504 DBD grafts. The following transplants were excluded: 85 retransplantations, 10 split-livers, 16 combined liver-kidney transplants and 77 transplants for acute hepatic failure. Two cases were excluded because of missing data and 3 recipients who died within 24 hours after transplant (all DBD grafts). This lead to an inclusion of 441 liver transplants: 115 DCD and 363 DBD liver transplants.

Baseline characteristics

Baseline characteristics are displayed in **Table 1**. Median donor age was 51 years and DCD donors were significantly younger (47 vs. 54 years; p<0.001). Median BMI was lower in the DCD donors as well (22.7 vs. 24.5; p=0.017). After correction for DCD transplantation the DRI was significantly lower in the DCD group (1.7 vs. 2.0; p<0.001). Median age of the recipients was 53 years and significantly higher in those who received a DCD graft (55 vs. 53 years; p=0.016). Both Child-Pugh and MELD-score prior to transplant were comparable in both groups. The cold ischemia time was 6.9h in the DBD group, compared to 6.5h in the DCD group (p=0.338). Recipient WIT was 28 minutes in both groups. The transfusion requirement was comparable for both groups as well. Postoperative peak serum AST was significantly higher in DCD recipients (2657 vs. 856 U/L; p<0.001).

Postoperative outcomes and the Comprehensive Complication Index

Postoperative outcome parameters in the first six months after liver transplantation are shown in **Table 2**. All in-hospital outcome parameters were comparable for DCD and DBD grafts. After six months the mortality rate was 11% in the DCD group, compared to 7% in the DBD group (p=0.152). The retransplantation rate was higher in DCD recipients (15% vs. 5%; p=0.001). A tendency towards more biliary complications was observed in the DCD group (34% vs. 26%; p=0.081). Furthermore, the incidence of ITBL in this period was significantly higher in this group (11% vs. 3%; p<0.001). More DCD recipients received a retransplant for ITBL as well (4% vs. 1%: p=0.031).

Table 2 - postoperative outcome parameters after liver transplantation with DCD and DBD grafts

In-hospital	DCD (n=115)	DBD (n=326)	Total (n=441)	P-value
≥1 severe complication (CD grade 3B) (%)	39 (34)	113 (35)	152 (35)	0.884
Postoperative ventilation days	0 (0-1)	0 (0-1)	0 (0-1)	0.117
Length of stay ICU (days)	3 (2-4)	3 (2-4)	3 (2-4)	0.557
Length of stay entire admission (days)	20 (15-28)	19 (15-29)	19 (15-29)	0.825
Retransplantation (%)	7 (6)	11 (3)	18 (4)	0.206
Death (%)	11 (10)	20 (6)	31 (7)	0.216
Within six months				
Readmission (%)	54 (47)	142 (44)	196 (44)	0.528
Death (%)	13 (11)	23 (7)	36 (8)	0.152
Biliary complications				
All biliary complications* (%)	39 (34)	83 (26)	122 (28)	0.081
Anastomotic biliary complications** (%)	21 (18)	59 (18)	80 (18)	0.969
Ischemic type biliary lesions (%)	13 (11)	9 (3)	22 (5)	<0.001
Retransplantation (%)				
Total	17 (15)	17 (5)	34 (8)	0.001
Hepatic artery thrombosis	5 (4)	9 (3)	14 (3)	0.372
Portal vein thrombosis	3 (3)	1 (1)	4 (1)	0.056
Primary non function	4 (3)	1 (1)	5 (1)	0.018
Ischemic type biliary lesions	5 (4)	3 (1)	8 (2)	0.031
Other	0	3 (1)	3 (1)	0.403

CD, Clavien Dindo; DBD, donation after brain death; DCD, donation after circulatory death; ICU, intensive care unit.
Continuous variables are presented in median and interquartile range.
* Include all biliary complications: ischemic type biliary lesions, anastomotic complications and treatment for incidental cholangitis without diagnosed underlying disease.
** Requiring at least endoscopic or percutaneous treatment.

The CCI at hospital discharge and six months after transplant is displayed in **Figure 1**. The median CCI at hospital discharge was 37.1 and comparable for DCD and DBD grafts (38.2 vs. 36.7; p=0.434). On the contrary, six months after transplant recipients in the DCD group had a significantly higher CCI (53.4 vs 47.2; p=0.041). To assess the origin of the overall difference at six months, all complications were divided into four categories: surgical (including hepatic artery thrombosis (HAT), rebleed, perforation), medical (PNF, kidney injury, rejection, diabetes mellitus (DM), myocardial infarction, stroke, etc.), biliary complications (including ITBL and anastomotic strictures) and infections. There were no significant differences in these subgroups for DCD and DBD grafts after six months, but a trend was observed for a higher CCI in the DCD groups for biliary (p=0.071) and medical (p=0.051) complications.

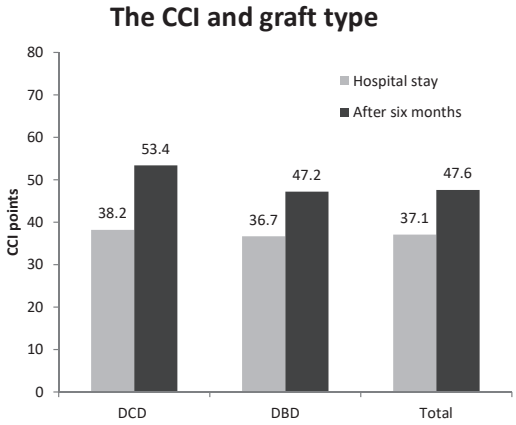

Figure 1 – CCI for recipients of DCD and DBD grafts at hospital discharge and six months after liver transplantation

Factors associated with a high Comprehensive Complication Index after six months

A multiple logistic regression model (**Table 3**) was used to identify donor, recipient and surgical factors associated with a complicated postoperative course (CCI >60 after six months). For example, one re-operation for bleeding (grade 3B) and re-admittance at ICU for respiratory sepsis (grade 4B) during hospital admittance and one endoscopic treatment for ITBL (grade 3A) and one course of antibiotics for a pneumonia after discharge (grade 2) lead to a CCI of 63.7 at six months. Three factors were associated with a CCI >60: Recipient BMI (OR 1.052; p=0.046), duration of recipient WIT (OR 1.032; p=0.008) and use of a DCD graft (OR 3.913; p=0.024).

Long term survival

The five-year estimated graft (71%) and patient (81%) survival are displayed in **Figure 2**. Recipients who received a DCD graft had a significantly lower five-year estimated graft survival (60% vs.75%; p=0.002). However, there was no significant difference observed in the estimated patient survival (DCD 75%; DBD 82%; p=0.090).

DISCUSSION

Our results show with use of the CCI that outcome of DCD and DBD liver transplantation is comparable during the index hospital stay, but that DCD recipients experience more complications in the long run. This study thus confirms the outcomes of other publications, reporting both similar and detrimental outcome in DCD liver transplantation and shows the dynamic increase of total burden of complications, depending

Table 3 - multiple logistic regression model to identify factors associated with a CCI >60 six months after liver transplantation

Recipient	Odds ratio	95% CI	P-value
Age	1.007	0.998-1.027	0.481
Gender	1.255	0.792-1.990	0.334
Body mass index	1.052	1.001-1.105	0.046
MELD score	1.018	0.992-1.045	0.168
Indication for transplant			
Biliary cirrhosis	1.000	.	0.231
Viral hepatitis	0.744	0.401-1.380	0.348
Alcohol related cirrhosis	0.944	0.488-1.825	0.863
Other	1.379	0.800-2.376	0.247
Donor			
Age	1.003	0.988-1.018	0.692
Gender	1.123	0.735-1.718	0.591
Body mass index	1.005	0.948-1.065	0.869
DCD graft	3.913	1.200-12.767	0.024
Surgical			
Cold ischemia time	1.091	0.983-1.211	0.102
Recipient warm ischemia time	1.032	1.008-1.056	0.008
>5 units RBC transfusion	1.440	0.896-2.313	0.132

CI, confidence interval; DCD, donation after circulatory death; MELD, model for end stage liver disease; RBC, red blood cells.

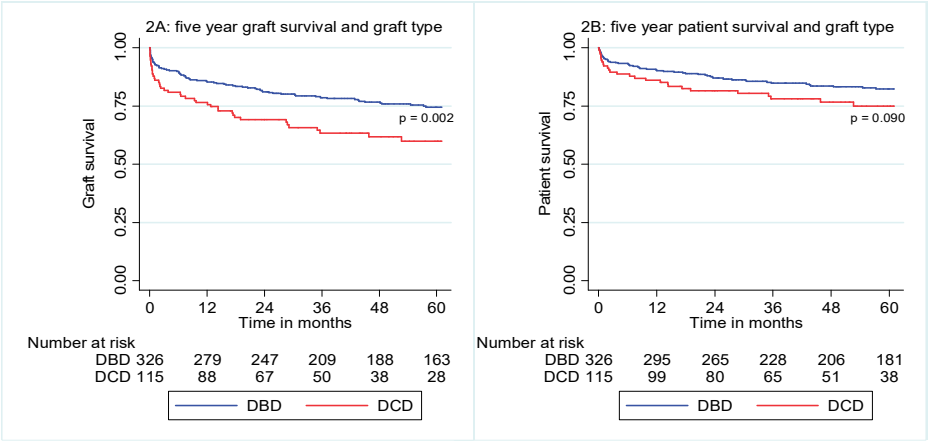

Figure 2 - Five year graft (A) and patient (B) survival after DCD and DBD liver transplantation

on the time frame that is analyzed (3,5,7,24,25). The novelty of our study lies in the method of comparing outcomes between DCD and DBD grafts. With the new CCI as a continuous variable, we can actually show the extent of difference in morbidity between the grafts and how the burden of complications increases over time.

The potential complications after DCD liver transplantation require a delicate selection of grafts and matching recipients. Known factors that impair outcomes of DCD grafts are both recipient and donor age and BMI, MELD-score, and prolonged cold and warm ischemic times (26,27). Our DCD and DBD cohorts show similar recipient characteristics with comparable MELD and Child-Pugh scores, previous medical history and even a higher recipient age in DCD recipients. The donor age limit of 60 years in DCD transplantation led to a lower donor age. Also, according to a protocol cut-off level of 28 for BMI in DCD donors to minimalize severely steatotic liver grafts, BMI was significantly lower in the DCD group. Importantly, cold and recipient warm ischemia times were comparable for DCD and DBD grafts.

The recently developed CCI is based on a formula used in the economic world, which incorporates multiple factors influencing the globalization of a corporation decision. With this formula all complications, weighted by severity, are integrated in a linear scale from 0 to 100. It facilitates reporting not only of the in-hospital morbidity, but also at various postoperative follow up moments, e.g. the six-months morbidity. In this study, the CCI proves again its easy applicability to longitudinal assessment of complications over time, as illustrated in the analysis of the six-months follow up. Also, the CCI correlates well with more traditional complication parameters, such as length of stay, grade 3 and higher complications and graft survival, which adds clinical relevance to this new global marker of morbidity. The major benefit of the CCI is that although the incidence of particular complications may be low, the accumulation of all complications and their severity is accounted for in the value of the CCI.

Our regression model identified DCD grafts as the main factor associated with a CCI of >60 after six months. We chose this cut-off because of the clinical example given in the results. Furthermore, Schlegel *et al* recently showed in a risk assessment of high MELD recipients that the median CCI for recipients with MELD >30 was 56 after one year (28). The CCI in this study correlated well with several preoperative risk scores for graft survival after liver transplantation as well. Other significant factors in our model included recipient BMI and prolonged recipient WIT. Obesity is a known factor in surgery for infectious complications and long-term outcomes, but results in liver transplantation are not conclusive and malnutrition is here an important factor

as well (29–31). Recipient WIT aggravates hepatic IRI, but recent studies focus more on the impact of the obligatory DWIT in DCD liver transplantation (32,33). DWIT is per definition not present in DBD donors, but this period is indirectly represented through the DCD graft factor in our multivariable model.

Parallel to the classical outcomes, the CCI showed no differences between DBD and DCD recipients during the index in-hospital stay, probably reflecting effective donor-recipient matching to balance the risks intrinsic to the use of a DCD graft. However, after six months more recipients in the DCD group developed biliary complications and required a retransplant. The progression of the CCI showed a similar picture: during the initial hospital admittance values were equal, but after six months the CCI was significantly higher for DCD graft recipients. The calculation of the CCI for the subgroups showed that mainly biliary and medical complications were more common and required more treatment in the DCD group within the first six months. Because only 22 recipients developed ITBL in this period, we did not create subgroup to evaluate the CCI for ITBL alone. The higher retransplantation rate at six months after DCD liver transplantation was predictive for a worse long-term outcome in our cohort and DCD recipients had a lower 5-year estimated graft survival. Earlier studies on this subject are not conclusive and several studies have reported impaired long-term graft survival for DCD grafts as well, while others showed results comparable with DBD grafts (8,34–36). The discrepancies between these studies is likely the result of variation in the selection of DCD donors and recipients between countries and transplant centers. The five-year patient survival was not inferior for DCD grafts in this study, probably due to our liberal retransplantation policy for patients with a failing graft due to ITBL. If ITBL as a late effect of the use of DCD liver grafts can be prevented, the outcome of DCD liver grafts can become truly comparable to that of DBD liver grafts. Hypothermic oxygenated and normothermic machine perfusion are currently under study to improve graft quality and show promising results in expanding the viable organ donor pool and decrease ITBL requiring retransplantation (37,38).

This study has several limitations. Data was collected retrospectively, but performed by only one individual, leading to a consistent interpretation and registration of complications. Our DCD and DBD cohorts are comparable for most of the pretransplant characteristics, but inherent to the use of high-risk grafts, some donor and recipient characteristics were significantly different, as described above. This reflects however the actual clinical situation in many institutions using DCD grafts, because of stricter donor and recipient selection criteria when DCD grafts are used. Therefore, we de-

cided not to balance the groups using propensity matching in this cohort. Recipients requiring retransplantation form a unique group: a 4A complication was scored for retransplantation and we terminated follow up after retransplantation, because we encountered a case with a retransplant for PNF and the recipient developed ITBL after retransplantation. Also, the CCI is originally developed for surgical complications, while in liver transplantation postoperative infections and medical complications are common as well.

In conclusion, with the use of the CCI this study provides a new view on postoperative complications and graft usage in liver transplantation. Patients receiving a DCD graft have a similar course during the postoperative hospital stay, but more complications occur thereafter in the first six months. Biliary complications such as ITBL, are major contributors to the morbidity of DCD liver transplantation. The following graft survival is impaired for DCD grafts as well. The novel graft persevering techniques, such as machine perfusion, are essential to improve the overall outcomes of DCD grafts.

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Chapter

Onset of donor warm ischemia time in DCD liver transplantation: hypotension or hypoxia?

Marit Kalisvaart
Jubi de Haan
Wojciech Polak
Diederik Gommers
Herold Metselaar
Jan IJzermans
Jeroen de Jonge

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SUMMARY

The aim of this study was to investigate the impact of hypoxia and hypotension during agonal phase of DWIT on hepatic IRI and complications in DCD liver transplantation. A retrospective single-centre study of 93 DCD liver transplants (Maastricht type III) was performed. DWIT was divided into two periods: agonal phase (from WoT until circulatory arrest) and asystolic phase (circulatory arrest until cold perfusion). During agonal phase, a drop to <80% in SpO2 was considered as the start of hypoxia (SpO2-Agonal) and a drop to <50 mmHg as the start of hypotension (SBP-Agonal). Peak postoperative AST level >3000 U/L was considered as severe hepatic IRI. SpO2 dropped within 2 minutes after WoT <80%, while the SBP dropped to <50 mmHg after 9 minutes, resulting in a longer SpO2-Agonal (13 minutes) than SBP-Agonal (6 minutes). In multiple logistic regression analysis, only duration of SpO2-Agonal was associated with severe hepatic IRI (p=0.006) and not SBP-Agonal (p=0.32). Also, recipients with long SpO2-Agonal (>13 minutes) had more complications with a higher CCI during hospital admission (43.0 vs 32.0; p=0.002) and 90-day graft loss (26% vs. 6%; p=0.01), compared to recipients with a short SpO2-Agonal (≤13 minutes). Furthermore, Cox proportional hazard modelling identified a long SpO2-Agonal as a risk factor for long-term graft loss (HR 3.30; 95% CI 1.15-9.48; p=0.03). In conclusion, the onset of hypoxia during agonal phase is related to severity of hepatic IRI and postoperative complications. Therefore, SpO2 <80% should be considered as the start of functional DWIT in DCD liver transplantation.

INTRODUCTION

DCD grafts are increasingly being used in liver transplantation and in some European countries more than one third of the deceased donor liver transplants are performed with DCD grafts (1–3). Nonetheless, the use of these marginal grafts is associated with various biliary and renal complications, resulting in inferior survival rates, compared to DBD grafts (4–11).

The inferior results are likely the consequence of the obligatory DWIT. This extra period of warm ischemia in DCD grafts is thought to aggravate hepatic IRI (12,13). In previous studies duration of DWIT has been linked to biliary complications and impaired graft survival (9–13). In general, DWIT consists of two periods (**Figure 3 – Chapter 2**): the agonal phase (from WoT until circulatory arrest) and asystolic phase (from circulatory arrest until cold perfusion). However, multiple definitions for DWIT are currently being used (14). The trajectory of agonal phase differs widely between donors and the exact moment of onset of hepatic tissue injury is still unknown. Furthermore, the lack of an uniform definition for the onset of DWIT compromises evaluation of its impact on recipient outcomes. Earlier studies, primarily focusing on the blood pressure during agonal phase, yielded varying results on its relation to postoperative complications (15–18). Abt et al showed that a slow decline in SBP during agonal phase is correlated with long term graft loss. Therefore, SBP is frequently used to mark the beginning of functional DWIT. However, from our clinical experience we have noticed that the peripheral oxygen saturation (SpO2) drops much quicker than SBP during the agonal phase. We therefore hypothesize that ischemic hepatic injury is more closely related to the onset of hypoxia, rather than hypotension during the agonal phase.

The aim of this study is first, to explore the relation between vital parameters during agonal phase and hepatic IRI and observe potential cut-off points for the start of functional DWIT. Secondly, we will explore the relation between the agonal phase and recipient outcome in DCD liver transplantation.

METHODS

This retrospective study was performed with approval of the Erasmus University Medical Centre Institutional Review Board (registration number: MEC-2014-670). All consecutive adult patients who underwent DCD liver transplantation in our centre

from July 2008 until March 2016 were included. All DCD grafts were retrieved from Maastricht type III donors. Exclusion criteria were retransplantation and liver transplantation for acute liver failure. None of the grafts were machine perfused. In the Netherlands, DCD donors older than 60 years of age or with a BMI >28 are regularly not considered for liver donation. Also, we allocate DCD livers preferably to recipients with HCC and/or a relatively low biological MELD-score of <20. The DRI was calculated to express graft quality (19). The amount of graft steatosis was assessed in biopsies taken after reperfusion. Data following duration and structure of different phases of DWIT were retrieved from the Eurotransplant database. An overview of DWIT is displayed in Figure 1A. The cut-off time points during agonal phase were considered at <80% for SpO2 (SpO2-Agonal) and <50 mmHg for SBP (SBP-Agonal). DCD grafts used in this study come from donors in the Netherlands only and are offered through the Eurotransplant waitlist, based on MELD status. In the Netherlands, WoT of the donor is on the ICU. After circulatory arrest, there is an obligatory 5 minutes waiting time, where after the donor is declared deceased and transported to the operation theatre (5-10 minutes) for organ procurement, using the super-rapid retrieval technique (20). The first flush (6-8 L) is performed with UW (University of Wisconsin) preservation fluid under a pressure of 200 mmHg via the aorta. The portal vein, hepatic artery and biliary tract are additionally flushed at the back-table with UW as well. Standard surgical technique included piggyback cavo-caval anastomosis with only incidental use of a portocaval shunt and the graft was routinely reperfused via portal vein. Duration of the surgical procedure, cold ischemia time, and recipient WIT, intraoperative blood loss with subsequent transfusion of red blood cell concentrates (RBC), and fresh frozen plasma (FFP) and end of surgery requirement of vasopressors were all recorded. The postreperfusion syndrome (PRS) was defined as a >30% drop in mean arterial pressure (MAP) in the 5 minutes after reperfusion, lasting at least 1 minute (21). The peak AST level in the first 72 postoperative hours was used as a marker for severity of hepatic IRI, a known marker for early allograft dysfunction (EAD) as well (22). The standard immunosuppression regime in our centre is based on the triple therapy strategy: prednisolone and MMF from day 0, basiliximab at day 0 and day 4 and introduction of tacrolimus on postoperative day 5. Duration of hospital and ICU admission, recipient and graft survival were all documented to assess postoperative morbidity and mortality. All major postoperative complications in the first year were documented. Major postoperative complications were defined as grade ≥ 3b by the Clavien-Dindo classification and the CCI was calculated at hospital discharge, 6 months and 12 months after transplantation (23,24).

Statistical analysis

Data were analysed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, NY, USA). The student's t test was used to compare normally distributed continuous variables. Nonparametric continuous variables were compared using the Mann-Whitney U test. To compare categorical variables the Chi-square test or Fisher's exact test were used. P-values <0.05 were considered statistically significant. Continuous variables were expressed as mean with standard deviation (SD) or median and inter-quartile range (IQR), where appropriate. A multiple logistic regression analysis was used to identify donor, DWIT and surgical risk factors associated with severe hepatic IRI (peak serum AST >3000 U/L). Two years graft and patient survival rates were estimated using Kaplan-Meier methods. A Cox proportional hazard model was used to identify donor, surgical and recipient factors associated with graft loss within the first two years after transplantation.

RESULTS

During the study period, 105 patients underwent DCD liver transplantation of whom 93 were included. Two cases were excluded because of re-transplantation and one recipient was transplanted for acute liver failure. In eight cases the duration of DWIT and donor's hemodynamic profile data was missing. In one case intraoperative data was not complete.

Baseline characteristics

Baseline characteristics are displayed in **Table 1**. Median recipient age was 57 years and 70% of the recipients were male. Viral hepatitis was the most common diagnosis of liver disease (27%), followed by alcohol-related cirrhosis (22%), and biliary cirrhosis (17%). Mean preoperative biological MELD score was 15. Median donor age was 47 years and 58% of the donors were male. The mean DRI was 2.6. Mean duration of total DWIT and asystolic phase were respectively 32 minutes and 16 minutes. Average duration of the total agonal phase was also 16 minutes. Of note, a variety in dispersion of the DWIT-phases was observed (**Table 1**): The SD and range in agonal phase were longer than in asystolic phase. SpO2 dropped already 2 minutes after WoT below 80%, while the SBP dropped below 50 mmHg after 9 minutes (**Figure 1**), resulting in a longer SpO2-Agonal (13 minutes) than SBP-Agonal (6 minutes). Also, the dispersion of SpO2-Agonal was greater than SBP-Agonal, with larger SD and range. The mean time between the drop in SpO2 and SBP was 7 minutes. The mean duration of cold ischemia time and RWIT was 6.6 hours and 29 minutes, respectively.

Table 1 - baseline donor, recipient and surgical characteristics in DCD liver transplantation

Donor	Total (n=93)	Donor warm ischemia (minutes)	Total (n=93)
Donor risk index	2.6 (0.46)	Total donor warm ischemia time	32 (32; 12-60)
Age (years)	47 (39-53)	Asystolic phase	16 (15; 7-28)
Male gender (%)	54 (58)	Agonal phase	
Body mass index	24.2 (22.0-26.0)	Total agonal phase	16 (15; 4-39)
serum AST (U/L)	46 (29-89)	SpO2-Agonal	13 (12; 2-38)
Graft steatosis		SBP-Agonal	6 (5; 1-17)
None	53 (57%)	Between SpO2 & SBP-Agonal	7 (6; 0-24)
1 - 10%	33 (36%)	**Transplant procedure**	
11 - 33%	4 (4%)	Operation time (hours)	7.9 (7.7; 6-12)
>33%	3 (3%)	Cold ischemia time (hours)	6.6 (6.4; 4-12)
Recipient		Recipient warm ischemia time (min)	29 (28; 17-61)
Age (years)	57 (49-63)	Blood loss (liters)	4.1 (3.2-6.2)
Male gender (%)	65 (70)	RBC transfusion (units)	3 (1-5)
Body mass index	26.5 (4.3)	FFP transfusion (units)	3 (0-6)
Etiology of liver disease (%)		Postreperfusion syndrome (%)	37 (40)
Biliary cirrhosis	16 (17)	Dose norepinephrine end of surgery (ug/kg/min)	0.30 (0.12-0.50)
Viral hepatitis	25 (27)	**Postoperative**	
Postalcoholic cirrhosis	20 (22)	EAD	56 (60%)
Other	32 (34)	Postoperative peak serum AST (U/L)	2287 (1305-4881)
Hepatocellular carcinoma (%)	31 (33)	Peak serum AST in categories	
LabMELD score	15 (7.8)	0 - 3000 U/L	53 (57)
Sodium (mmol/L)	138 (134-140)	>3000 U/L	40 (43)
Medical history (%)		AST = aspartate aminotransferase; EAD, early allograft dysfunction; FFP, fresh frozen plasma; MELD, model for end stage liver disease; RBC, red blood cells; SBP, systolic blood pressure. Continuous variables are displayed as mean (standard deviation) and median (IQR) where appropriate. Values of DWIT and intraoperative periods are displayed as follows: mean (median; range).	
Hypertension	19 (20)		
Coronary artery disease	2 (2)		
Diabetes mellitus	29 (31)		

Duration of ischemia periods and hepatic ischemia/reperfusion injury

More than half of the recipients developed EAD (60%) according to the Olthoff criteria (22), due to the relatively high peak AST levels (median 2287 U/L) in the first days after DCD liver transplantation. Therefore, this parameter was not considered suitable to quantify hepatic IRI. Instead, we used a cut off of 3000 U/L in peak AST in the first 72h after transplantation. The peak AST was >3000 U/L in 43% of the recipients. Univariable analysis of risk factors associated with severe hepatic IRI (**Table 2A**) showed that duration of total agonal phase was significantly associated to a peak AST >3000 U/L (OR 1.08; 95%CI 1.01-1.14; p=0.01), but the correlation for SpO2-Agonal was even stronger (OR 1.11; 95%CI 1.01-1.20; p=0.004). No relation was observed for SBP-Agonal and severe hepatic IRI (p=0.41). Multivariable analysis of the same

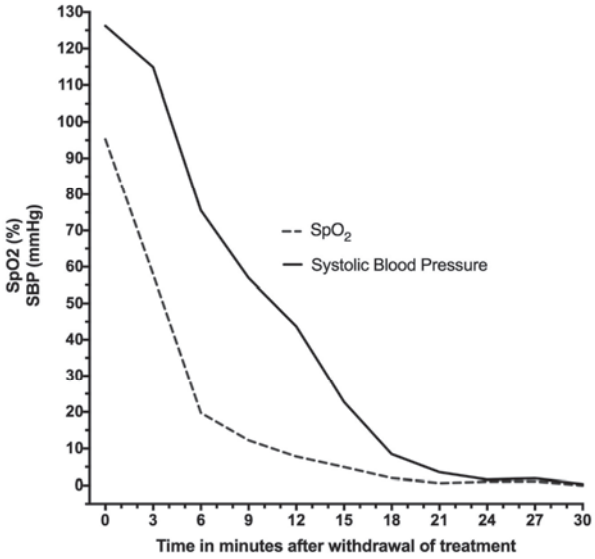

Figure 1 – The course of SpO2-Agonal and SBP-Agonal after withdrawal of treatment of the liver organ donors.

risk factors was used to correct for potential confounders in relation to severe hepatic IRI. Two multivariable analyses were performed, both with all factors of the univariable analysis and either with the SpO2-Agonal (**Table 2B**) or SBP-Agonal (**Table 2C**) period. In table 2B agonal phase was split into the time before and after the drop <80% in SpO2 and in table 2C agonal phase was equally split using the drop in SBP <50 mmHg. **Table 2B** shows that only the SpO2-Agonal is related to severe hepatic IRI (p=0.006) and not the period before the drop in SpO2 (p=0.26). On the contrary, Table 2C shows that the duration of the SBP-Agonal period was not an independent factor (p=0.32), while the period between the drop in SpO2 and the drop in SBP was significantly related to severe hepatic IRI (hypoxic agonal phase) (p=0.003).

Duration of SpO2-Agonal and clinically relevant outcome parameters

Early postoperative complications

Table 3 displays the recipient outcome for duration of SpO2-Agonal. After visualization of LOESS plots, the SpO2-Agonal was divided into two groups at the mean of 13 minutes. Recipients with a long SpO2-Agonal had significantly more postoperative complications and a longer length of stay, for both ICU and hospital admission. The CCI was measured at hospital discharge, six months and one year after transplantation and at each moment the CCI was significantly higher in recipient with a long

Table 2 - Univariable and multivariable analysis of risk factors associated with severe hepatic ischemia/reperfusion injury in DCD liver transplantation

2A. Univariable analysis				2B. Multivariable analysis SpO2-Agonal				2C. Multivariable analysis SBP-Agonal			
Donor	**OR**	**95% CI**	**P-value**	***Donor***	**OR**	**95% CI**	**P-value**	***Donor***	**OR**	**95% CI**	**P-value**
Age (years)	1.03	1.00 - 1.06	0.086	Age (years)	1.05	0.98 - 1.12	0.10	Age (years)	1.05	0.98 - 1.12	0.20
Female gender	0.67	0.29 - 1.53	0.34	Female gender	0.43	0.13 - 1.43	0.10	Female gender	0.51	0.15 - 1.74	0.29
Body mass index	1.06	0.95 - 1.18	0.30	Body mass index	0.87	0.72 - 1.06	0.19	Body mass index	0.88	0.73 - 1.06	0.19
Donor risk index	0.75	0.30 - 1.85	0.53	Donor risk index	0.51	0.08 - 3.42	0.37	Donor risk index	0.48	0.07 - 3.43	0.46
Graft steatosis				*Graft steatosis*				*Graft steatosis*			
No steatosis	1.00			No steatosis	1.00			No steatosis	1.00		
Steatosis 1-10%	2.54	1.04 - 6.22	0.04	Steatosis 1-10%	2.57	0.83 - 7.92	0.09	Steatosis 1-10%	2.43	0.78 - 7.63	0.13
Steatosis >10%	5.29	0.93 - 30.11	0.06	Steatosis >10%	9.17	1.32 - 63.94	0.02	Steatosis >10%	8.80	1.26 - 61.32	0.03
DWIT				***DWIT***				***DWIT***			
Total agonal phase	1.08	1.01 - 1.14	0.01	*Agonal phase*				*Agonal phase*			
SpO2-Agonal	1.11	1.04 - 1.20	0.004	Before SpO2-Agonal	0.88	0.70 - 1.10	0.26	Before SpO2-Agonal	0.89	0.71 - 1.11	0.30
SBP-Agonal	1.04	0.94 - 1.15	0.41	SpO2-Agonal	1.14	1.04 - 1.26	0.006	SBP-Agonal	1.07	0.94 - 1.23	0.32
.	.	.	.	.	.	.	.	Between SpO2 & SBP-Agonal	1.19	1.06 - 1.34	0.003
Asystolic phase	1.05	0.96 - 1.15	0.32	Asystolic phase	1.08	0.99 - 1.13	0.37	Asystolic phase	1.11	0.97 - 1.28	0.13
Intra-operative				***Intra-operative***				***Intra-operative***			
Cold ischemia time	0.97	0.69 - 1.35	0.84	Cold ischemia time	1.09	0.73 - 1.70	0.63	Cold ischemia time	1.09	0.71 - 1.69	0.69
RWIT	1.05	1.00 - 1.11	0.07	RWIT	1.06	0.99 - 1.13	0.07	RWIT	1.06	0.99 - 1.14	0.10
RBC transfusion	0.97	0.87 - 1.10	0.65	RBC transfusion	0.93	0.84 - 1.13	0.75	RBC transfusion	0.91	0.77 - 1.08	0.27

Figure 2A: Univariable analysis of factors associated with severe hepatic ischemia/reperfusion injury (peak postoperative AST >3000 U/L).
Figure 2B: Multivariable analysis with agonal phases splitted at the cut-off of saturation drop below 80%.
Figure 2C: Multivariable analysis with agonal phases splitted at the cut-off of systolic blood pressure below 50 mm Hg.
CI, confidence interval; DWIT, donor warm ischemia time; OR, odds ratio; RBC, red blood cells; RWIT, recipient warm ischemia time.

SpO2-Agonal. Also, recipients in the long SpO2-Agonal group experienced more 90-day graft loss (26% versus 6%; p=0.01). Three recipients had postoperative PNF of the graft requiring retransplantation and all of them had a long SpO2-Agonal. Surprisingly, no correlation was observed between duration of SpO2-Agonal and development of biliary complications in the first year after transplantation.

Long term graft loss

The median follow up was 2.2 years. The estimated 2-year graft and patient survival was 79% and 86%, respectively. **Figure 2** shows the 2-year graft survival for recipients with a short and long SpO2-Agonal. Recipients with a long SpO2-Agonal had the worst survival rate (70% versus 87%; p=0.03). No difference was observed in

Table 3 - Duration of SpO2-Agonal and recipient outcomes after DCD liver transplantation.

Length of SpO2-Agonal	<13 minutes (n=47)	≥13 minutes (n=46)	Total (n=93)	p-value
In-hospital				
≥ 1 Major postoperative complication*	10 (21%)	25 (54%)	35 (385)	0.001
Length of ICU admission (days)	2 (2-3)	3 (2-6)	2 (2-4)	0.02
Length of hospital admission (days)	16 (14-22)	22 (17-29)	18 (15-26)	0.009
Comprehensive Complication Index (median)				
In-hospital	32.0	43.0	38.1	0.002
Six months	46.0	58.4	48.2	0.006
One year	48.0	62.8	59.7	0.008
90-day graft loss	3 (6%)	12 (26%)	15 (16%)	0.01
Retransplantation - HAT	2 (4%)	3 (7%)	5 (5%)	
Retransplantation - PNF	0	3 (7%)	3 (3%)	
Retransplantation - ITBL	0	1 (2%)	1 (1%)	
Death	1 (2%)	5 (11%)	6 (6%)	
Biliary complications in first year				
Overall biliary complications**	17 (36%)	18 (39%)	35 (38%)	0.77
Anastomotic strictures***	12 (26%)	12 (26%)	24 (26%)	0.95
ITBL	6 (13%)	5 (11%)	11 (11%)	0.78
Retransplantation for ITBL	1 (2%)	2 (4%)	3 (3%)	0.62

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

** Including all biliary complications requiring at least medical treatment (i.e. antibiotics / ursodeoxycholic acid).

*** Requiring at least endoscopic treatment.

HAT, hepatic artery thrombosis; ICU, intensive care unit; ITBL, ischemic-type biliary lesions; PNF, primary non function.

two-year patient survival for recipients with a short (92%) and long (80%) SBP-Agonal (p=0.12). The Cox proportional hazard model (**Table 4**) for graft loss in the first two years after transplantation showed that a long SpO2-Agonal leads to a three-fold increase in hazard ratio (HR) for graft loss (HR 3.30; 95% CI 1.15–9.48; p=0.03). Increasing recipient BMI was the other independent factor is this model (HR 1.13; 95% CI 1.02–1.26; p=0.02).

DISCUSSION

This study provides new insight in the impact of vital parameters during the agonal phase on hepatic IRI in DCD liver transplantation. Our findings confirm the hypothesis that ischemic hepatic injury is more closely related to the onset of hypoxia, rather than hypotension. Similarly, in regular medical practice we fear hypoxia for damaging a patient's organs, while a short period of severe hypotension is tolerated

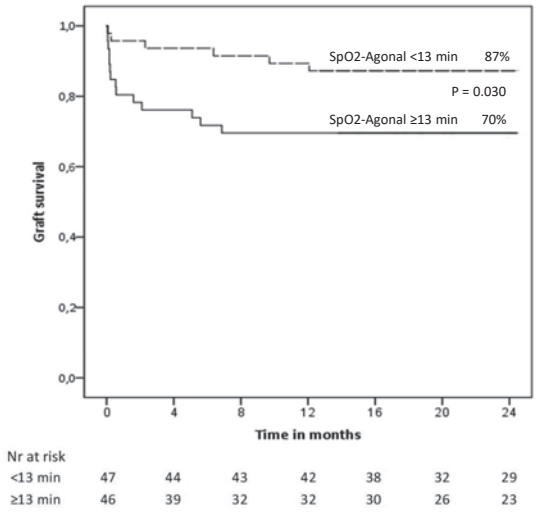

Figure 2 - Kaplan Meier curve for duration of SpO2-Agonal and graft survival after DCD liver transplantation.

Table 4. Cox proportional hazard model for graft loss after DCD liver transplantation.

Recipient	HR	95% CI	P-value
Age	1.01	0.96 - 1.07	0.63
Body mass index	1.13	1.02 - 1.26	0.02
LabMELD	0.99	0.92 - 1.08	0.97
Hepatocellular carcinoma	0.28	0.07 - 1.15	0.08
Donor			
Donor risk index	0.91	0.30 - 2.75	0.87
Graft steatosis			
None	1.00		
1-10%	1.84	0.70 - 4.79	0.21
>10%	4.11	0.77 - 22.01	0.09
Ischemia periods			
DWIT - SpO2-Agonal			
<13 minutes	1.00		
≥13 minutes	3.30	1.15 - 9.48	0.03
DWIT - asystolic phase	1.06	0.94 - 1.20	0.33
Cold ischemia time	1.02	0.95 - 1.08	0.64
Recipient warm ischemia time	1.23	0.89 - 1.71	0.20

CI, confidence interval; DWIT, donor warm ischemia time; HR, hazard ratio; MELD, model for end stage liver disease.

much better. Therefore, we hypothesized hypoxia would be more detrimental to DCD donor livers than hypotension during the agonal phase and our results indicate that the drop in SpO2 to <80% is much more relevant to assess the potential severity of hepatic IRI. Moreover, a prolonged SpO2-Agonal is associated with more postoperative complications and long-term graft failure.

Previous studies have used many different definitions for DWIT and consequently, the duration of an acceptable DWIT in these studies ranges from 10 to 35 minutes (15,17,25–27). The study by Abt et al showed that a quick drop in SBP after WoT was associated with an improved graft survival (15). Hong et al identified a period of MAP <60 mmHg before circulatory arrest longer than 20 minutes increased the risk for graft loss (17). Of note, the course of SpO2 was not considered in these studies. In a study of Firl et al the trajectory of agonal phase was divided into three categories by the course of SpO2 and MAP; a quick decline, a slow gradual decline, and a relatively long stable phase with initial good vital parameters with a quick decline just before circulatory arrest (16). The authors were not able to form strong conclusions donors with the worst prognosis, but in donors with a slow decline, only the course

of MAP was associated with graft loss. The course of SpO2 was not associated with graft loss, but is should be noted that pulse oximetry measurement of SpO2 is not reliable below a value of 80% (28–30). A more recent multicentre-study by Coffey et al investigated the relation between potential cut-offs of SpO2 and MAP and a composite endpoint consisting of early and late complications (31). Only the cut-off of SpO2 <60% was associated with postoperative complications, but these results were not confirmed in a multivariable analysis. Important pitfalls of this study are the low SpO2 cut-off of 60%, the uniform outcome that weakens potential effect on different complications and the lack of an analysis of the relation between DWIT and hepatic IRI.

In many countries, the cut-off for discarding a DCD liver is 30 minutes of functional DWIT (start of agonal phase at SBP<50 mm Hg until cold perfusion) and we also analysed these periods with this cut-offs for SBP. The cut-off for SpO2 was set at 80%, because the accuracy of pulse oximetry decreases significantly with arterial haemoglobin saturation levels below 75-80%. Using these cut-offs, the onset of hypoxia was on average almost directly after WoT and seven minutes earlier than hypotension, leading to a much longer SpO2-Agonal than SBP-Agonal period. In the multivariable analysis, only duration of SpO2-Agonal was associated with severe hepatic IRI. More importantly, the period between the drop in SpO2 and SBP (hypoxic agonal phase) was actually the period related to severe hepatic IRI in this multivariable model, suggesting that an agonal phase starting with a long hypoxic period is the driving force of the extra hepatic IRI in DCD grafts. These results are supported by studies investigating shock livers suggesting that hypoxia is an important cause for hepatic injury, even without hemodynamic shock (32). The increased hepatic IRI with the use of DCD grafts has previously been linked to impaired outcomes (13,33,34). We found that recipients of a graft with a SpO2-Agonal period longer than 13 minutes had more major complications, a higher CCI and more graft loss in the first 90 days. Moreover, the three recipients with PNF all had a SpO2-Agonal of ≥13 minutes. Considering the sum of SpO2-Agonal (13 min) and asystolic phase (16 min) is 29 min, we would advise to set the new cut-off for functional DWIT at 30 minutes.

The severity of hepatic IRI and complications has a multifactorial origin. Steatotic grafts are more vulnerable to IRI and graft steatosis was a significant factor in our multivariable analysis as well (36,37). We also observed a trend for increased RWIT, a known factor to increase hepatic IRI (38). Remarkably, the duration of the asystolic phase was not significant, this might be caused by the relative narrow dispersion of this phase, reflecting the legal five minutes no-touch period, transport of the

donor and cannulation time of the dedicated organ retrieval team, thus almost standardizing this period in all donors. Cold ischemia was not a factor, which could be explained by the relatively short cold ischemia time (mean <6.5h). The molecular pathways that could potentially explain our results regarding the importance of hypoxia have been previously studied before in renal transplantation by Damman et al (39). In this human study, transcriptomics of donor biopsies during retrieval and transplantation followed by functional pathway analysis showed that pathways related to prolonged and worsening deprivation of oxygen were associated with delayed graft function in DCD grafts. These pathways were already upregulated before organ retrieval and included metabolic pathways related to hypoxia and the complement-and-coagulation cascades. After reperfusion, these pathways were related to delayed kidney graft function. Furthermore, an experimental study in a rat liver transplant model with simulated prolonged WIT by Zhang et al confirmed that these complement-associated pathways are an important factor in the severity of hepatic IRI (40). To our knowledge, such analyses of prolonged warm ischemia, have not yet been performed in human liver transplantation. However, these studies confirm that hypoxia is an important factor in the warm phase of IRI. Interestingly, the duration of SpO2-Agonal was not related to development of biliary complications in our study. It is known that the biliary tree responds differently to ischemia than hepatocytes and previous experimental studies suggested that they are better resistant to hypoxia, but more susceptible to reoxygenation injury (41,42). Taner et al studied the impact of DWIT on ITBL and only observed a relation between the duration of asystolic phase and development of ITBL (10). This implicates that the biliary tree is less affected by hypoxia or hypotension during the agonal phase, compared to the full no-flow ischemia during the asystolic phase. Despite there was no relation between the duration of SpO2-Agonal and biliary complications, the 90-day outcomes and long-term survival rates were worse for grafts with a long SpO2-Agonal, due to an increased rate of HAT and PNF.

There are several limitations to this study. The retrospective design of this study means that recipient perioperative management varies, but our institution has a well-implemented liver transplant protocol and over 1000 liver transplants done. Donor data was retrieved from Eurotransplant, which has a well-organized database. However, in the first years of DCD organ retrieval, not all DWIT-data was available and we had to exclude eight cases. Peak serum AST levels are a surrogate marker for hepatic IRI, also depending on the weight of the donor liver. The only alternative option would be a histological assessment, but this would be subject to the interpretation by different pathologists. We specifically not use the classic Olthoff-criteria

for EAD, as we have observed that the median peak AST levels is above 2000 U/L in the majority of the DCD recipients. This study is reflecting the practice in the Netherlands, which can be different from other countries.

We believe our study provides strong evidence that hypoxia, rather than hypotension, marks the beginning of hepatic tissue injury in DCD grafts. We advise transplant professionals to consider functional DWIT as the period between SpO2 <80% and start of cold perfusion, irrespective of blood pressure. Duration of hypoxia during the agonal phase over 13 min could identify potential poor-quality grafts and thereby enable the transplant surgeons to engage in application of graft-improving methods such as machine perfusion.

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Chapter

The UK DCD Risk Score: A new proposal to define futility in DCD liver transplantation

Andrea Schlegel
Marit Kalisvaart
Irene Scalera
Richard Laing
Hynek Mergental
Darius Mirza
Thamara Perera
John Isaac
Philipp Dutkowski
Paolo Muiesan

Adapted from: Journal of Hepatology 2018;68(3):456–464.

SUMMARY

In this study, we provide a new prediction model for graft loss in DCD liver transplantation. Using UK national DCD database, a risk analysis was performed in adult recipients of DCD liver grafts in UK between 2000 and 2015 (n=1153). A new risk score was calculated (UK DCD Risk Score) on the basis of regression analysis, and validated using the UNOS-database (n=1617) and our own DCD liver transplant database (n=315). Finally, the new score was compared with two other available prediction systems, the DCD risk scores from UCLA and Kings-College-Hospital, London. Seven strongest predictors of DCD graft survival were identified: functional donor warm ischemia, cold ischemia, recipient MELD, recipient age, donor age, previous liver transplantation, and donor BMI. A combination of these risk factors (*UK DCD Risk Score*) stratified best recipients in terms of graft survival in the entire UK DCD database as well as in the UNOS and in our own DCD population. Importantly, the *UK DCD Risk Score* significantly predicted graft loss due to PNF or IC in the futile group (>10 score points). The new prediction model demonstrated a better C statistic of 0,79, compared to the two other available systems (0,71 and 0,64; respectively). The *UK DCD Risk Score* is a reliable tool to detect high risk and futile combinations of donor and recipient factors in DCD liver transplantation. It is simple to use and offers a great potential to better decide which DCD graft should be rejected or may benefit from functional assessment and further optimization by machine perfusion.

INTRODUCTION

Driven by organ scarcity and the need to decrease waiting list mortality, liver transplant professionals worldwide have expanded the donor organ pool (1). In this context, livers from DCD donors are increasingly used for transplantation and several countries implemented DCD programs (2–4). However, DCD organs may carry further risk due to the additional donor warm ischemia leading potentially to a higher rate of severe complications, such as PNF and ITBL (5). Specific donor and recipient risk factors are therefore critically evaluated by transplant centres (6–9). For example, graft cold ischemia, donor age and donor BMI or recipient MELD score have all been reported to impact on graft survival (7). Careful selection of grafts and recipients appears therefore decisive for outcome. Data analysis from Kings College Hospital (KCH) underline this fact, showing excellent low rates of ITBL (2.5%), when the overall risk is low and WIT is kept short with an average of functional DWIT of 16 minutes (10,11). Such results stand in contrast to other European centres and to previous analyses of US data (12–14), which report longer donor warm ischemia times with higher incidence of ITBL and graft loss (15). The combination of too dangerous risk originating from three sources, e.g. donor, graft and recipient is however unclear.

The intention of this study was therefore to search for an easily applicable score system, based on a few independent donor and recipient key factors, with the highest possible accuracy in prediction of complications and graft loss after DCD liver transplantation.

METHODS

Study Design and Patients

After approval by the National Health Service (NHS) institutional review board in the UK, records of all adult (≥18 years) DCD recipients transplanted for chronic liver disease were extracted from an NHS Blood and Transplant (NHSBT) Analysis and Research file from January 2000 through December 2015 (national UK cohort) (16). Two other DCD cohorts were used for validation. First, adult DCD cases were extracted from the United Network for UNOS database from December, 1987 through September, 2010, after approval by the University of Washington institutional review board (UNOS cohort). And second, we analysed our own institutional DCD database, which included patients transplanted from January 2005, to December 2015 (Birmingham cohort). To reduce confounding variables, paediatric and partial transplants (split

and living donor liver transplantation), DBD and combined liver transplants, were excluded. Recipients with lost follow up were excluded from the analysis. Patients with exception points (e.g., hepatocellular cancer within Milan criteria) were included in the analysis. Importantly, pretransplant MELD score was calculated without inclusion of those exception points (laboratory MELD). The primary outcome measured for regression analysis was one-year graft survival (least missing entries). The final analysis included 1153 patients. The two validation cohorts, UNOS and Birmingham, consisted of 1861 and 315 DCD transplant cases. 0We defined ITBL in this analysis on the basis of radiological findings, e.g. intrahepatic or hilar biliary strictures and dilatations, occurring in the absence of hepatic artery stenosis (HAS) or thrombosis (HAT), portal vein thrombosis, chronic ductopenic rejection, or recurrent primary sclerosing cholangitis. Overall incidence of biliary complications, extrahepatic strictures, ITBL and bililary leakages for our DCD transplants from Birmingham are described in detail in Suppl. Table 5.

Statistical Analysis

To establish a clinically valid regression model, we choose parameters, which are easily available for surgeons at each DCD transplant centre worldwide. Our aim was, therefore, to combine donor, graft, and recipient factors in one practical score system on post-transplant graft survival. All factors should be easily available prior to transplantation. Accordingly, significant variables with impact on outcome after DCD liver transplantation (with a p-value<0.25) were considered further were selected (6 metric, 1 dichotomous), for example, donor age, functional DWIT, cold ischemia, laboratory MELD score, recipient age and retransplantation (17–20). In an attempt to increase information on graft quality, donor BMI was also included as surrogate marker of steatosis and other metabolic disorders (21). Functional DWIT in UK is defined as time between SBP below 50 mmHg and cold aortic organ flush (22).

For candidate predictors with less than 60% missing values (e.g. recipient lab MELD score pre-transplant (32/1153; 2.8% missing), functional DWIT (464/1153; 40.2%) and cold ischemia of the graft (89/1153; 7.7% missing), we performed multiple imputation (MI) using multivariate normal regression, imputing a total of 20 datasets (n=24213) (23). This method assumes that the data are missing at random MAR, which appears as a less restrictive assumption than that required by complete case analysis and multivariate normal regression has been shown to be valid whether or not all imputed variables follow a normal distribution (24). In each of the imputed datasets we used a backward stepwise approach for the multivariable logistic regression with p-values ranging between <0.001 and >0.05 as inclusion and exclusion thresholds,

respectively. The strongest predictors for one-year graft survival after DCD liver transplantation, that were selected in 75% to 80% of the imputation models were included in the final multivariable model. Backward elimination is generally preferred as an automated predictor selection procedure because it takes correlations among predictors into calculation (23,25–31). The imputed data sets were combined and the point system was developed to enhance clinical applicability according to the Framingham risk scheme (**Table 2**) (32). For each predictor, the median of all values below (Midpoint W_1reference – W_7reference) and above the threshold has been calculated. The Midpoint for the cohort below each threshold (W1-7reference) is subtracted from the midpoint of all values above the threshold. The factor β is multiplied with the difference (Wij-Wireference), separately for each factor (32).

Performance and validation of the new prediction model

The performance of the prediction model in the derivation cohort was evaluated by examining measures of discrimination and calibration. Discrimination is the ability of the risk score to differentiate between patients who do and do not experience an event (in our case, the occurrence of graft loss after DCD liver transplantation). This measure was quantified by calculating the area under the curve (AUC) of the receiver operating characteristic statistic. Calibration reflects the agreement between predicted probabilities from the model and observed outcomes. We used the Hosmer–Lemeshow test to statistically determine the extent of agreement between the predicted and the observed probabilities. We performed an internal validation using a bootstrapping procedure. This approach uses the entire data in order to develop the prediction model and in addition accounts for model overfitting or uncertainty compensating for overoptimism in the final prediction model. The bootstrapping in the current study was performed using 500 bootstrap resamples, each time selecting variables and developing a model within the sample. The new prediction model was compared to available systems from UCLA (DCD risk score UCLA) (17) KCH named as DCD Risk Index (DCD-RI) (Suppl. Table 4) (33). Furthermore, the model was validated externally in the UNOS database and internally, in our own population from Birmingham, where we specifically evaluate the predictive value of our new score, excluding retransplantations. Statistical analysis was performed using IBM SPSS Statistics version 23 (IBM Corporation, Armonk, NY, USA).

RESULTS

Are there differences between the 3 study populations from UK, UNOS and Birmingham?

Following eligibility criteria, 1153 cases were available from the UK database, and 1863 were selected from the UNOS database. Our institutional database included 315 DCD transplants. Median follow-up accumulated to 865 days in the UK, as compared to 600 days in US and 936 days in our population. No differences were found between the three populations in terms of recipient age (median 55 vs. 54 vs. 58 years), recipient BMI (27.0 vs. 27.4 vs. 27.0 kg/m^2), donor BMI (median 25.0 vs. 25.1 vs. 25 kg/m^2) and cold ischemia time (median 7.1 vs. 7.0 vs. 7.0 hours). Major differences (UK data vs. UNOS vs. own data) were noted regarding donor age (median 49 vs. 34 vs. 51 years) and functional DWIT (median 17 vs. 14 vs. 17 min). In the UNOS cohort, centre dependent variations exist on the time of initiation of donor warm ischemia. Most US centres, however, define it also as functional DWIT,

Table 1 - Donor, graft, and recipient characteristics in the 3 DCD cohorts in the UK, UNOS, and in Birmingham.

Donor & graft characteristics	UK (n=1153)	UNOS (n=1861)	Birmingham (n=315)
Age (years)	49 (35–59)	34 (21–47)	51 (36–62)
Body mass index (kg/m2)	25 (23–28)	25.1 (22.0–29.1)	25 (22.7–27.7)
Total donor warm ischaemia time (min)	27 (22–31)	-	27 (22–32)
Functional warm ischaemia time (fDWIT) (min)	17 (14–20)	14 (9–21)	17 (14–21)
No. of donors with fDWIT >20 min	254 (22%)	364 (19.6)	82 (26)
No. of donors with fDWIT >30 min	40 (3.5%)	124 (6.7)	13 (4.1)
Asystolic warm ischaemia time (min)	13 (11–15)	-	12 (10–14)
Cold ischaemia time (h)	7.1 (6–8.2)	7 (5.3–9)	7 (5.7–8.1)
No. of grafts with CIT >6 h	853 (74%)	1145 (61.5%)	222 (70.5%)
Recipient characteristics			
Age (years)	55 (48–61)	54 (49–60)	58 (51–64)
Body-Mass-Index (kg/m2)	27 (24–30)	27.4 (23.8–31.2)	27 (24–30)
lab-MELD score	15 (11–19)	16.4 (10.8–23.5)	13 (9–17)
UKELD score	53 (50–57)	-	53 (49–56)
BAR–Score	5 (3–8)	7 (3–10)	5 (3–7)
Follow-up (days)	865 (364–1704)	600 (160–1355)	936 (426–1602)

Data presented as median and IQR for continuous variables or as number and percent for categorical variables.

BAR, Balance of Risk Score; BMI, Body-Mass-Index; CIT, cold ischaemia time; DCD, donation after circulatory death; fDWIT: IQR, interquartile range; MELD, model of end-stage liver disease; UKELD, United Kingdom model of end-stage liver disease; UNOS, United Network for Organ Sharing.

initiated after a specified period of time during sustained hypotension (systolic blood pressure < 50mmHg)(14). The BAR-score at transplantation was slightly higher in UNOS compared to the other cohorts (median 5 vs. 7 vs. 5 points), corresponding to the slightly higher MELD score in UNOS regions when compared to the European population (median 15 vs. 16.4 vs. 13 points)(18). In addition, the percentage of retransplantations at the time of DCD transplant was comparable in UK and UNOS, while all candidates in our own population were primary transplants (11.3% vs. 11.3% vs. 0%, **Table 1**).

Which are the key prediction factors for graft survival after DCD liver transplantation?

Initial calculations were performed in the large DCD database from UK and tested, in a next step, in the UNOS and our institutional DCD population. We evaluated 43

Table 2. Development of the new prediction score based on multivariate regression analysis.

Parameter	Category	Regression Coefficient ßi	p-value	Reference Value Wi (Midpoint)	ßi x (Wij-Wireference)	Risk Score
Donor age	≤ 60 yr	0.084	*0.001*	46 (W_1reference)	0	0
	> 60 yr			66	1.688	**2**
Donor BMI	£ 25 kg/m^2	0.519	*0.0001*	23 (W_2reference)	0	0
	> 25 kg/m^2			28	2.598	**3**
Functional DWIT	≤ 20 min	0.341	*<0.0001*	15 (W_3reference)	0	0
	21-30 min			24	3.069	**3**
	> 30 min			32	5.797	**6**
Cold ischemia time	no	0.791	*0.001*	5.5 (W_4reference)	0	0
	yes			7.7	1.74	**2**
Recipient age	≤ 6h	0.241	*0.0001*	52 (W_5reference)	0	0
	> 6h			64	2.892	**3**
Recipient lab MELD	≤ 25	0.109	*0.0001*	14 (W_6reference)	0	0
	> 25			30	1.744	**2**
Retransplantation	No	8.571	*<0.001*	0 (W_7reference)	0	0
	Yes			1	8.571	**9**
Total Score Points						**0-27**

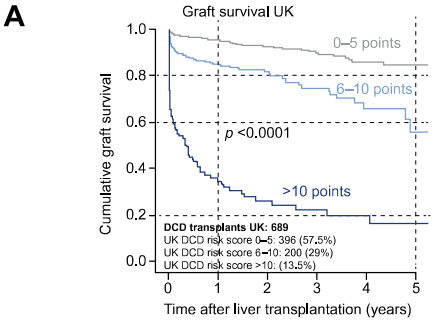
The imputed data sets were combined and the point system was developed according to the Framingham risk scheme. For each predictor, the median of all values (Midpoint W1reference – W7reference) below and above the threshold has been calculated. The Midpoint for the cohort below each threshold (W1-7reference) is subtracted from the midpoint of all values above the threshold. The factor b is multiplied with the difference (Wij-Wireference), separately for each factor to develop the score points.32 BMI, body mass index; CIT, cold ischaemia time; fDWIT: functional donor warm ischaemia time; MELD, model of end-stage liver disease; yr, years; min, minutes; h, hours; b, regression coefficient.

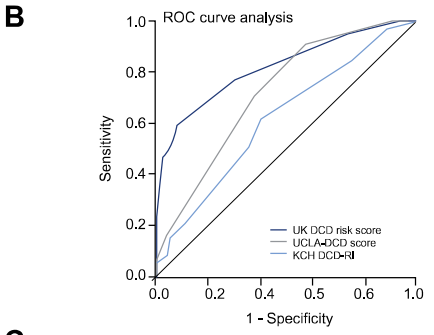
candidate predictors. The median number of missing values per candidate was 6 (IQR 2.5-9.5). Five variables were excluded, due to more than 60% missing values, which involved AST, donor albumin, agonal donor phase, arterial donor pressure and recipient hospital status at transplant. Among the 38 remaining candidate variables 28 had complete data and 10 contained <60% missingness and were subjected to the multiple imputation procedure. The full multivariable prediction model based on the backward stepwise approach and revealed seven parameters as significant predictors for graft loss: donor age and BMI, functional DWIT, cold ischemia time, recipient age, MELD score and previous liver transplantation (**Table 2**).

Recipient BMI was not identified as independent predictor. Using the significant regression coefficients, the UK-DCD-Risk score was calculated with a range from 0 to 27 points reflecting an exponential increase in one-year graft loss. The strongest predictor was retransplantation (0 or 9 points), followed by functional donor warm ischemia (0 or 3 or 6 points), recipient age (0 or 3 points), donor BMI (0 or 3 points), donor age (0 or 2 points), cold ischemia time (0 or 2 points), and lab MELD (0 or 2 points; **Table 2**). Internal validation yielded a c-statistic of 0.79 (95%CI: 0.744-0.839) and the p-value for the Hosmer and Lemeshow goodness of fit test ranged from 0.143 to 0.506 in the 20 imputed data sets, corresponding to a well-calibrated model. Internal validation of the model using bootstrapping procedure revealed minimal optimism of 0.00005 (95%CI: 0.00574 to 0.064).

How does the newly developed risk score compare to other available prediction systems?

In an attempt to compare predictability of outcome, c-statistics of other score systems were assessed in the UK database referred to graft survival. For both available prediction models in DCD liver transplantation, the UCLA-DCDscore (17) and the KCH-DCD-RI from Kings College Hospital(33), the areas under the receiver operator characteristic curves (AUC) were 0.71 and 0.64, respectively (**Figure 1**). These findings were confirmed by a superior AUC for the new DCD model in our own cohort in Birmingham (AUC=0.754), compared to the UCLA-DCD score (AUC=0.639) and the KCH-DCD RI (AUC=0.583) (Suppl. Figure 3). Accordingly, the new UK-DCD-Risk score showed a more significant decrease in graft survival per increasing score cluster (0–5, 6–10, >10; **Figure 1**), when compared to both other models. Of note, the BAR score was also inferior in prediction of graft survival after DCD liver transplantation in this dataset (Suppl. Figure 1). Additional analysis showed that cumulative 1-year graft survival per each single score point followed an exponential increase for the new score model above a certain threshold of approximately 10 points (**Figure 3**). Both





C

Scores	Area	SE	Asymptotic sig.	95% CI
UK DCD risk score	0.79	0.024	<0.0001	0.744–0.839
KCH DCD-RI	0.64	0.027	<0.0001	0.562–0.668
UCLA-DCD score	0.71	0.024	<0.0001	0.663–0.757

Figure 1 - Five-year graft survival according to the UK DCD Risk Score and ROC curve analysis (UK cohort).

(A) Kaplan–Meier survival plots were developed for graft survival according to the three risk classes of the new score model. Comparisons between groups were made using the log-rank test. Discrimination is the ability of the risk score to differentiate between patients who do and do not experience graft loss. This measure was quantified by calculating the AUC statistic. (B) This is displayed in the ROC curve. (C) The table compares the AUC values of the new model with previously described DCD scores from UCLA and KCH. AUC, area under the receiver-operating-characteristic curve; DCD, donation after circulatory death; DCD-RI, donation-after-circulatory-death risk index; KCH, King's College Hospital; ROC, receiver operating characteristic; SE, standard error; UCLA, University of California, Los Angeles.

other DCD scores failed to predict cumulative graft loss at each single score point (**Figure 3**). Of note, UK DCD Score grouping at this threshold (≤10 vs. >10) splitted graft survival best as compared to the two other scores, that is, DCD Score from UCLA (≤4 vs. >4) and DCD-RI from KCH (≤4 vs. >4) (**Figure 3**). The newly developed score showed a very good positive prediction value of 71% and 86.1% in UK and UNOS. The specificity was 0.95.

A

Score	Cohort	Score threshold	n	Positive predictive value*	False omission rate*	Delta	C statistic
UK DCD risk score	UK	≤10	596		13.4%	57.5%	0.79
		>10	93	71.0%			
	UNOS	≤10	1,380		22.7%	63.4%	0.72
		>10	237	86.1%			
KC DCD-RI	UK	≤4	501		17.2%	27.1%	0.64
		>4	97	44.3%			
UCLA-DCD score	UK	≤4	572		19.2%	37.9%	0.71
		>4	42	57.1%			

*: Graft loss above the threshold
*: Graft loss below threshold

B Prediction of one year graft loss by different DCD scores

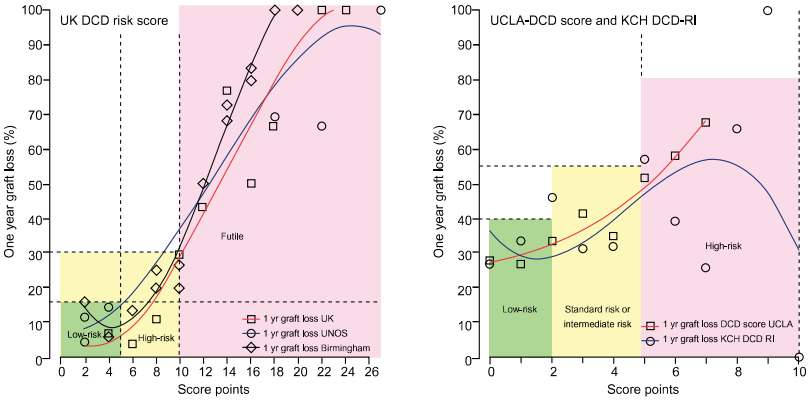
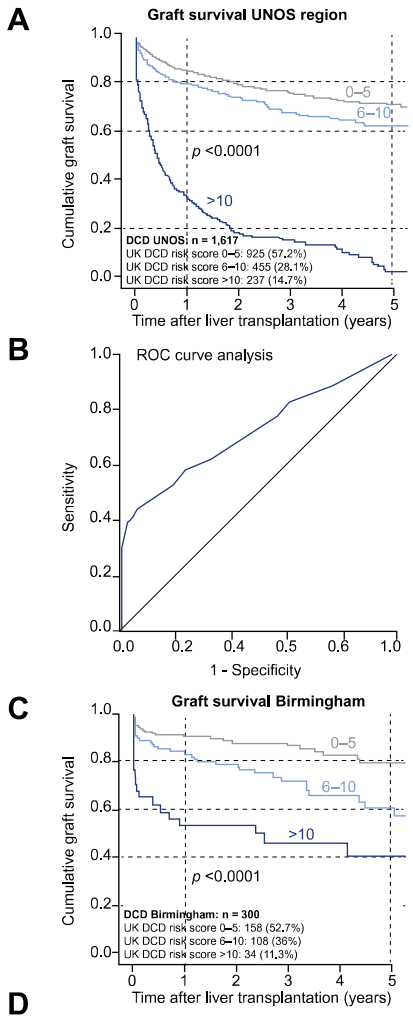


Figure 2 - Prediction of graft loss by the UK DCD Risk Score in all three cohorts, compared to other available DCD risk models.

(A) The positive predictive value and false omission rate are shown together with the specificity of three different score models. (B) Survival plots highlight the percentage of grafts, which are lost with increasing score points, for the new prediction model in three different cohorts (UK, UNOS, and Birmingham) compared to two existing models in the UK. DCD, donation after circulatory death; DCD-RI, donation-after-circulatory-death risk index; KCH, King's College Hospital; UCLA, University of California, Los Angeles; UNOS, United Network for Organ Sharing.

Is the new prediction model valid in other DCD cohorts?

External validation in the UNOS database, confirmed the excellent discrimination between acceptable outcome below the threshold of 10 points and significantly lower graft survival in the futile group with more than 10 UK DCD Score points (**Figure 1&3**). Of note, recipients in the futile group (>10 points) experienced a very high risk for graft loss of more than 60% in one year and > 80% in 5 years. In addition, candidates in the high-risk group (>5 to 10 score points) lost the graft in 18% and 40% in one and five years, compared to the lowest risk group (0-5 points), where 5% and 15% of DCD livers were lost in one and five years, respectively (**Figure 1&2**). Importantly, UK DCD Score ranking was also useful in our own cohort, where the strongest predictor, re-transplantation, was not available (*p<0.0001*; **Figure 2**). Exclusion of cases with a UK DCD risk score of more than 10 points from a DCD liver transplant due to expected poor prognosis would refer to 13.4% of patients in the UK (93/689), to 14.7 % of cases in the US (237/1617) and to 11.4% of cases in our population (34/300) (**Figure 1&2**).



D

Scores	Area	SE	Asymptotic sig.	95% CI
UK DCD risk score UNOS region	0.722	0.015	<0.0001	0.693–0.750

Figure 3 - Validation of the UK DCD Risk Score in the UNOS region and Birmingham. Kaplan–Meier survival plots for graft survival were developed to validate the new model in the UNOS DCD cohort.
Comparisons between groups were made using the log-rank test. (B) In addition, graft survival in our local DCD liver-transplant cohort in Birmingham is displayed. The AUC statistic, obtained from the UNOS cohort, parallels the values we received from the development cohort in the UK. (C) The table demonstrates a similar AUC in the UNOS cohort, when compared to the UK cohort. AUC, area under the receiver-operating-characteristic curve; DCD, donation after circulatory death; ROC, receiver operating characteristic; UNOS, United Network for Organ Sharing.

How does the new score correlate with post-transplant complications and morbidity?

While numerous risk factors have been described previously for DCD liver transplantation, prediction of ischemic cholangiopathy (IC) or also primary non-function (PNF) remains unsolved. We were therefore particularly interested to identify a combination of key factors with significant impact on graft loss due to IC or PNF. Donor-recipient

constellations with more than 10 score points (futile group) experienced significantly more graft loss due to PNFs and IC in both large DCD cohorts in UK and UNOS (*p<0.0001; p=0.0001*; **Table 4**). In addition, recipients in the high risk and futile group developed significantly more often vascular complications, e.g. HAT, leading to graft failure in numerous cases in all cohorts (**Table 4**). In order to evaluate liver function and other important post-transplant complications and general morbidity, we analysed our institutional DCD cohort in accordance with the three clusters of

Table 3. Potential combinations of additional key risk factors providing a UK DCD Risk Score ≤10 or >10 points.

	Potential combinations of risk factors
DCD Risk Score ≤10 points	No retransplantation [0] + recipient MELD ≤25 points [0] + recipient age ≤60 yr [0] + cold ischaemia time >6 h [2] + functional donor warm ischaemia >20 to ≤30 min [3] + donor age ≤60 yr [0] + donor BMI ≤25 [0]
	No retransplantation [0] + recipient MELD >25 points [2] + recipient age ≤60 yr [0] + cold ischaemia time ≤6 h [0] + functional donor warm ischaemia time >30 min [6] + donor age ≤60 yr [0] + donor BMI ≤25 [0]
	No retransplantation [0] + recipient MELD >25 points [2] + recipient age >60 yr [3] + cold ischaemia time >6 h [2] + any functional donor warm ischaemia >20 to ≤30 min [3] + donor age ≤60 yr [0] + donor BMI ≤25 [0]
	No retransplantation [0]+ any functional donor warm ischaemia up to ≤30 min [0–6] + any donor age [0–2] + any recipient MELD [0–2]
	No retransplantation [0]+ functional donor warm ischaemia >20 to ≤30 min [0–3] + any donor age [0–2] + any donor BMI [0–3] + any recipient MELD [0–2]
	Retransplantation [9] + recipient MELD ≤25 points [0] + recipient age ≤60 yr [0] + cold ischaemia time ≤6 h [0] + functional donor warm ischaemia ≤20 min [0] + donor age ≤60 yr [0] + donor BMI ≤25 [0]
DCD Risk Score > 10 points	No retransplantation [0] + recipient MELD >25 points [2] + recipient age >60 yr [3] + cold ischaemia time >6 h [2] + functional donor warm ischaemia >20 to ≤30 min [3] + donor age >60 yr [2] + donor BMI ≤25 [0]
	No retransplantation [0] + recipient MELD >25 points [2] + recipient age ≤60 yr [0] + cold ischaemia time >6 h [2] + functional donor warm ischaemia >30 min [6] + donor age >60 yr [2] + donor BMI ≤25 [0]
	No retransplantation [0] + recipient MELD >25 points [2] + recipient age >60 yr [3] + cold ischaemia time >6 h [2] + functional donor warm ischaemia >30 min [6] + donor age >60 yr [2] + donor BMI ≤25 [0]
	Retransplantation [9] + recipient MELD >25 points [2] + recipient age ≤60 yr [0] + cold ischaemia time ≤6 h [0] + functional donor warm ischaemia ≤20 min [0] + donor age ≤60 yr [0] + donor BMI ≤25 [0]
	Retransplantation [9] + recipient MELD >25 points [2] + recipient age ≤60 yr [0] + cold ischaemia time >6 h [2] + functional donor warm ischaemia >20 to ≤30 min [3] + donor age >60 yr [2] + donor BMI ≤25 [0]

BMI, body mass index; CIT, cold ischaemia time; DCD, donation after circulatory death; fDWIT, functional donor warm ischaemia time; h, hours; MELD, Model of end stage; liver disease; min, minutes; UKELD, United Kingdom model of end-stage liver disease; yr, years.
*Numbers in parenthesis correspond to DCD Risk Score points.

Table 4. Aetiology of graft loss according to the UK DCD Risk Score in the UK, UNOS, and Birmingham DCD cohorts.

Outcome parameter	Overall	Low risk (0-5 points)	High risk (6-10 points)	Futile (>10 points)	p value low vs. high risk	p value high risk vs. futile
UK (n=)	689	396	200	93		
Cause of graft failure						
PNF	29 (4.2%)	2 (0.5%)	2 (1.0%)	25 (26.9%)	0.605	0.001
HAT	14 (2.0%)	1 (0.3%)	4 (2.0%)	9 (9.7%)	0.045	0.005
ITBL	26 (3.8%)	5 (1.3%)	6 (3.0%)	15 (16.2%)	0.194	0.001
One-year graft survival (%)	85%	96%	85%	37%	<0.001	<0.001
UNOS (n=)	1617	925	455	237		
Cause of graft failure						
PNF	87 (5.4%)	21 (2.3%)	12 (2.6%)	54 (22.8%0	0.709	<0.001
HAT	32 (2.0%)	7 (0.7%)	2 (0.4%)	23 (9.7%)	0.726	<0.001
ITBL	58 (3.6%)	9 (1.0%)	4 (0.9%)	45 (19.0%)	1	<0.001
One-year graft survival (%)	75%	85%	80%	33%	<0.001	<0.001
Birmingham (n=)	300	158	108	34		
Cause of graft failure						
PNF	9 (3.0%)	2 (1.3%)	2 (1.9%)	5 (14.7%)	1	0.009
HAT	17 (5.7%)	7 (4.4%)	6 (5.6%)	4 (11.8%)	0.775	0.133
ITBL	19 (6.3%)	5 (3.2%)	7 (6.5%)	7 (20.6%)	0.236	0.041
One-year graft survival (%)	85%	91%	85%	53%	<0.001	<0.001

DCD, donation after circulatory death; HAT, hepatic artery thrombosis; IC, ischaemic cholangiopathy; PNF, primary non-function; UNOS, United Network for Organ Sharing.

the new prediction model. Increasing risk is transmitted by increasing score points resulting in significantly impaired early liver function (INR day one: 1.5 vs. 1.7 vs. 2.0, *p=0.0001*, *p=0.0005*; Suppl. Figure 4) and higher liver enzyme release (median peak ALT during first week after transplant (787 vs. 1116 vs. 1906, *p=0.0004; p=0.0002)*. In addition, ICU and hospital stay after DCD transplantation increased significantly from low risk to futile score group (Suppl. Figure 4). Overall post-transplant morbidity was summarized by the CCI (34), where the new DCD prediction score was associated with a significantly increased median CCI throughout the three risk groups (*p<0.0001*, *p=0.0093;* Suppl. Figure 4).

DISCUSSION

Our search for a new score to optimize justice and utility for DCD liver allocation has led to several new findings. First, by combining a few major donor and recipient parameters, we developed a simple score system which best stratifies recipient graft loss after DCD liver transplantation, as compared to other recent developed prediction concepts. Second, this score proved to be highly discriminatory for PNF and ITBL in both, the national UK (NHSBT) and US (UNOS) databases. Third, our calculations represent the largest analysis of DCD liver transplant recipients. Fourth, the new model correlates well with post-transplant morbidity throughout all three risk clusters.

Risk assessment in liver transplants has been repeatedly addressed by several studies but is usually limited to the main endpoints mortality or graft loss. In addition, it appears arbitrary which thresholds and which combinations of parameters in a critical ill patient predict outcome. Currently, the most convincing calculations of risk prediction in liver transplantation base on a relatively limited number of key parameters leading to varying score points according to their regression coefficients. The sum of such scores expresses the total risk up to a risk threshold (balance principle) (18,19,35). However, while risk scores have been implemented in DBD liver transplantation, their application in DCD liver transplants is limited, as the DCD population is by far smaller in most countries, and parameters depicting higher graft injury are less well defined. Importantly, graft loss due to PNF and ITBL is a major and much more relevant problem in DCD liver transplantation in contrast to DBD liver transplantation, and justifies from our point a new effort to find a formula of their prediction, based on a national analysis.

Minimizing the sum of risk is an old principle in surgery, and many transplant professionals automatically aim to allocate DCD livers to low MELD primary liver transplant candidates (10,22). This established approach has led to a significantly improved early patient survival after DCD grafting (12,22). However, the precise impact of different donor/recipient combinations on graft failure is unclear. Two groups have developed models to predict the likelihood of graft survival after DCD liver transplantation (17,33). In one detailed analysis, Khorsandi et al identified six donor, graft and recipient factors to predict graft failure after transplantation by the DCD-RI, which combines functional DWIT, duration of donor hepatectomy, cold ischemia time, recipient MELD, underlying liver disease and retransplantation (33). DCD transplant cases are not equally distributed to the three risk clusters of this score from KCH. In contrast to the lowest risk group, which includes only a very small number of DCD

transplant cases (11.5%; 69/598), almost 40% are allocated to the highest risk group (235/598) and majority of DCD transplantations are classified into the "standard" risk group (50%; 294/598). Based on the number of score points allocated to each case at parameter cut-offs, the KCH DCD – RI showed inferior prediction in terms of graft loss. For example, one main risk factor in DCD liver transplantation, the functional DWIT, contributes to only 1 score point, when ranging above 25 minutes (53/677 in UK DCD cohort, 7.8%). According to this score, majority of DCD livers receive zero points for an already prolonged functional DWIT of up to 25 minutes (624/677; 92.2% of UK DCD cohort). A similar picture occurs from the parameter cold ischemia time, where the maximum of only 1 parameter point is distributed, when exceeding 10 hours. In UK only, a few DCD grafts experience such long storage times (67/1153; 5.8 %). In contrast, majority of points (three) are allocated to recipients with hepatitis C virus infection, alcohol related liver disease, cryptogenic liver cirrhosis or Budd-Chiari syndrome as indication for liver transplantation. Such parameters, alone may not predict graft loss based on DCD-related complications (PNF and ITBL), but due to recurrence of the underlying disease. In addition, three score points are allocated to DCD liver recipients with an elevated lab MELD of >25 points, which appears only in a limited number of DCD transplantations in UK (59/691; 8.5%). Thus, the DCD-RI from KCH seems less suitable to stratify post-transplant graft survival when compared to the new UK DCD risk score.

Another risk model has been calculated to identify risk combinations, the UCLA-DCD Score, being a combination of 6 variables, e.g. donor HBV-core antibody positivity and functional warm ischemia (MAP <60mmHg to flush), cold storage time, recipient MELD, recipient BMI, retransplantation and underlying liver disease in the recipient (17). The highest predictive factor with 3 points was HCV positivity combined with HCC in this score, which may end up predicting graft loss due to HCV recurrence rather than PNF or ITBL. Secondly, the impact of Hepatitis C positivity will change in the near future due to the direct-acting antiviral medications (36). In addition, important predictors of early graft failure, e.g. functional DWIT contributed only with one score point. An additional score factor, selected by the authors of the UCLA-DCDs core, published in 2011, was donor HBV-core antibody positivity, which is expressed in only very few cases in the UK DCD cohort (14/1153; 1.2%). Thus, the UCLA-DCD score was also inferior in predicting graft survival (Figure 3). To develop a meaningful prediction model, ideally this should base on clinically relevant score parameters, which are of great importance in all countries with DCD liver transplantation (35,37,38).

In contrast to the above-mentioned calculations, our prediction model discriminated best in terms of graft survival below and above a cut-off of 10 (**Table 4; Figure 1-4**). Based on this, donor - recipient combinations with more than 10 points are exposed to a high risk of graft loss due to clinically relevant and DCD related complications, such as PNF or IC (**Table 4**). In addition, the new model significantly correlates with post-transplant morbidity.

Our model has also shortcomings. First, we failed to include graft steatosis in our calculation due to limited donor liver biopsies in the UK. Nevertheless, a correlation of BMI and steatosis has been shown in a recent study (39), and we have been able to demonstrate donor BMI as independent predictor. Second, most DCD recipients in UK have a low MELD score at time of transplantation (median lab MELD 15 points), because DCD liver allocation is excluded from MELD allocation. Third, cut-off definitions for higher risk based on graft survival deterioration with however further impact on complications. For example, candidates in the high-risk group (6 -10 score points) are exposed to significantly higher rate of graft loss due to IC. This high-risk group would therefore, benefit from a graft treatment prior to implantation (40). In this context, machine perfusion approaches such as hypothermic oxygenated perfusion (HOPE) have been previously shown to reduce reperfusion injury and intrahepatic biliary complications after transplantation of human DCD livers (41–43). In addition, ex-situ graft evaluation during normothermic or hypothermic machine liver perfusion might be a useful tool to test liver viability, though better markers of graft function during these approaches are urgently needed (44–46).

One of the advantages of UK DCD risk score is that it derives from objective factors, readily available at the time of an organ offer, with the exception of warm and cold ischemia. However, as the liver retrieval starts and the functional DWIT becomes available, the score can be recalculated with a fairly precise prediction of cold ischemia. At this stage, the score values may suggest a different decision regarding the use of the graft and/or the choice of a different local recipient. In the future, we will calculate the score system at organ offer. When donor/recipient combinations result in more than 10 points, we will either try to find a different recipient to decrease the overall risk and secondly perform graft treatment by machine perfusion prior to implantation. In addition, future models should also consider the amount of graft steatosis and the impact on the score threshold whether to accept a DCD liver for a certain recipient or not (47). Though more reliable methods of fat quantification are urgently needed, analysis of graft biopsies provide additional information on the amount of steatosis and therefore help to decide which DCD liver to accept or

how to adapt the preservation technique for a specific risk combination. Due to the fact that important risk parameters, e.g. WIT, are frequently missing or incomplete in nationwide databases, further multicentre cohort studies are required to refine existing and new scoring systems. More specifically, an outcome study including DCD transplants from multiple centres in Europe and US, has been initiated and will enable us to first provide another validation of our new model and second to demonstrate the effective relation between the number of risk points per DCD case and specific complications in the recipient.

In summary, we believe that the UK DCD Risk Score may be a very practical and new guidance for allocation of a specific organ to a recipient, and helps also to decide which DCD liver requires processing with a new preservation technology, including machine perfusion. This would potentially help to implement such expensive approaches more specifically.

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Part II

Graft quality and renal complications after liver transplantation

Chapter

The kidney at risk in liver transplantation recipients: Review of the literature

KIDNEY PROBLEMS IN LIVER TRANSPLANTATION

Renal complications are an important issue after liver transplantation. Many patients with ESLD awaiting a liver transplant have renal impairment and they are at risk for short- and long-term renal problems afterwards. AKI is frequently observed in the early postoperative phase and is the result of several donor, recipient and surgical risk factors. A significant proportion of the recipients will be in need of peri-operative RRT and not all of them have a full recovery of kidney function and will develop CKD, and in some cases ESRD, requiring long-term RRT or kidney transplantation. Considering the growing pre-transplant renal problems due to the 'sickest-first' allocation policy in many countries and the increased use of marginal grafts in liver transplantation, the rate of renal complications is likely to increase. In this chapter we will discuss the renal problems in patients with ESLD and risk factors for development of AKI and CKD after liver transplantation.

Renal failure in cirrhosis

Patients with ESLD will frequently present with AKI due to ascites with subsequent volume depletion or episodes of spontaneous bacterial peritonitis. On the other hand, some have a slower decrease in kidney function over the years of developing decompensated cirrhosis. The majority of kidney function in ESLD is thought to be functional (rather than damage) and related to hemodynamic disbalances (1). The portal hypertension in cirrhosis causes primary arterial vasodilatation in the splanchnic circulation, leading to a reduction in the systemic vascular resistance. Increased cardiac output can compensate for this reduction initially, but in advanced stages of cirrhosis the systemic vascular resistance will be so much reduced the cardiac compensation is not sufficient (**Figure 1**). This will lead to hypovolemia and subsequent activation of vasoconstrictor systems will keep up the arterial blood pressure, but will impair the kidneys, leading to more ascites and renal vasoconstriction and hypoperfusion (2). There are four types of renal failure in ESLD: (I) the hepatorenal syndrome (HRS), (II) hypovolemia-induced renal failure, (III) parenchymal renal disease and (IV) drug-induced renal failure (1). HRS is the far most common form and can be divided into HRS-type 1 or HRS-AKI and HRS-type-2. HRS-type 1 classically presents like AKI within several days which usually responds well to medical therapy with vasoconstrictors (i.e. terlipressin), and is often related to a precipitating factor, such as spontaneous bacterial peritonitis, gastrointestinal haemorrhage or acute-on-chronic liver failure (3). HRS-type 2 has a more gradual decrease in kidney function (>two weeks) and often severe ascites, resistant to diuretic therapies (4). The MELD-score was developed in 2000 to predict mortality in patients undergoing transjugular

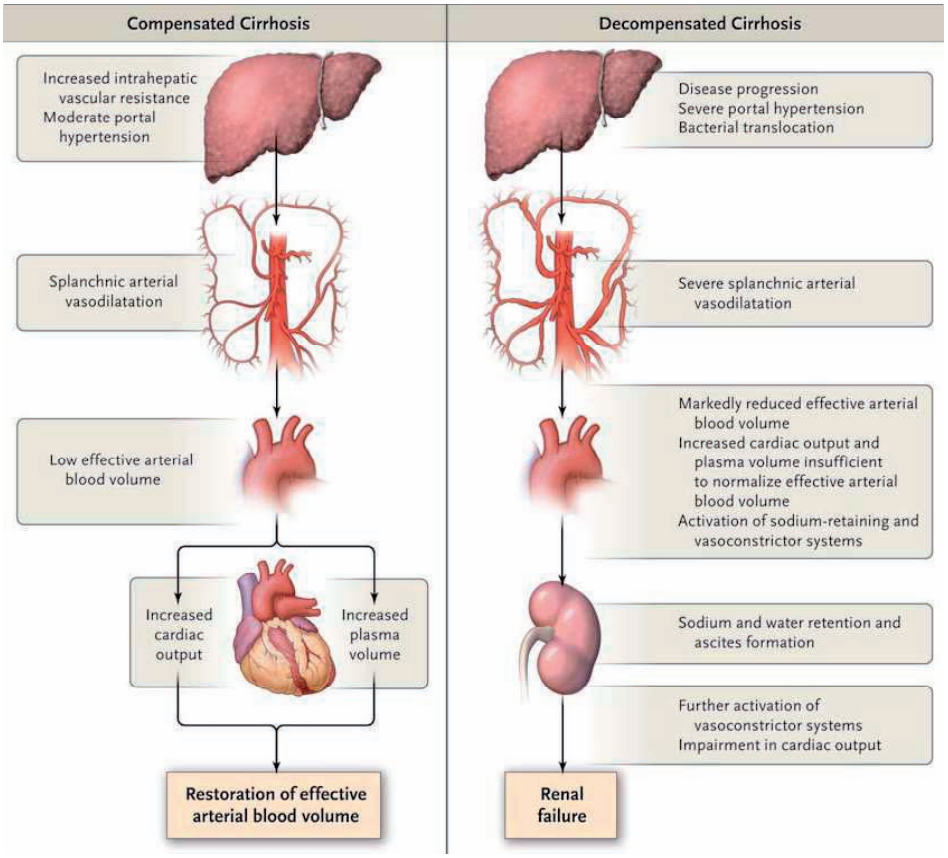

Figure 1 – Pathogenesis of circulatory abnormalities and renal failure in cirrhosis.
From Ginès et al, New Eng J Med, 2009 (1).

intrahepatic portosystemic shunt for refractory ascites and soon this prediction model was implemented in the US and many European countries to allocate grafts for liver transplantation (5–7). The MELD-score consists of three pillars: coagulopathy (INR), impaired bilirubin metabolism (serum bilirubin), and renal dysfunction (serum creatinine). A patient gets additional points, when he or she is in need of regular RRT, acknowledging the importance and predictive value of renal dysfunction in patients on the liver transplant waiting list. As a result, patients undergoing a liver transplant have more frequently severe renal dysfunction over the last years, increasing the risk for renal problems after the liver transplantation (8–10).

Acute kidney injury after liver transplantation

Several centres have reported their experience with AKI after liver transplantation, with incidence rates ranging from 24% to 85% (11–24). This wide variance is partly

Table 1 – Criteria based on serum creatinine levels to classify AKI after liver transplantation.

Criteria	Stages	Year
RIFLE (25)	§ Risk: increased creatinine x 1.5 **OR** decreased GFR >25% from baseline § Injury: increased creatinine x 2.0 **OR** decreased GFR >50% § Failure: increased creatinine x 3.0 **OR** decreased GFR >75% **OR** creatinine level ≥354 µmol/L with an acute rise of ≥ 44.2 µmol/L § Loss: complete loss of renal function for >4 weeks (renal replacement therapy) § End stage renal disease: no recovery of kidney function	2004
AKIN (26)	§ Stage 1: increased creatinine x 1.5 **OR** ≥26.4 µmol/L from baseline (within 48h) § Stage 2: increased creatinine x 2.0 § Stage 3: increased creatinine x 3.0 **OR** creatinine level ≥354 µmol/L with an acute rise of ≥ 44.2 µmol/L **OR** requiring renal replacement therapy	2007
KDIGO (27)	§ Stage 1: increased creatinine x 1.5 (within 7 days) **OR** ≥26.4 µmol/L (within 48h) from baseline § Stage 2: increased creatinine x 2.0 § Stage 3: increased creatinine x 3.0 **OR** creatinine level ≥354 µmol/L with an acute rise of ≥ 44.2 µmol/L **OR** requiring renal replacement therapy	2012

the result of the criteria used to classify AKI. **Table 1** gives an overview of the criteria used for AKI over the last years. Officially, AKI is defined by either an increase in serum creatinine levels or decrease or loss of urine output. However, since serum creatinine levels are most widely available and more accurately measured than urine output in the daily practice, almost all studies only use the creatinine levels to define AKI after liver transplantation. The Kidney Disease Improving Global Outcomes (KDIGO)-criteria are considered as the most up-to-date and is used in most of the recent literature.

Risk factors for acute kidney injury

Postoperative AKI is the result of the combination of donor, graft, recipient and surgical risk factors. Furthermore, the early use of nephrotoxic immunosuppression after the liver transplant increases the risk for AKI (15,17,22,28). The peri-operative practice in liver transplantation is very diverse worldwide and the numerous studies evaluating factors associated with AKI identified different risk factors. The literature (US National Library of Medicine - PubMed online database) since 2000 was screened for factors associated with development of AKI (acute kidney injury or acute renal failure) after liver transplantation. An overview of independent risk factors (using multivariable regression analysis) that were identified in *at least two* single-centre experiences were included, which is shown in **Table 2**. The preoperative MELD-score was identified by most previous studies, other recipient factors included the serum creatinine, a raised BMI and history of DM. The use of DCD grafts and a longer recipient WIT during the transplant have an impact on AKI development as well.

Table 2 – Factors associated with development of AKI after liver transplantation

	Risk factor	References
Donor / Graft	§ Use of a DCD graft	Leithead 2012 (29), Doyle 2015 (30)
	§ Duration of recipient warm ischemia time	Leithead 2014 (21), Barreto 2015 (14), Park 2015 (15), Zongyi 2017 (28)
Recipient	§ Preoperative serum creatinine	Gallardo 2004 (11), Sanchez 2004 (31), O'Riordan 2007 (12), Wadei 2016 (32), Zongyi 2017 (28)
	§ Raised BMI	Iglesias 2010 (17), Park 2015 (15), Hilmi 2015 (33)
	§ Non-Caucasian race	Contreras 2002 (34), Iglesias 2010 (17)
	§ Diabetes mellitus	Utsumi 2013 (22), Hilmi 2015 (33)
	§ Viral hepatitis	Barreto 2015 (14), Wadei 2016 (32)
	§ APACHE-II-score	Gallardo 2004 (11), Zhu 2010 (35)
	§ MELD-score	Sanchez 2004 (31), Zhu 2010 (35), Klaus 2011 (36), Utsumi 2013 (22), Romano 2013 (23), Kim 2014 (37), Park 2015 (15)
	§ Child-Pugh-score	Iglesias 2010 (17), Fonseca-Neto 2011 (20), Hilmi 2015 (33)
	§ Blood urea nitrogen	Contreras 2002 (34), Sanchez 2004 (31)
	§ Hypoalbuminemia	Chen 2011 (19), Park 2015 (15), Cabezuelo (38)
Surgical	§ Operation time	Park 2015 (15), Wadei 2016 (32)
	§ Blood loss	Utsumi 2013 (22), Hilmi 2015 (33), Park 2015 (15), Zongyi 2017 (28)
	§ Red blood cell transfusion	Contreras 2002 (34), Gallardo 2004 (11), Chen 2011 (19), Park 2015 (15), Wadei 2016 (32)
	§ Inotrope/vasopressor requirement	Sanchez 2004 (31), Chen 2011 (19)
	§ Postreperfusion syndrome	Fonseca-Neto 2011 (20), Park 2015 (15)
Post-operative	§ Calcineurin inhibitor (overexposure)	Iglesias 2010 (17), Utsumi 2013 (22), Park 2015 (15), Zongyi 2017 (28)
	§ Transaminase peak	Contreras 2002 (34), Leithead 2014 (21), Rahman 2017 (39), Jochmans 2017 (40)
	§ Prolonged dopamine use	Cabezuelo (38), Zongyi 2017 (28)

Furthermore, blood loss with subsequent transfusion requirements and the use of vasopressors were identified by multiple centres. As expected, overexposure to calcineurin inhibitors was associated with AKI.

Graft quality and acute kidney injury

The Birmingham group showed a simultaneous increase in incidence of AKI after liver transplantation with the evolving use of marginal grafts, and especially with DCD grafts (21,29). In both studies, the postoperative release of transaminases was an independent factor associated with AKI, representing the severity of hepatic IRI as the link between graft quality and development of AKI. Interestingly, in a subgroup

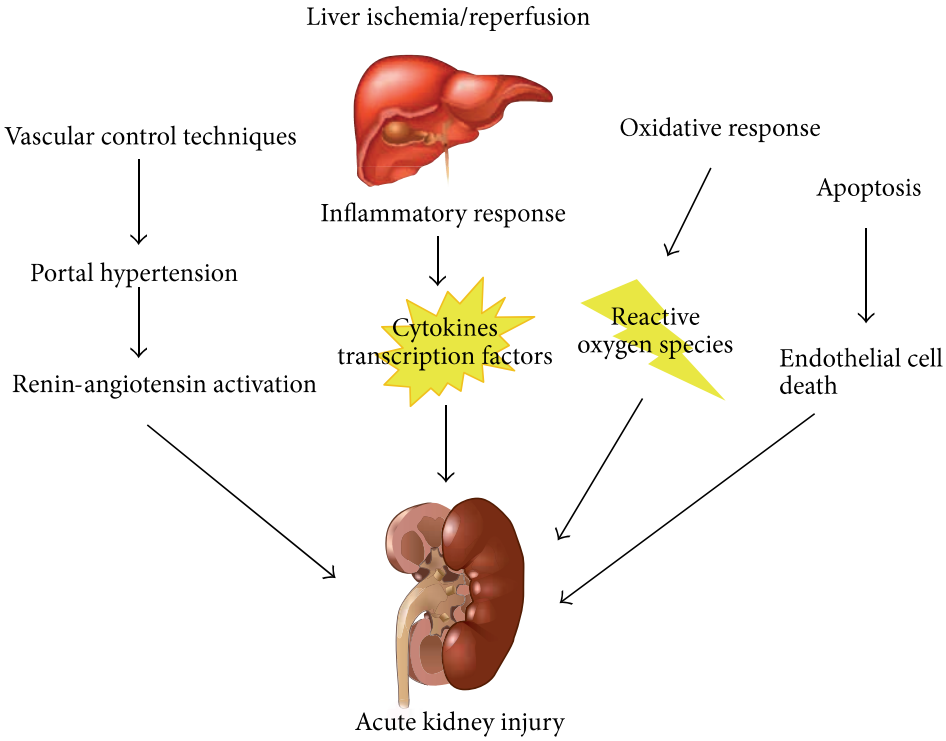

Figure 2 – overview of the mechanism causing AKI after hepatic ischemia/reperfusion injury
From Nastos *et al*, Oxidative Medicine and Cellular Longevity, 2014 (49).

analysis of DBD grafts, these peak transaminase levels were also higher in recipients with post-transplant AKI (41). Several factors impact on graft quality. Grafts from older donors, steatotic graft and DCD grafts are known to be more susceptible to hepatic IRI, as are grafts who experience longer cold and warm ischemia times (42–47). It is known that hepatic IRI induces a systemic inflammatory response similar as seen in sepsis (48). The subsequent release of pro-inflammatory cytokines and reactive oxygen species causes renal injury (**Figure 2**) (49). Although the pathogenesis between hepatic IRI and development of AKI is not fully understood yet, there is evidence that the release of these cytokines (including TNF-α) leads to dysregulation of endothelial adhesion molecules and renal endothelial cell apoptosis, which promotes leukocyte recruitment in the interstitial space, causing renal injury (50–52). The reactive oxygen species released by activated neutrophils cause direct renal damage and recruitment of leukocytes like monocytes and macrophages further aggravate the oxidative injury in the kidney (53).

Acute kidney injury biomarkers

Over the last years, several serum and urine biomarkers for the prediction for AKI after liver transplantation have been identified. The most common cause of AKI in this setting is acute tubular necrosis. Therefore, markers of acute tubular injury, including kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), and interleukin-18 (IL-18) are the main subject of interest (54). In a recent study evaluating the relationship between postreperfusion gene expression, serum mediator and development of postoperative AKI revealed that a combination of endothelin-1 (ET-1) and IL-18 expression was highly predictive for AKI (55).

Acute kidney injury in relation to other outcomes after liver transplantation

Development of AKI after liver transplantation does not stand on its own. Due to the intense relation with hepatic IRI, recipients developing (severe) AKI are also likely to experience other complications, including infections and the need for a reoperation in the first days after liver transplantation with an prolonged admission in intensive care and in the hospital (11,19,34,39). Postoperative AKI in general is related with increased use of hospital resources and costs and previous studies have also linked AKI to an increased risk for graft loss (19,28,56,57). Furthermore, there is a clear relation between AKI and recipient mortality on the short and long-term, especially in recipients that require RRT in the early postoperative phase (12,14,34–36). Although most recipients recover from the direct renal damage in the following months after liver transplantation, Ojo *et al* already reported in 2003, that postoperative AKI requiring RRT is a risk factors for development of CKD, which was later confirmed by several single-centre experiences (29,32,33,58).

Development of chronic kidney disease after liver transplantation

In the US nationwide study from Ojo and colleagues including 69.321 recipients of non-renal organs, the 5-year cumulative incidence of severe CKD (eGFR <30 ml/min/1.73 m^2) was 18% for liver transplantation recipients (58). This is relatively high, compared to recipients of heart (11%) and lung (16%) transplants, even though liver transplant recipients require less nephrotoxic immunosuppression. Other studies reported observed overall CKD (eGFR <30 ml/min/1.73 m^2) in 39% up to 78% of the recipients (16,33,59,60). Severe CKD and ESRD incidence rates reached from 6% to 18% and 1% to 12%, respectively (16,32,33,60–63). **Figure 3** shows the course of renal function over 25 years after liver transplantation in a US large cohort with more than 1.000 recipients (60). This study (and several others) has shown that recipients who develop CKD after liver transplantation have an increased mortality-risk, especially when the eGFR drops <30 ml/min/1.73 m^2 (58,64,65).

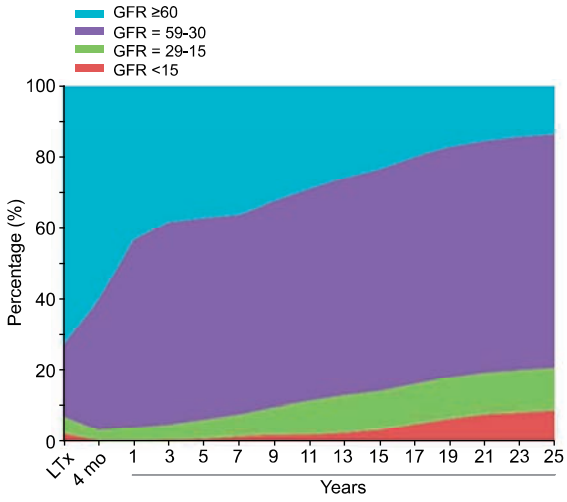

Figure 3 – Course of renal function after liver transplantation
From Allen et al, Journal of Hepatology, 2014 (60).

Risk factors for CKD

Similar to AKI after liver transplantation, CKD has a multifactorial origin. Sharma *et al* introduced the three-hit model with risk factors contributing to the development of post-transplant CKD (**Figure 4**) (66). The first hit is the combination of pre-transplant renal impairment due to HRS, glomerulonephritis, comorbidities such

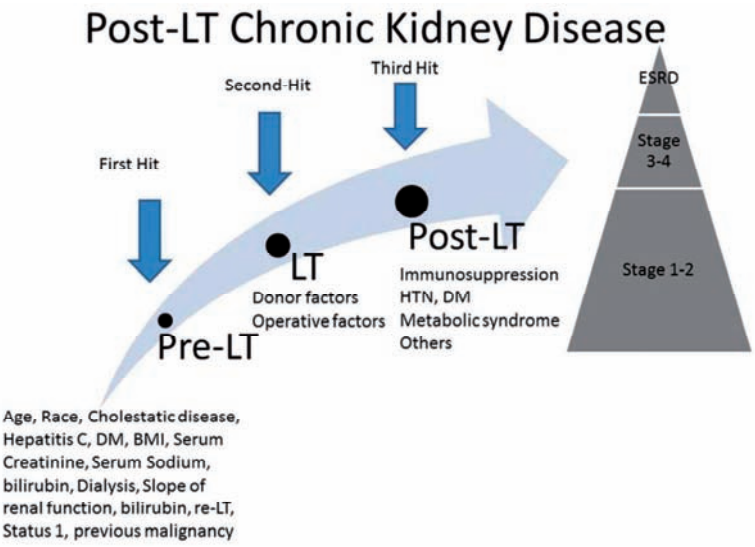

Figure 4 – the three-hit model of risk factors for development of CKD after liver transplantation
From Sharma et al, Advances in Chronic Kidney Disease, 2015 (66).

as hypertension and DM, and additional acute tubular necrosis due to episodes of sepsis (1,58,59,67–70). The second hit happens peri-operatively: blood loss and hypotensive episodes during the transplant procedure and postoperative complications such as infections, bleeding and biliary complications further impact on kidney function. As described above, the use of marginal grafts increase the severity of hepatic IRI with subsequent AKI, potentially increasing the risk for renal impairment on the long-term. The third hit is the result of immunosuppression that not only has direct nephrotoxic consequences, but long-term use of calcineurin inhibitors and steroids also increase the risk for post-transplant metabolic syndrome. This syndrome and its individual components DM and hypertension have a further negative impact on kidney function (66,71,72).

Recovery of renal function after liver transplantation

Up to one fourth of the recipients with AKI require RRT in the first weeks after liver transplantation (12,21,37,73). This group is a mix of those who have with pre-transplant renal failure, those who have a difficult transplant procedure and/or postoperative complications. Recovery of renal function ranges from 70% to 98%, which mostly depends on the duration of RRT prior to liver transplantation (62,64,69,74–76). Other risk factors for non-recovery of kidney function include recipient age, MELD-score and pre-existing DM (69,74–76). In a study with 155 patients requiring post-transplant RRT the average duration until recovery was 33 days and after one year 83% was not dialysis dependent anymore (74). It should be noted that in most countries there is a thorough and careful selection of patients who are likely to not recover from their renal failure and they are offered a simultaneous liver and kidney transplant (68,77).

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Chapter

The postreperfusion syndrome is associated with acute kidney injury following DBD liver transplantation

Marit Kalisvaart
Jubi de Haan
Dennis Hesselink
Wojciech Polak
Bettina Hansen
Jan IJzermans
Diederik Gommers
Herold Metselaar
Jeroen de Jonge

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SUMMARY

AKI is frequently observed after DBD liver transplantation and associated with impaired recipient survival and chronic kidney disease. Hepatic IRI is suggested to be an important factor in this process. PRS is the first manifestation of severe hepatic IRI directly after reperfusion. We performed a retrospective study on the relation between hepatic IRI and PRS and their impact on AKI in 155 DBD liver transplant recipients. Severity of hepatic IRI was measured by peak postoperative AST levels and PRS was defined as >30% decrease in MAP ≥1 minute <5 minutes after reperfusion. AKI was observed in 39% of the recipients. AKI was significantly more observed in recipients with PRS (53 vs. 32%; p=0.013). Median peak AST level was higher in recipients with PRS (1388 vs. 771U/L; p<0.001). Decrease in MAP after reperfusion correlated well with both severity of AKI (p=0.012) and hepatic IRI (p<0.001). Multiple logistic regression identified PRS as an independent factor for postoperative AKI (OR 2.28; 95% CI 1.06-4.99; p=0.035). In conclusion, PRS reflects severe hepatic IRI and predicts AKI after DBD liver transplantation. PRS immediately after reperfusion is an early warning sign and creates opportunities to preserve postoperative renal function.

INTRODUCTION

AKI is a common complication after liver transplantation with a reported incidence of 20 to 78% (1–6). Recipients developing AKI have an impaired short- and long-term survival and an increased risk to CKD (3–8). Furthermore, it is associated with an increased length of hospital stay, utilization of resources and costs of care (9). The etiology of AKI after liver transplantation is multifactorial and not completely understood. Besides pre-operative renal function, known risk factors include severity of liver disease (MELD score), recipient age and co-morbidities such as DM and hypertension (3,7,10). After liver transplantation calcineurin inhibitor nephrotoxicity is considered to be a major risk factor for renal failure (11–13). Next to these classic risk factors, graft characteristics are increasingly being recognized as contributors to the development of AKI after liver transplantation(14). A comparative study by Leithead *et al.* showed that AKI was more frequent when DCD grafts were used (54%) compared to DBD grafts (32%) (1). The obligatory extra DWIT in DCD liver transplantation appears to be responsible for this higher incidence of AKI. However, the majority of liver transplantations with deceased donors are still performed using DBD grafts (15,16). With AKI developing in one third of DBD recipients, it remains an important complication after DBD liver transplantation. Hepatic IRI, giving rise to a systemic inflammatory response similar as seen in sepsis and multi-organ failure, is the predominant factor associated with postoperative AKI after DBD liver transplantation (2,17–20).

Currently, graft biopsies at reperfusion and peak serum AST levels until 72 hours after liver transplantation are being used to quantify the severity of hepatic IRI (21–23). These diagnostic modalities do not allow early recognition nor quantification of hepatic IRI at reperfusion during the operation. However, early identification of severe hepatic IRI is pivotal to facilitate possible preventive measures to preserve renal function. An early indicator of severe hepatic IRI in liver transplantation is PRS. PRS is characterized by a decrease in systemic vascular resistance, hypotension, impaired cardiac output and an increased pulmonary vascular resistance directly after reperfusion (24). This hemodynamic phenomenon has an incidence between 12% and 55% and is associated with higher in-hospital mortality (25–29). Knowing hepatic IRI is a risk factor for development of postoperative AKI and PRS is a manifestation of severe hepatic IRI, recognition of PRS could allow early identification of recipients at risk for AKI after DBD liver transplantation. Paugam-Burtz *et al.* has demonstrated in a retrospective study about the consequences of PRS that this phenomenon is associated with an increase of postoperative severe renal dysfunction (eGFR < 30ml/

min) (25). However, this relatively small study did not focus on postoperative renal injury and did not investigate a linear correlation between hemodynamic instability after reperfusion and the severity of postoperative renal injury.

The aim of our study was first to analyze the effect of PRS on the development of postoperative AKI and second to explore the relation between hemodynamic instability after reperfusion and the severity of hepatic IRI in DBD liver transplantation.

METHODS

This retrospective study was performed with approval of the Erasmus University Medical Center Rotterdam Institutional Review Board (MEC-2014-670). All consecutive patients who underwent first DBD liver transplantation in our center from July 2008 until October 2014 were included. Exclusion criteria were liver transplantation for acute liver failure, urgent retransplantation in the first week of follow up, living-donor liver transplantation, combined liver-kidney transplantation and AKI diagnosed in the week prior to liver transplantation.

Recipient baseline characteristics at time of admission (age, gender, etiology of liver disease, HCC, serum sodium, creatinine, bilirubin, and INR) were collected. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease Study 4-variable equation: 186 x (serum creatinine (umol/L) / 88,4)$^{-1,154}$ x age (in years)$^{-0,203}$ x 0,742 (if woman) x 1,212 (if black), as was MELD score (30). Relevant medical history (hypertension, insulin dependent diabetes mellitus (IDDM) and coronary artery disease) was recorded. Presence of ascites, either diuretic controlled or refractory, was evaluated by historic evidence of ascites by CT-scan (31). The DRI, was calculated to assess graft quality (32). During surgery recipient' hemodynamics were monitored using an intra-arterial and pulmonary artery catheter. Continuous infusion of norepinephrine was adjusted to target a MAP of 70 mmHg. Hypotension after reperfusion was treated with single-dose administration of epinephrine. Infusion of norepinephrine was registered throughout surgery. The regular fluid regimen during surgery consisted of the combined use of crystalloids, hydroxyethyl starches, transfusions of RBC, FFP and platelets. Albumin 20% (200cc) was administered to every recipient at the end of surgery. Standard surgical technique included piggyback cavocaval anastomosis without the use of a portocaval shunt or veno-venous bypass. Reperfusion was induced after portal anastomosis before completion of arterial and biliary anastomosis. Length of cold ischemia time

and WIT, intraoperative blood loss with subsequent transfusion were recorded. MAP was documented at start of the surgical procedure, prior to reperfusion, lowest MAP in the first 5 minutes following reperfusion and then 15, 30, 45 and 60 minutes after reperfusion. PRS was defined as a decrease of >30% in MAP lasting at least one minute within the first five minutes after reperfusion (24). The peak serum AST level in the first 72 postoperative hours was used as a marker for hepatic IRI. This parameter has previously been used to describe the effect of hepatic IRI on AKI in liver transplantation by Leithead *et al* and is used as a marker for EAD as well (33–35). Postoperative serum creatinine levels were collected daily in the first week after liver transplantation. AKI was defined according to AKIN criteria (36): stage 1; ≥1.5 times baseline serum creatinine level or an increase of 26.5 umol/L above baseline, stage 2; >2 times baseline level, and stage 3; >3 times baseline level or requirement of RRT all within 48 hours. Postoperative AKI was divided into mild AKI (AKIN stage 1) and severe AKI (AKIN stage 2&3).

The postoperative immunosuppression regimen changed during the study period. In the first 3.5 years the regimen included tacrolimus from postoperative day 0 (target trough serum concentration (C_0) of 8-12 ug/L), prednisolone for 3 months and intravenous basiliximab (20mg) on postoperative day 0 and 4. In the last three years tacrolimus introduction was delayed until day 5 and MMF was added from day 0 and withdrawn after adequate concentrations of tacrolimus were reached. The immunosuppression was adjusted in case of impaired renal function or infection. As a surrogate marker for potential tacrolimus nephrotoxicity, the highest trough serum tacrolimus level (C_0) in the first postoperative week was recorded. Length of hospital and ICU stay, recipient and graft survival up to one year were documented. All major postoperative complications during hospitalization were documented where major postoperative complications were defined as grade ≥ 3a by the Clavien Dindo classification (37).

Statistical analysis

Data were analyzed using IBM SPSS Statistics version 21 (IBM Corporation, Armonk, NY, USA). Continuous variables were tested for normality with the Shapiro-Wilk test. The student's t test and the one-way analysis of variance (ANOVA) were used to compare normally distributed continuous variables. Nonparametric continuous variables were compared using the Mann-Whitney U test. To compare categorical variables the Chi-square test or the Fisher's exact test were used. P-values < 0.05 were considered statistically significant. Continuous variables were expressed as mean with standard deviation (SD) or median and interquartile range (IQR), where appropriate. Long-

term survival rates were estimated using Kaplan-Meier methods. Recipient, donor and peri-operative variables with a p-value <0.1 in univariable analysis were included in a multiple logistic regression model to identify predictors for postoperative AKI.

RESULTS

During the study period 206 patients underwent first DBD liver transplantation of which 155 were included. Forty-eight recipients were excluded: 36 for acute liver failure, three with retransplantation within the first week of follow up, three living donor liver transplants, four combined liver-kidney transplantations and two recipients who developed AKI prior to liver transplantation. Additionally three recipients were excluded because of incomplete intraoperative data.

Recipient, graft, and surgical characteristics

Table 1 shows the preoperative recipient and graft characteristics. Two third (68%) of the recipients were men and median age was 54 years. The majority of etiology of liver diseases could be divided into three groups: biliary cirrhosis (39%), viral hepatitis (19%), and post-alcoholic cirrhosis (15%). Median lab MELD score was 16 and median preoperative recipient' creatinine levels were 72 umol/L. Surgical characteristics are displayed in **Table 2**. Median length of cold ischemia and WIT was respectively 6.2 hours and 28 minutes. Median blood loss during surgery was 4.0 liters. PRS was observed in 53 (34%) of the recipients and the mean decrease in MAP post-reperfusion was 14 mmHg. Median average dose of continuous norepinephrine infusion was 0.17 ug/kg/min. The peak postoperative serum AST level was 930 U/L. The induction of tacrolimus therapy was in 65 (42%) of the recipients before the fifth postoperative day. The median highest peak C_0 in this group was 6.4 ug/L.

Postoperative acute kidney injury

Sixty-one recipients (39%) developed AKI in the first week, of whom 71% developed AKI within the first two days. Of this 61 recipients, 47 recipients (77%) developed mild AKI (AKIN stage 1) and 14 recipients (23%) developed severe AKI (AKIN stage 2: 8 recipients; AKIN stage 3: 6 recipients). RRT was required in 5 of the 61 recipients (8%) during their hospital stay. To identify factors associated with postoperative AKI an univariable and subsequent multiple logistic regression analysis was performed. Preoperative serum creatinine and eGFR, length of cold ischemia and WIT, RBC transfusion, occurrence of PRS and average dose of norepinephrine infusion during surgery were associated with the development of postoperative AKI as shown in

Table 1 - Preoperative graft and patient characteristics in liver transplantation

Characteristics	n = 155
Graft	
Donor risk index	1.81 (0.36)
Recipient	
Age (years)	54 (43-60)
Male gender (%)	105 (68)
Etiology of liver disease (%)	
Viral hepatitis	30 (19)
Biliary cirrhosis	61 (39)
Postalcoholic cirrhosis	23 (15)
Other	41 (27)
Hepatocellular carcinoma (%)	40 (26)
LabMELD score	16 (10-21)
Creatinine (umol/L)	72 (59-91)
eGFR (ml/min/1.73m2)	89 (66-117)
Sodium (mmol/L)	138 (134-141)
Ascites (%)	
None	69 (45)
Diuretic controlled	47 (30)
Refractory ascites	39 (25)
Medical history (%)	
Hypertension	16 (10)
Coronary artery disease	9 (6)
Hepatorenal syndrome	12 (8)
Diabetes mellitus	
None	119 (77)
None insulin dependent	10 (7)
Insulin dependent	26 (17)

eGFR = estimated glomerular filtration rate; MELD = model for end stage liver disease. Continuous variables are displayed as mean (standard deviation) and median (interquartile range) where appropriate.

Table 3. These factors were subsequently included in the multiple logistic regression analysis, shown in the right section of **Table 3**. Duration of cold ischemia (OR 1.302; 95% CI 1.067-1.590; p=0.009), length of WIT (OR 1.064; 95% CI 1.002-1.130; p=0.030), and the occurrence of PRS (OR 2.283; 95% CI 1.061-4.915; p=0.035) were significantly associated with the development of postoperative AKI.

Table 2 – Intra- and postoperative characteristics for patients undergoing liver transplantation

Characteristics	n = 155
Intraoperative	
Cold ischemia time (hours)	6.2 (5.3-7.5)
Warm ischemia time (min)	28 (25-33)
Blood loss (liters)	4.0 (2.5-6.0)
RBC transfusion (units)	3 (1-6)
Hemodynamics	
MAP start operation (mmHg)	89 (16)
MAP decrease at reperfusion (mmHg)	14 (21)
Postreperfusion syndrome (%)	53 (34)
MAP hour 1 postreperfusion (mmHg)	68 (8)
Inotropics- norepinephrine	
Average dose in surgery (ug/kg/min)	0.17 (0.10-0.25)
Postoperative	
Postoperative peak serum AST (U/L)	930 (637-1752)
Postoperative tacrolimus use	
Highest C_0 in first week (ug/L)	3.5 (<1.4-7.3)
Start before day 5 postoperative (%)	65 (42)
Highest C_0 if start < day 5 (n=65) (ug/L)	6.4 (4.1-11.1)

AST = aspartate aminotransferase; MAP = mean arterial pressure; RBC = red blood cells . Continuous variables are displayed as mean (standard deviation) and median (interquartile range) where appropriate.

Impact of postreperfusion syndrome and hepatic IRI on development of AKI

Postoperative AKI was significantly more observed in recipients with PRS: 28 (53%), compared to 33 (32%) in recipients without PRS (p=0.013). The mean decrease in MAP after reperfusion was 14 (SD 21) mmHg and significantly more pronounced in recipients with AKI (19 (SD 23) vs. 10 (SD 19) mmHg; p=0.007). In addition, a significant larger decline in MAP was observed with increasing AKI severity (p=0.012) (**Figure 1**). To assess the relation between PRS and hepatic IRI, postoperative peak serum AST levels were compared between recipients with and without PRS. Recipients experiencing PRS had higher peak AST levels (median 1388 U/L; IQR 785-3027) compared to recipients without PRS (median 771 U/L ; IQR 601-1362) (p<0.001). Furthermore, in recipients with PRS the postoperative peak AST levels increased with severity of AKI (p=0.009) whereas there was no relation between severity of hepatic IRI and AKI in recipients without PRS (p=0.814). After stratification of the postoperative peak AST levels (**Figure 1**), there was also a significant correlation between the decrease of MAP at reperfusion and the severity of hepatic IRI (p<0.001).

Table 3 - Univariable and multiple logistic regression analysis of variables associated with acute kidney injury

	Univariable analysis			Multiple logistic regression analysis		
	Odds ratio	**95% CI**	**p-value**	**Odds ratio**	**95% CI**	**p-value**
Recipient						
Age	0.990	0.962 - 1.018	0.462	.	.	.
Male gender	0.627	0.309 - 1.275	0.197	.	.	.
Etiology of liver disease						
Viral hepatitis	1.000			.	.	.
Biliary cirrhosis	0.976	0.386 - 2.469	0.958	.	.	.
Postalcoholic cirrhosis	1.538	0.502 - 4.718	0.451	.	.	.
Other	2.100	0.972 - 5.569	0.136	.	.	.
LabMELD score	1.030	0.990 - 1.072	0.138	.	.	.
Serum creatinine	1.008	1.000 - 1.016	0.047	1.004	0.995 - 1.014	0.348
eGFR	0.992	0.993 - 1.001	0.082	.	.	.
Sodium	1.008	0.948 - 1.072	0.806	.	.	.
Medical history						
Refractory ascites	1.360	0.610 - 3.030	0.452	.	.	.
Hypertension	1.623	0.575 - 4.582	0.361	.	.	.
Coronary artery disease	1.249	0.322 - 4.848	0.748	.	.	.
Hepatorenal syndrome	2.307	0.697 - 7.633	0.171	.	.	.
IDDM	1.191	0.816 - 4.513	0.135	.	.	.
Graft						
Donor risk index	1.093	0.440 - 2.713	0.849	.	.	.
Intraoperative						
Cold ischemia time	1.244	1.035 - 1.494	0.020	**1.302**	**1.067 - 1.590**	**0.009**
Warm ischemia time	1.083	1.027 - 1.142	0.003	**1.064**	**1.002 - 1.130**	**0.030**
RBC transfusion	1.134	1.045 - 1.230	0.003	1.090	0.988 - 1.203	0.085
Hemodynamics						
MAP start operation	0.985	0.965 - 1.006	0.161	.	.	.
Postreperfusion syndrome	2.342	1.186 - 4.624	0.014	**2.283**	**1.061 - 4.915**	**0.035**
MAP hour 1 postreperfusion	1.012	0.978 - 1.047	0.500	.	.	.
Average dose norepinephrine						
≤ 0.17 ug/kg/min	1.000			.	.	.
> 0.17 ug/kg/min	1.783	0.928-3.424	0.082	1.137	0.541 - 2.392	0.735
Postoperative tacrolimus use						
Highest C_0 level in first week	0.959	0.896 - 1.026	0.221	.	.	.
Start < postoperative day 5	0.749	0.388 - 1.448	0.749	.	.	.

CI = confidence interval; eGFR = estimated glomerular filtration rate; IDDM = insulin dependent diabetes mellitus; MAP = mean arterial pressure; MELD = model for end stage liver disease; RBC = red blood cells.

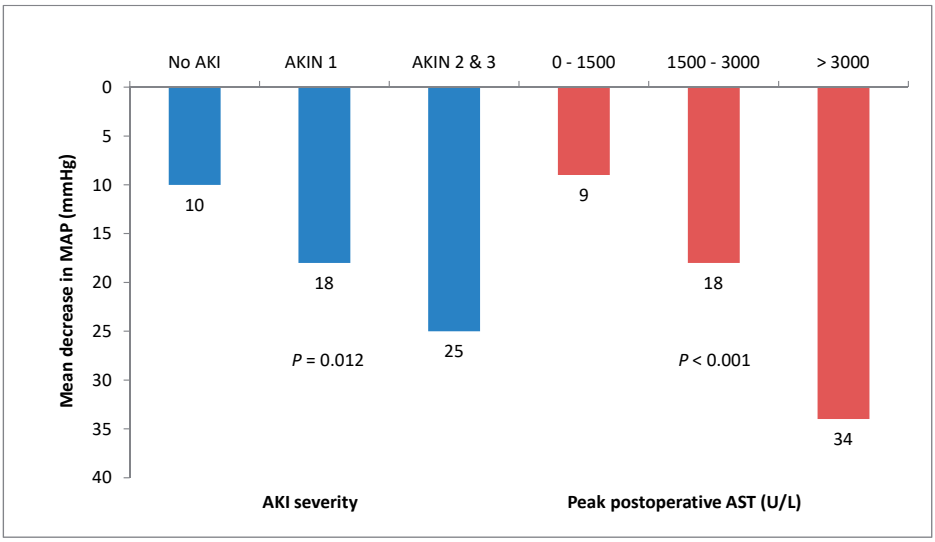
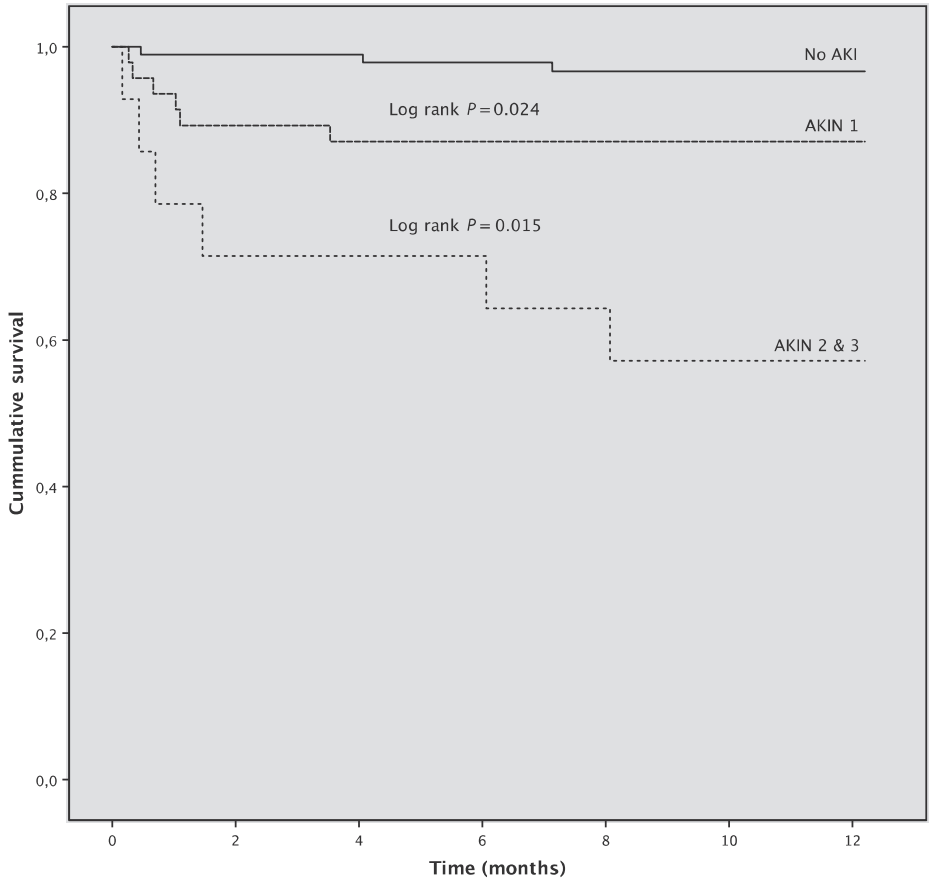


Figure 1 - Decrease in mean arterial pressure after reperfusion for severity of acute kidney injury (left) and hepatic ischemia reperfusion injury (right)

Consequences of AKI

Median length of ICU stay was 3 (IQR 2-5) days in recipients with AKI, compared to 2 (IQR 2-3) days in the control group (p=0.003). Furthermore, median length of hospital stay was one week longer in recipients with AKI with 24 (IQR 19-35) days, compared to 17 (IQR 14-27) days in the control group ($p < 0.001$). In-hospital morbidity correlated well with AKI severity: the occurrence of at least one major postoperative complication increased with severity of AKI: 30 (32%) of recipients without AKI had a major complication, compared to 31 (66%) for recipients with mild AKI and 12 (86%) with severe AKI ($p<0.001$). In-hospital mortality increased with severity of AKI as well: two (2%) of the recipients without AKI died during hospital stay, compared to five (11%) and four (29%) recipients with respectively mild and severe postoperative AKI ($p<0.001$). The impact of AKI on recipient survival continued until one year postoperative, which is displayed in **Figure 2**. Overall estimated one-year recipient survival was 88% and decreased with AKI severity (overall log-rank $p<0.001$). Moreover, the mild AKI group had a significantly lower survival compared to the control group (log rank p=0.026). The overall estimated one-year graft survival was 84% with no difference in graft survival observed between recipients with no AKI (91%) and mild AKI (80%) (p=0.072).



Number of recipients at risk

No AKI	94	89	88	83	76	69	67
AKIN 1	47	40	39	38	36	35	33
AKIN 2 & 3	14	10	10	10	9	8	8

Figure 2 - Kaplan-Meier survival table for recipient survival, divided in groups of severity of acute kidney injury

DISCUSSION

In this study we observed AKI in 39% of DBD graft recipients, which is in line with earlier published studies (1–6). Confirming our hypothesis, AKI was more frequently observed in recipients who experienced PRS during liver transplantation. We also showed that the decrease in MAP immediately after reperfusion correlated well with severity of AKI. Multivariable analysis identified PRS as an independent risk factor for AKI: if recipients experienced PRS, the odds of developing AKI showed a more than two-fold increase. The other subject of our hypothesis was the relation between the

severity of hepatic IRI and the extent of hemodynamic instability after reperfusion and that PRS is an early manifestation of severe hepatic IRI. Our results confirm this hypothesis: the decrease in MAP directly after reperfusion correlated well with post-operative peak AST levels and recipients with PRS had significant higher peak AST levels as well. Leithead *et al.* previously observed a relationship between hepatic IRI and AKI when DBD grafts are used (35). Our results do not only confirm this theory, but also provide new insight in this process. The extent of hepatic IRI is displayed by the amount of hemodynamic instability after reperfusion and severe hepatic IRI with subsequent occurrence of PRS can be predictive for the development of postoperative AKI.

AKI after liver transplantation has previously been related to the use of marginal organs, such as DCD grafts (1,14). Paugam-Burtz *et al* was the first to describe a relation between PRS and postoperative renal failure (25). Fonseca-Neto *et al* described a positive correlation between PRS and AKI in liver transplantation performed using conventional surgical technique without veno-venous bypass (38). In a cohort of living-donor liver transplant recipients Park *et al.* correlated the occurrence of PRS with postoperative AKI as well (39). However, we are the first to describe a dose-effect relationship between the extent of hemodynamic instability and the severity of renal injury. In our cohort, the decrease in MAP correlated well with the severity of postoperative AKI (**Figure 1**). Ekser *et al.* demonstrated in patients requiring combined liver-kidney transplantation, that delaying the kidney transplantation beyond 48 hours after liver transplantation, yielded significantly better renal outcomes than simultaneous kidney (40). This contributes to our hypothesis that renal function is especially vulnerable in the first hours after reperfusion, a phase characterized by hemodynamic instability and the release of pro-inflammatory cytokines caused by hepatic IRI.

AKI after liver transplantation is multifactorial of origin and in our study PRS occurred in 46% of recipients with postoperative AKI. So others factors are likely to contribute to development of AKI as well. Therefore, we performed a multiple logistic regression analysis. PRS was an independent factor associated with AKI in this model with an odds ratio of 2.3. In previous studies numerous preoperative factors are ascribed to influence development of AKI, such as age, female sex, severity of liver disease and renal function (3,7,10). The preoperative creatinine levels were significantly higher in the AKI group in the univariate analysis. However, this factor was not a significant contributor to the development of AKI in the multiple logistic model, which is in line with numerous previous studies (7,35,41). Next to PRS, several factors

related to the transplant procedure were identified. We described that increase of both cold and warm ischemia periods contributes to postoperative AKI when DBD grafts are used. These ischemia periods are both known factors to worsen hepatic IRI , but only WIT has earlier been linked to development of AKI (21,42–44). To our knowledge, duration of cold ischemia has not been related to AKI before. Liver transplantation is high risk surgery with substantial blood loss which increases the risk for periods of hypotension, a classical risk factor for AKI after major surgery (45,46). In our univariable logistic regression the influence of blood loss during surgery on AKI is illustrated by the requirement of RBC transfusion, but in the multiple logistic regression model with other factors, RBC transfusion requirement was not contributing to AKI. Tacrolimus has an important role in the immunosuppression after liver transplantation and is known for its nephrotoxicity (11–13). Over two third of the recipients developed AKI within the first two postoperative days, but in the majority of patients (58%) the introduction of tacrolimus was delayed until day 5. Of the recipients who received tacrolimus before day 5, the highest trough levels (C_0) were relatively low and comparable in recipients with and without AKI. This could explain why exposure to tacrolimus did not influence AKI development in our model. Furthermore, the fluid regimen during surgery might also contribute to the development of AKI. The usage of HES during liver transplantation has been linked with postoperative AKI. However this effect was only seen when it was not combined with albumin (45). Although HES is regularly used in our perioperative regimen, we consider its effect on AKI limited since all patients routinely received intravenous albumin.

This study has several limitations; the retrospective design has its inherent shortcomings. One third of the recipients were excluded, but our strict exclusion criteria yielded a more homogenous population where preoperative renal injury was minimal. The majority of the excluded recipients were transplantations for acute liver failure and retransplantations. The retransplantation rate in our cohort is relatively high, due to the increased use of DCD grafts. The retrospective design of this study could have given rise to varying perioperative management, but our institution has a well-implemented transplant protocol. Opposed to earlier studies, we assessed renal injury using serum creatinine levels only during the first week. This shorter and fixed period prevents confounders such as surgical and infectious complications that occur beyond the first postoperative week. Moreover, we strictly followed AKIN-criteria, being the most sensitive AKI definition at present. To our knowledge our study is the first to link PRS to the development of AKI after DBD liver transplantation using these strict AKIN criteria.

The importance of inclusion of recipients with relatively mild AKI (AKIN stage 1) is underlined by the observed morbidity and increased mortality. Recipients who developed mild AKI did experience more major complications and hospital mortality was higher compared to the control group. This effect carried on as reflected in the impaired one-year survival in recipients with mild AKI. Given the impact of AKI on recipient outcomes in our study, there is an obvious need to prevent renal injury. Protection against hemodynamic instability after reperfusion using vasopressor agents could be a feasible preventive intervention. Ryu *et al*. studied the effect of vasopressor therapy before reperfusion on the occurrence of PRS (47). Occurrence of PRS decreased with vasopressor pretreatment, being either phenylephrine or epinephrine, compared to placebo. However, this relatively small study did not investigate postoperative renal injury and no difference in-hospital mortality or hospital length of stay was observed. These results raise the question whether PRS, or hepatic IRI in general should be the focus of an intervention to preserve postoperative renal function. Other effects of hepatic IRI associated with renal injury – such as the release of pro-inflammatory cytokines – are not reflected by PRS (20,48,49). The exact effects of hepatic IRI on the kidney are complex, but experimental murine studies showed renal injury characterized by renal tubular necrosis, inflammatory changes and interstitial capillary endothelial apoptosis (50). These inflammatory responses of the kidney to hepatic IRI suggest that solely preventing the hemodynamic instability after reperfusion might not be enough to preserve renal function. Other ways to reduce hepatic IRI are surgical techniques such as initial arterial reperfusion or stepwise portal reperfusion are suggested to improve hemodynamic stability after reperfusion. This could also prevent the sudden release of cold components and pro-inflammatory cytokines in the systemic circulation and perhaps limit hepatic IRI. Other emerging strategies to minimalize hepatic IRI are hypothermic or normothermic oxygenated perfusion of the graft. These preservation methods have already shown in animal models to reduce postoperative biliary injury and immune response in DCD liver transplantation (51–54). In this setting the occurrence of PRS could also be used as an early measurement of hepatic IRI. Furthermore, the occurrence of PRS allows early identification -within minutes after reperfusion- of recipients at risk for AKI and enables early preventive measures to minimize additional renal injury after reperfusion. This could trigger adjustment of postoperative fluid management, delayed introduction of calcineurin inhibitors and other nephrotoxic medication.

In conclusion, our study confirmed that AKI is an important complication after DBD liver transplantation as even mild postoperative AKI has a substantial impact on recipient outcomes including survival. Hepatic IRI is a known factor to influence

development of AKI and the severity of hepatic IRI correlates with the extent of hemodynamic instability after reperfusion during transplantation. PRS, as a reflection of severe hepatic IRI, is predictive for development of AKI and allows early identification of patients at risk and could create opportunities to limit postoperative renal injury.

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Chapter

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

Marit Kalisvaart
Andrea Schlegel
Ilaria Umbro
Jubi de Haan
Irene Scalera
Wojciech Polak
Jan IJzermans
Darius Mirza
Thamara Perera
John Isaac
Anna Paolo Mitterhofer
Jeroen de Jonge
Paolo Muiesan

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SUMMARY

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

INTRODUCTION

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

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

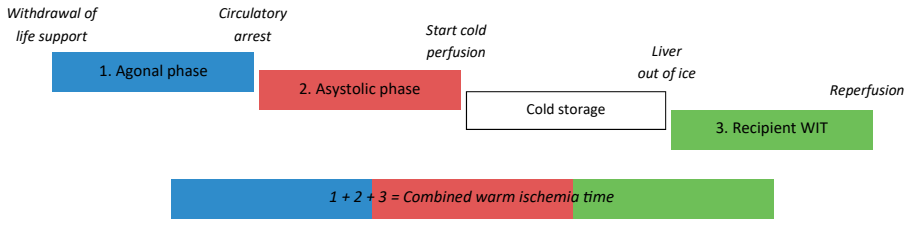

Figure 1 - Definition of combined warm ischemia time in DCD liver transplantation

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

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

METHODS

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

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

Table 1: Clinical characteristics of DCD liver transplantation recipients

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

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

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

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

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

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

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

Statistical analysis

Data were analyzed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, New York, USA). Continuous variables were tested for normality with the Shapiro-Wilk test. The student's t test and the one-way analysis of variance were used to compare normally distributed continuous variables. Nonparametric continuous variables were compared using the Mann-Whitney U test. To compare categorical variables, the Chi-square test or the Fisher's exact test were used. P-values < 0.05 were considered statistically significant. Continuous variables were expressed as mean with standard deviation (SD) or median and IQR, where appropriate. Categorical variables were expressed in quantities and percentages. To relate donor-, recipient-, and peri-operative factors to the development of severe AKI after DCD liver transplantation, a multiple logistic regression analysis was performed with all clinical relevant factors. Long-term survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests.

RESULTS

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

Recipient, donor, and perioperative characteristics in both centers

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

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

Development of acute kidney injury

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

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

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

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

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

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

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

Warm ischemia time and acute kidney injury

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

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

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

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

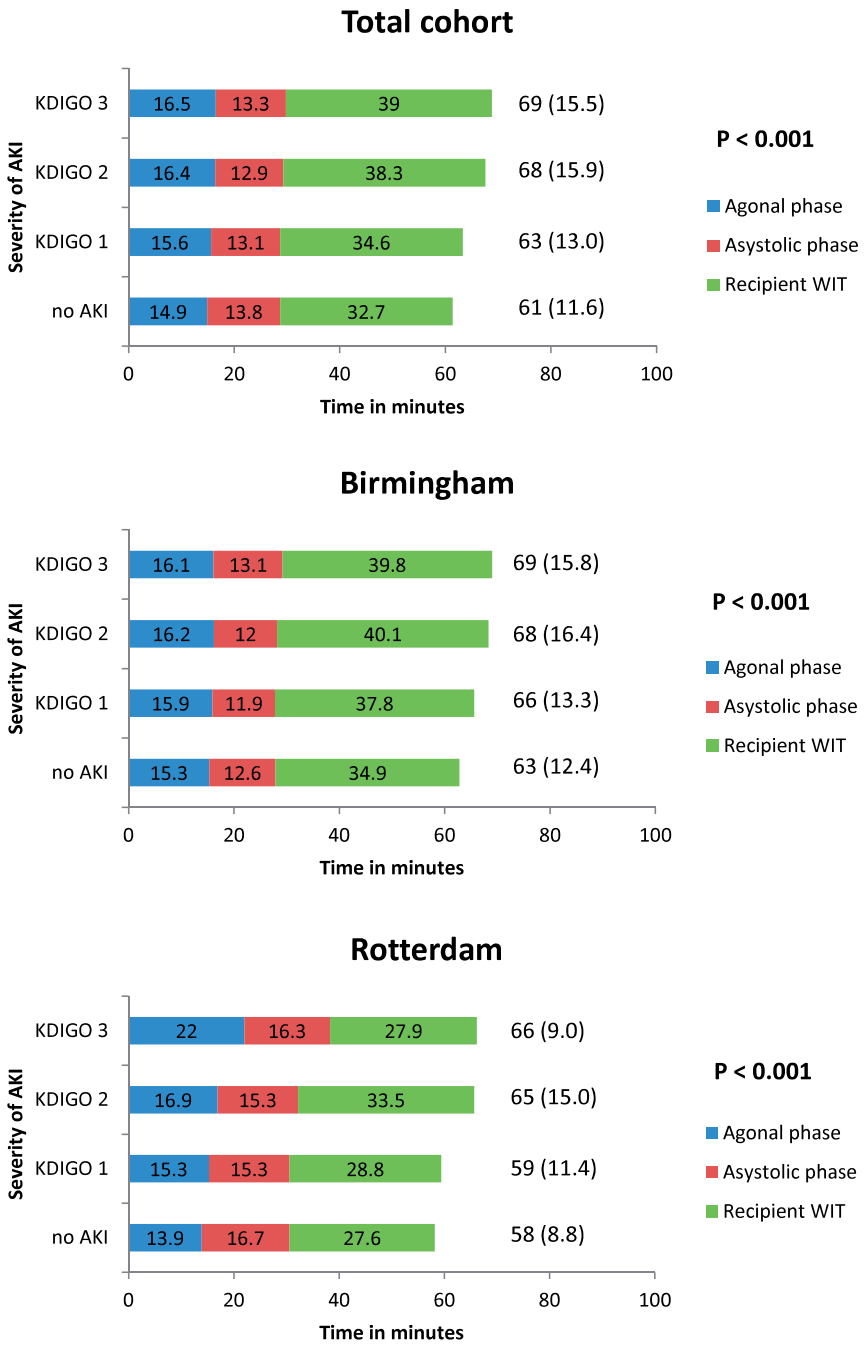

Figure 2 - Mean length of total warm ischemia time and severity of acute kidney injury after DCD liver transplantation

Multivariable analysis for development of severe acute kidney injury

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

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

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

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

Hepatic IRI, graft-related outcomes and acute kidney injury

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

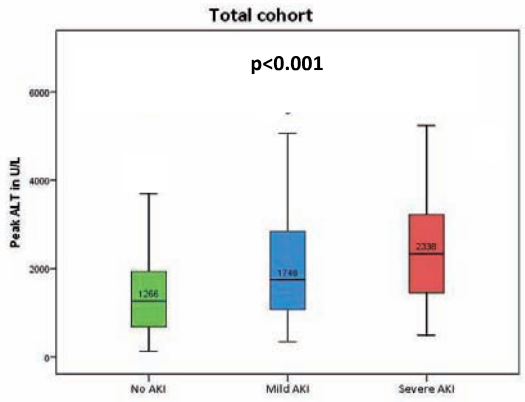

Figure 3 - hepatic IRI and acute kidney injury after DCD liver transplantation

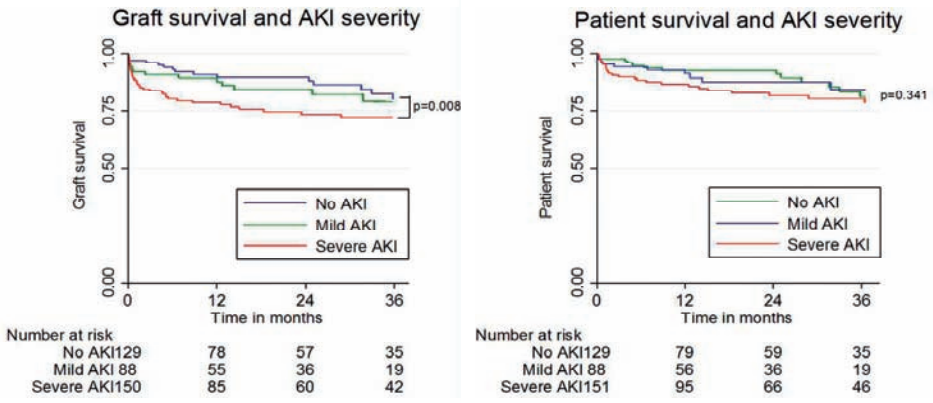

Figure 4 - Acute kidney injury and estimated 3-year graft (left) and recipient (right) survival and severity of acute kidney injury after DCD liver transplantation

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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Chapter

The AKI Prediction Score: A new prediction model for acute kidney injury after liver transplantation

Marit Kalisvaart
Andrea Schlegel
Ilaria Umbro
Jubi de Haan
Irene Scalera
Wojciech Polak
Jan IJzermans
Darius Mirza
Thamara Perera
John Isaac
Anna Paolo Mitterhofer
Jeroen de Jonge
Paolo Muiesan

Submitted.

SUMMARY

AKI is a frequent complication after liver transplantation and associated with impaired long-term survival rates, CKD and higher costs. Although numerous risk factors have been previously identified, their cumulative impact on the development of AKI remains unknown. Our aim was therefore to design a new model to predict the frequency of post-transplant AKI. Risk analysis was performed in all patients undergoing primary liver transplantation in two centres (n=1230). A new model to predict severe AKI (KDIGO stage 2/3) was calculated based on weight of the factors in a multivariable regression analysis according to the Framingham risk scheme. Overall, 34% of the patients developed severe AKI, including 18% requiring postoperative RRT. Five factors were identified as strongest predictors: both recipient and donor BMI, DCD graft use, FFP requirements, and recipient WIT, leading to 0-25 score points with an AUC of 0.70 for the new AKI Prediction Score. Three risk classes were identified: low, intermediate and high risk. Recipients with intermediate/high risk had impaired long-term graft survival and more postoperative complications assessed with the CCI. Furthermore, severe AKI was less frequently observed if such recipients received a renal-sparing immunosuppression, compared to those receiving calcineurin inhibitor based immunosuppression (29 vs. 45%; p=0.007). In conclusion, The AKI Prediction Score is a new and reliable instrument to identify recipients at risk for severe post-transplant AKI. This score is readily available at end of the transplant procedure, creating opportunities to engage in renal-sparing immunosuppression and early RRT.

INTRODUCTION

AKI is a common issue after liver transplantation and various studies have reported incidence rates up to 60% (1–4). Post-transplant AKI is associated with the development of CKD and impaired survival rates (5–8). Furthermore, patients requiring temporary RRT have a prolonged hospital stay with subsequent requirement of more resources and higher costs (9). Several donor, recipient and surgical factors have been linked to a higher risk for the development of AKI after liver transplantation. Recipient factors include BMI, pre-transplant MELD-score and history of DM or hypertension (1,6,7). Older and steatotic grafts or DCD donors have been associated with a higher rate of post-transplant AKI (1,10,11). The duration of cold and warm ischemia and transfusion requirements are further known risk factors (12,13). Finally, postoperative events and the use of nephrotoxic immunosuppression can further aggravate the damage to the kidneys (14–17).

The multifactorial origin of AKI complicates the prediction of who is particularly at risk to develop AKI and who will also require RRT. The introduction of the 'sickest first' allocation following the MELD-score prioritizes patients with impaired pre-transplant renal function and such patients are more vulnerable for the additional renal damage during and after liver transplantation (18). In addition, the evolving use of marginal grafts over the last years, including DCD and steatotic grafts, increases the risk for AKI (1). Such livers are more prone to increased hepatic IRI and previous studies have shown that severe hepatic IRI is associated with hemodynamic instability and development of AKI after liver transplantation (12,13,19,20). The numerous risk factors for AKI and its significant impact on hospital resources and short- and long-term outcomes, highlights the need for a standardized prediction model for AKI after liver transplantation surgery. Therefore, the aim of this study is to develop a novel prediction score to identify recipients at risk for post-transplant AKI at the earliest possible point after liver transplantation.

METHODS

Data collection

The liver transplantation cohorts of the Queen Elizabeth Hospital in Birmingham, UK (2007-2015) and the Erasmus MC University Medical Centre in Rotterdam, the Netherlands (2008-2014) were used to develop this new prediction model. All consecutive adult patients who underwent primary, orthotopic deceased-donor liver

transplantation were included and their medical records were retrospectively assessed. Exclusion criteria were retransplantation, super-urgent transplantation, RRT prior to liver transplantation, domino transplants, combined liver-kidney transplantation, and machine perfusion of the graft.

Study parameters

Donor, graft, recipient and surgical characteristics that were assessed as risk factor for AKI are highlighted in **Table 1**. The DRI and MELD-score were calculated according to previous studies (21,22). Post-transplant AKI was defined according to the KDIGO criteria (23): an increase in serum creatinine by ≥26.5 μmol/L within 48 hours or an increase in creatinine to ≥ 1.5 times baseline within the first 7 postoperative days. AKI was classified into 3 stages: stage 1, increase ≥26.5 μmol/L or increase of 1.5-1.9-fold from baseline; stage 2, increase of 2-2.9-fold; stage 3, increase >3-fold or increase in serum creatinine to ≥354 μmol/L or initiation of RRT. The peak serum AST level in the first 72 hours was used as surrogate marker for hepatic IRI (12). EAD was defined according to the Olthoff criteria (24). Based on the Clavien-Dindo Classification, all postoperative complications requiring treatment during initial hospital admission were collected and used to calculate the CCI to comprehend all postoperative morbidity (25,26). A major postoperative complication was defined as a Clavien-Dindo Classification grade ≥3b complication.

Transplant practice and immunosuppression

DCD grafts are retrieved using the super-rapid donor cannulation technique (27). The preferred preservation fluid for liver procurement in both centres is heparinized UW solution under pressure via the aorta. In the UK, the portal system is also cannulated and flushed in the donor with 1 litre UW solution. Additional back-table flush is performed in both centres, including a flush of the biliary tract. The standard implantation technique in both centres include the piggyback cava anastomosis (classic or side-to-side cavo-cavostomy) without the use of veno-venous bypass. A portocaval shunt is performed in selected cases. Triple-therapy immunosuppression after liver transplantation is the standard of care in both centers. In Birmingham, the regimen consists of Tacrolimus, Azathioprine or MMF and prednisolone, all introduced at day 0. Prednisolone therapy is generally discontinued after three months. A renal-sparing protocol is considered in recipients with impaired renal function and consists of either low-dose Tacrolimus introduction combined with MMF or delayed introduction of Tacrolimus (on day 3-5) with MMF and Basiliximab on day 0 and 4. In Rotterdam, the immunosuppression regimen was modified during the study period. Until 2012 patients received a triple-therapy regimen of Tacrolimus, MMF and pred-

Table 1 - Donor, recipient and surgical characteristics in the two cohorts.

Donor / Graft characteristics	Birmingham (n=1009)	Rotterdam (n=221)	P-value
Age (years)	52 (41-62)	53 (44-62)	0.207
Body mass index (kg/m^2)	25.8 (23.1-28.7)	24.4 (22.5-26.6)	<0.001
Donor risk index	1.8 (1.5-2.2)	2.2 (1.8-2.5)	<0.001
DCD graft (%)	285 (28%)	67 (30%)	0.537
Recipient			
Age (years)	55 (47-62)	54 (44-61)	0.107
Male gender (%)	650 (64%)	148 (67%)	0.472
Body mass index (kg/m^2)	26.6 (23.6-30.4)	25.5 (23.1-28.9)	0.001
Etiology of liver disease (%)			<0.001
ALD	276 (27%)	39 (18%)	
Hepatitis C	225 (22%)	31 (14%)	
Hepatitis B	40 (4%)	21 (10%)	
NASH	97 (10%)	30 (14%)	
PSC	118 (12%)	56 (25%)	
PBC	131 (13%)	11 (5%)	
AIH	30 (3%)	9 (4%)	
Other	92 (9%)	24 (11%)	
Hepatocellular carcinoma (%)	260 (26%)	63 (29%)	0.402
Biological MELD-score	13 (9-17)	16 (9-21)	<0.001
Serum creatinine (µmol/l)	78 (63-96)	76 (61-95)	0.271
Serum sodium (µmol/l)	138 (135-140)	138 (134-140)	0.775
Medical history (%)			
Hypertension	169 (17%)	18 (8%)	0.001
Diabetes mellitus	265 (26%)	57 (26%)	0.885
Surgery			
Cold ischemia time (hours)	7.8 (6.5-9.3)	6.4 (5.6-7.5)	<0.001
Recipient warm ischemia time (min)	38 (33-43)	28 (25-34)	<0.001
RBC transfusion (units)	2 (0-4)	3 (1-6)	<0.001
FFP transfusion (units)	6 (2-10)	4 (0-7)	<0.001

Continuous variables are displayed as median and interquartile range.
AIH, auto-immune hepatitis; ALD, alcohol-related liver disease; DCD, donation after circulatory death; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

nisolone (for three months), which were all introduced at day 0. From 2012, induction of tacrolimus is delayed until day 5, MMF is started on day 0 and Basiliximab given on day 0 and 4. Target peak trough levels for tacrolimus range between 7-10 ug/L during the first months in both centres.

Development of the new risk score

To establish a clinically relevant risk score we decided to develop a prediction model, that is directly available at the end of the transplant procedure to timely adapt the immunosuppression regimen and consider early RRT. Therefore, we only include preoperative donor and recipient parameters and surgical factors, including the duration of ischemia periods. Previous studies, including our own, have shown, that mainly AKI grade 2 and 3 have an impact on long-term survival and the development of CKD (5,12,28). We have therefore chosen severe AKI (KDIGO stage 2 and 3) as outcome variable of our prediction model. As our own centre data was used, all datasets were complete and compensatory methods for missing values were not required. All significant factors in univariable regression analysis ($p<0.25$) were considered further. A backward stepwise approach was used for the multivariable logistic regression with a p-value >0.05 as exclusion threshold (29). Backward elimination is generally preferred as an automated predictor-selection procedure because it takes correlations among predictors into calculation (30). The five strongest predictors were included in the final model. A point system for the score was developed according to the Framingham risk scheme (31). For each predictor, the median of all values below (midpoint W_1 reference – W_1 reference) and above the threshold was calculated. The midpoint for the cohort below each threshold ($W_{1–7}$ reference) was subtracted from the midpoint of all values above the threshold. The factor β was multiplied with the difference (W_{ij}–$W_{ireference}$), separately for each factor (31,32).

Performance and validation of the new prediction model

The performance of the prediction model was evaluated by examining discrimination (ability to discriminate between patients who do and do not experience the event [severe AKI]) and calibration (the agreement between predicted probabilities from the model and observed outcomes). Discrimination was quantified by calculating the area under receiver-operating-characteristic curve (AUC) statistic. The Hosmer-Lemeshow test was used to determine the extent of agreement between the predicted and observed probabilities. We performed an internal validation using a bootstrapping procedure. This approach uses the entire data to develop the prediction model and accounts for model over fitting or uncertainty compensating for over optimism in the final prediction model. The bootstrapping in the current study was performed using 500 bootstrap resamples, each time selecting variables and developing a model within the sample.

Additional statistics and ethical approval

Data were analysed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, New York, USA).Graphical images were constructed using GraphPad Prism version 7.04 (GraphPad Software, La Jolla, CA, USA). Median and IQR were used to analyse continuous variables and comparisons were made using the Mann-Whitney U test. Categorical variables were expressed in quantities and percentages. To compare categorical variables, the Chi-square test or the Fisher's exact test were used. Long-term survival rates were estimated using Kaplan-Meier methods, with comparisons between groups performed using log-rank tests. Completeness, plausibility and validity of the data were independently verified (by MK & AS), including objective review of all historical medical charts. All patients with complete data records were included. The local regulatory board approval was obtained prior to study initiation and database/chart review in Birmingham and Rotterdam.

RESULTS

Baseline characteristics and development of AKI

Overall, 1230 recipients were included in this study: 1009 recipients from Birmingham and 221 recipients from Rotterdam. The donor, recipient and surgical characteristics in both centres are displayed in **Table 1**. The recipient age was comparable in both centres (median Birmingham 55; Rotterdam 54 years; p=0.107) and the recipient BMI was significantly higher in Birmingham (26.6 vs. 25.5; p=0.001). The most common aetiologies of liver disease in Birmingham were alcohol-related liver disease (27%) and hepatitis C (22%), while in Rotterdam primary sclerosing cholangitis (25%) was the most common indication for transplantation, followed by alcohol-related liver disease (18%) (**Table 1**). Recipients in Rotterdam presented with a higher lab MELD-score (median 16 vs. 13; p<0.001). Importantly, no differences were observed

Table 2 - Development of AKI after liver transplantation in both cohorts.

	Birmingham (n=1009)	Rotterdam (n=221)	Total (n=1230)	P-value
No AKI	420 (42%)	129 (58%)	549 (45%)	
Overall AKI	589 (58%)	92 (42%)	681 (55%)	<0.001
AKI stage 1	195 (19%)	57 (26%)	252 (21%)	
AKI stage 2	134 (13%)	24 (11%)	158 (13%)	
AKI stage 3	260 (26%)	11 (5%)	271 (22%)	
Severe AKI (stage 2/3)	394 (39%)	35 (16%)	429 (25%)	<0.001
RRT	207 (21%)	6 (3%)	213 (18%)	<0.001

AKI, acute kidney injury; RRT, renal replacement therapy

for serum creatinine levels (median Birmingham 78 µmol/l; Rotterdam 76 µmol/l; p=0.271) prior to liver transplantation. Donors in both centres were of same age, but had a significantly higher BMI in Birmingham (median 25.8 vs. 24.4; p<0.001). In Birmingham, 28% of the transplants were performed with DCD grafts, compared to 30% in Rotterdam (p=0.537). Both the duration of cold ischemia (median 7.8 vs. 6.4 hours; p<0.001) and recipient WIT (38 vs. 28 min; p<0.001) was longer in Birmingham. Overall, 681 (55%) of the recipients developed AKI (**Table 2**), which was more common in Birmingham (58% vs. 42%; p<0.001). Additionally, in Birmingham liver recipients developed more severe form of AKI (39% vs. 16%; p<0.001) and required postoperative RRT more frequently (21% vs. 3%; p<0.001).

Key predictors of severe AKI and the new AKI Prediction Score

Initially, we evaluated 18 donor, recipient and surgical factors and the multivariable logistic regression identified the following five parameters as significant predictors for development of severe AKI: donor BMI (as a surrogate marker for graft steatosis), the use of a DCD graft, recipient BMI, the duration of recipient WIT (implantation

Table 3 - Multivariable logistic regression analysis for development of CKD after liver transplantation. The imputed data sets were combined and the point system was developed according to the Framingham risk scheme. For each predictor, the median of all values (Midpoint W_1reference – W_7reference) below and above the threshold has been calculated. The Midpoint for the cohort below each threshold ($W_{1\text{-}7}$reference) is subtracted from the midpoint of all values above the threshold. The factor βi is multiplied with the difference (W_{ij}-$W_i$reference), separately for each factor to develop the score points.

Parameter	Category	Regression Coefficient βi	*p-value*	Reference Value Wi (Midpoint)	βi x (Wij-Wireference)	Risk Score
Donor BMI	≤ 25 kg/m^2	0	*0.030*	22.85 ($W_{2\text{reference}}$)	0	0
	> 25 kg/m^2	-0.306		28.26	3.111	**3**
DCD graft	no	0	*<0.001*	0 ($W_{3\text{reference}}$)	0	0
	yes	-0.801		1	0.801	**1**
Recipient BMI	≤ 30 kg/m^2	0	*0.002*	24.93 ($W_{6\text{reference}}$)	0	0
	> 30 kg/m^2	-0.477		32.6	3.659	**4**
No. of FFP	≤ 3 U	0		0 ($W_{7\text{reference}}$)	0	0
	4 - 6 U	-1.103	*<0.001*	5	5.515	**6**
	7 - 10 U	-0.948	*<0.001*	8	7.584	**8**
	> 10 U	-0.636	*0.002*	16	10.176	**10**
Recipient WIT	≤ 36 min	0	*<0.001*	30 ($W_{9\text{reference}}$)	0	0
	> 36 min	-0.616		42	7.392	**7**
Total Score Points						**0-25**

β, regression coefficient; DCD, donation after circulatory death; FFP, fresh frozen plasma; Recipient WIT, recipient warm ischemia time.

time) and FFP requirements during the transplant procedure (**Table 3**). Both donor and recipient age, preoperative MELD-score and serum creatinine levels were not identified as predictors. The score points, calculated for the AKI Prediction Score ranged from 0 to 25 points (**Table 3**). The strongest predictor was FFP requirements (0, 6, 8, or 10 points), followed by the duration of recipient WIT (0 or 7 points), recipient BMI (0 or 4 points), donor BMI (0 or 3 points) and the use of DCD graft (0 or 1 point). The C-statistic of this model was 0.70 (**Figure 1A**) and the Hosmer-Lemeshow Goodness-of-fit test had a p-value of 0.664. In addition, the cumulative incidence of severe AKI increased exponentially with each single point of the AKI Prediction Score and recipients were divided into three risk groups: low risk (0-10 points), intermediate risk (11-20 points) and high risk (>20 points). Sixty-two percent of the recipients in the high risk group developed severe AKI, compared to 39% and 23% in the intermediate and low risk group, respectively (**Figure 1B**).

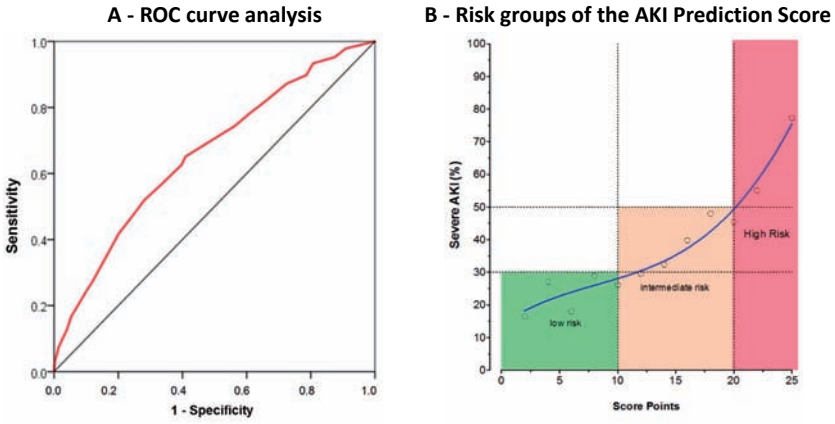

Figure 1- (A) ROC curve analysis and (B) The incidence of severe AKI according to the risk groups of the AKI Prediction Score.

The AKI Prediction Score and other outcomes

Hepatic IRI and subsequent early dysfunction of the graft are a known risk factors for AKI and we therefore analysed these outcomes according to the AKI Prediction Score. As expected, the surrogate marker for severity of hepatic IRI, peak serum AST levels increased significantly with the score (**Table 4**). In addition, EAD was observed in 32%, 40%, and 62% in the low, intermediate, and high risk group, respectively ($p<0.001$). Importantly, primary non-function (PNF) was more frequently observed in the higher risk groups (low 1%; intermediate 2%; high risk 5%; $p=0.002$). And 90-day graft loss occurred more often in recipients with an overall higher risk (low

Table 4 - Other recipient outcomes after liver transplantation in relation to the risk groups of the AKI Prediction Score.

AKI Prediction Score	Low risk	Intermediate risk	High risk	P-value
Peak AST (n=1098)	753 (304-1550)	1048 (374-2218)	1684 (886-3040)	<0.001
Early allograft dysfunction	175 (32%)	229 (40%)	70 (62%)	<0.001
Primary non function*	4 (1%)	11 (2%)	6 (5%)	0.002
Severe complication**	129 (24%)	170 (30%)	46 (41%)	0.001
Comprehensive Complication Index***	20.9 (0-40.5)	22.6 (8.7-43.3)	24.2 (8.7-48.1)	0.007
Comprehensive Complication Index >30	187 (34%)	218 (38%)	52 (46%)	0.049
ICU length of stay	2 (2-4)	3 (2-6)	4 (2-7)	<0.001
Hospital length of stay	12 (8-18)	11 (8-18)	13 (9-23)	0.264
90-day graft loss	38 (7%)	37 (7%)	18 (16%)	0.002

* Severe graft dysfunction resulting in death or retransplantation within 7 days after initial liver transplantation
** Defined as a Clavien Dindo classification grade 3B complication in the first 30 postoperative days
*** Comprehensive Complication Index in the first 30 postoperative days
Continuous variables are displayed as median and interquartile range.
AST, aspartate transaminase; ICU, intensive care unit; INR, international normalized ratio

7%; intermediate 7%; high risk 16%; p=0.002). An additional analysis of postoperative complications showed that recipients with a higher AKI Prediction Score had more severe complications (low 24%; intermediate 30%; high risk 41%; p=0.001). In accordance with the increasing overall risk, the sum of early post-transplant complications, as expressed by the 30-day CCI was significantly higher in the groups with

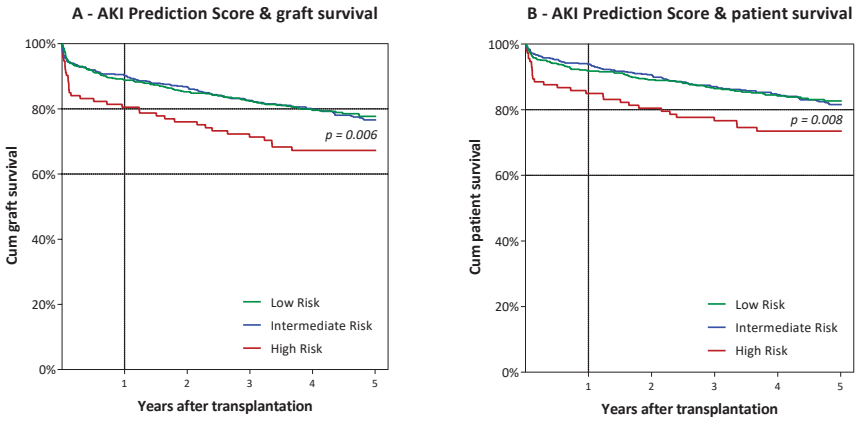

Figure 2 - AKI Prediction Score risk groups and (A) graft survival and (B) patient survival after liver transplantation.

more risk (low 20.9; intermediate 22.6; high risk; 24.2; p=0.007). The long-term graft and patient survival followed a similar pattern. The 5-year graft survival for recipients in the low (79%) and intermediate risk (77%) group was similar, while recipients in the high risk group has a significant lower graft survival (67%) (p=0.006) (**Figure 2A**). Comparable results were observed for patient survival after 5 years (low 83%; intermediate 82%; high risk 74%; p=0.008) (**Figure 2B**).

AKI Prediction Score and Immunosuppression

The majority of recipients (81%) received a Tacrolimus-based immunosuppression from day 0 and 19% started with a renal-sparing treatment with MMF from day 0 and Basiliximab on day 0 and 4 combined with a delayed introduction of Tacrolimus on day 3-5 (**Table 5**). Overall, recipients with a standard immunosuppression protocol had better pre-transplant kidney function (eGFR 89 vs. 77 mL/min/1.73 m^2; p<0.001). This difference was even more pronounced in recipients classified as intermediate and high risk (>10 score points; eGFR 87 vs. 67 mL/min/1.73 m2; p<0.001). We analysed the differences in immunosuppression for recipients with a low risk for AKI (0-10 points) compared to recipients with intermediate and high risk (>10 points). In the Low Risk group, significantly less recipients experience AKI when they received the renal sparing regimen (11 vs. 30%; p<0.001). A similar pattern was observed comparing the intermediate/high risk group (29% vs. 45%; p=0.007).

Table 5 - Development of acute kidney injury after liver transplantation according to immunosuppression regimens in different AKI Prediction Score risk groups.

	Pre-transplant eGFR (mL/min/1.73 m2)	No/mild AKI	Severe AKI	p-value*
All recipients (n=1227)				<0.001
Tacrolimus based	89	607 (61%)	389 (39%)	
MMF / Basiliximab / Delayed Tacrolimus	77	194 (83%)	40 (17%)	
AKI Prediction Score: 0-10 points				<0.001
Tacrolimus based	91	276 (70%)	118 (30%)	
MMF / Basiliximab / Delayed Tacrolimus	85	136 (90%)	16 (11%)	
AKI Prediction Score: >10 points				0.007
Tacrolimus based	87	331 (55%)	721 (45%)	
MMF / Basiliximab / Delayed Tacrolimus	67	58 (71%)	24 (29%)	

* p-value displays the statistical difference in severe AKI between the three immunosuppression regimens.

DISCUSSION

Acute kidney injury in the setting of liver transplantation is increasingly recognized and in focus by many. We here present a new prediction model for the development of AKI after liver transplantation and show the following new findings. First, the AKI Prediction Score is an easy-to-use tool to assess the individual recipient risk of AKI development based on the combination of donor, recipient and surgical risk factors. Second, the new model stratifies recipients into three risk groups (low, intermediate and high risk) and correlated well with an impaired graft function and overall post-transplant morbidity and graft loss. Third, the AKI Prediction Score is readily available at the end of the transplant procedure, enabling doctors to use the score to avoid further nephrotoxicity through immunosuppression and therefore reduce complications and improve general outcomes.

Post-transplant AKI is an important risk factor for development of CKD, especially if candidates require RRT in the early post-transplant phase (5,6,10). Recipient with AKI also have an increased mortality-risk in the short and long-term (33–36). The high incidence of this complication and its multifactorial origin motivated us to develop a new score, that identifies recipients at risk for AKI combining donor, recipient and surgical characteristics. The strongest predictor in the new score was the requirement of FFP transfusions during the transplant procedure. This was not unexpected, as significant blood loss during the hepatectomy phase is common recipients which carry a higher risk due to coagulopathy and portal hypertension. Additionally, coagulopathy and fibrinolysis represent severe hepatic IRI and impaired early graft function (24,37,38). Other predictors in our model with negative impact on graft function and subsequent kidney injury were a higher donor BMI, a prolonged implantation of the graft (recipient WIT) and the use of a DCD graft. Such three factors have been previously identified as risk factors for AKI after liver transplantation by many (1,16,17,39). Donor BMI serves as surrogate marker of graft steatosis and fatty livers are known to be more susceptible to hepatic IRI (40–42). Prolonged graft implantation times impair the graft function further and lead to a more severe AKI, expectedly (1,12,39). In addition, the obligatory DWIT in DCD liver transplantation aggravates the injury to these grafts and the subsequent risk for postoperative AKI (8,28,43). The fifth predictor in the model was obesity of the recipient (BMI >30 kg/m^2), which is a known factor to increase the risk for postoperative AKI in general and after liver transplantation (6,16,44,45). Although preoperative recipient serum creatinine levels and MELD-score have been identified as risk factors for AKI after liver transplantation in previous studies, they were found to predict AKI not significant predictors our

cohort. This could be the result of a generally increased use of marginal grafts in our centres, which might have a more significant impact on AKI (1). Also, recipients with HRS (with subsequent elevated serum creatinine levels) prior to liver transplantation, may improve their kidney function in the early period after liver transplantation.

In addition to the 25-point system of the score, we stratified the recipients into three clusters of low, intermediate and high risk, where severe AKI was observed in 23%, 39% and 63%, respectively. Post-transplant AKI was often simultaneously observed with severe hepatic IRI, EAD and other complications (10,13–15,19). Such results by others were also paralleled by our study. Graft injury and initial graft function, displayed by transaminase release and incidence of EAD, worsened with increasing score points. In addition, the incidence of PNF and other complications increased with higher risk in the AKI Prediction Score. Second, the overall postoperative morbidity was also in parallel with the risk of the three clusters with significantly more severe complications and a higher CCI during hospital stay. Third, recipients with a low and intermediate risk had comparable long-term graft and recipient survival rates, while recipients with a high risk according to the AKI Prediction Score, had impaired graft and recipient survival rates.

This is not the first score to predict the risk for AKI after liver transplantation, however it is the largest deceased-donor population and the first score assessing the impact of graft quality. In 2017, Park and colleagues developed a risk score for recipients of a living-donor transplant in a Korean centre (16). In general, the incidence of AKI is lower with the use of living-donor grafts and only 8% of the recipients had severe AKI in this cohort. This is in line with our results, where we observed that higher risk grafts induce more AKI in the recipients. In addition, the Park model required ten variables, including postoperative serum glucose levels and the type of immunosuppression regimen. Another risk score form a large Chinese multicentre study mostly included DCD grafts (17). The authors developed here a model involving postoperative predictors, such as the immunosuppression protocol and dopamine treatment. Importantly, both models used the old RIFLE-criteria (and not the most recent KDIGO-criteria) to classify AKI, which appear not as standard over the last years. However, both scores achieved a slightly better discrimination in the C statistic, however direct comparison appeared difficult, since too many variables were required and our score did not include any postoperative predictors. This is a major advantage of our AKI Prediction Score, as it is directly available at the end of the transplant procedure. This allows the physicians to adapt the immunosuppression in recipients with a higher risk of severe AKI. Our results showed that in recipients with more than score 10 points who receive

a renal sparing immunosuppression regimen had significantly less often severe AKI compared to recipients receiving a standard regimen with Tacrolimus starting at day 0. Another opportunity with timely AKI risk prediction is early initiation of RRT, given there is evidence that in critically ill patients an early initiation of RRT reduces mortality and fosters early recovery of renal function (46).

Our prediction model has several limitations. First, we only used five predictors in our score and a larger, more heterogeneous cohort might have identified more predictors. However, our two-centre cohort of two European centres yielded more than 1200 recipients and due to the use of our own datasets, we did not have any missing values, as common in large national registries. The five predictors are likely available in every transplant centre and cohort, making this score simple and easy to use. Next, severe AKI (KDIGO stage 2 & 3) is an arbitrary threshold, however previous studies have shown that severe forms of AKI have the strongest relation with long-term survival rates and development of CKD (5,12,28). Third, it should be noted we excluded recipients with severe pre-transplant renal dysfunction requiring RRT. Such recipients have a different risk pattern of post-transplant kidney problems and depending on their origin of their renal failure, they either receive peri-operative RRT or receive a combined liver-kidney transplant. Therefore, we considered these recipients not suitable as a baseline cohort to develop a new score.

In conclusion, the AKI Prediction Score is a new and simple tool to support transplant professionals in assessing the risk for recipients who develop AKI with good discriminative characteristics. This new model is highly predictive for other postoperative issues, including impaired graft function and severe complications. The great advantage of this score is that it is timely available at the end of the transplant procedure to avoid further postoperative renal injury.

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Chapter 10

Chronic kidney disease after liver transplantation: Can we safely use extended criteria grafts?

Marit Kalisvaart
Andrea Schlegel
Palak Trivedi
Keith Roberts
Darius Mirza
Thamara Perera
John Isaac
Jeroen de Jonge
James Ferguson
Paolo Muiesan

Submitted.

SUMMARY

The increased use of ECD grafts has been associated with AKI after liver transplantation. However, the relation between graft quality and development of CKD remains unknown. Our aim was therefore to identify risk factors for CKD and all adult patients (2007-2015) transplanted for end-stage liver disease at our centre were assessed. Long-term renal function was divided into three groups: no CKD (eGFR≥60), mild CKD (eGFR30-59), severe CKD (eGFR<30). Marginal DBD grafts (donor age >70 years and BMI >35 kg/m^2 or cold ischemia time >12h) and DCD grafts were considered as ECD grafts. Overall, 926 recipients were included and 43% received an ECD graft (15% marginal DBD; 28% DCD). After five years, 35% developed CKD, severe CKD and end-stage renal disease occurred in only 2% and 1%, respectively. The incidence of CKD was comparable for all three graft groups (standard 36%; marginal DBD 29%; DCD 35%; standard vs. marginal DBD p-value 0.164; standard vs. DCD p-value 0.801). None of the ECD criteria were identified as independent risk factors in a cox proportional hazard model for CKD. Risk factors included recipient age, female gender and preoperative kidney function. Furthermore, recipients who had severe post-transplant AKI (KDIGO stage 2/3) had a 1.8-fold increased risk to develop CKD. Long-term kidney function of recipients with severe AKI depended on the recovery in the first postoperative weeks. In conclusion, there is no direct relation between the use of ECD grafts and CKD after liver transplantation. Caution should be taken in recipients who experience severe AKI, regardless of graft type.

INTRODUCTION

Short-term renal dysfunction is a common issue after liver transplantation. The majority of liver recipients recover from the postoperative AKI, despite the frequent need for temporary renal RRT (1). However, a significant proportion of recipients develop CKD, resulting in ESRD requiring RRT or kidney transplantation years after liver transplantation. In a previous large study two thirds of the recipients developed CKD after ten years, but there is a wide range between studies due to the different criteria used for CKD and variations in duration of follow-up (2). In addition, CKD is associated with higher mortality rates and increased costs, where further analysis of causes and underlying mechanisms appears essential (2–6).

Three main stages during liver transplantation have previously been recognized to impact on long-term postoperative kidney function: (I) pre-transplant renal failure in end-stage liver disease, (II) additional kidney injury during the transplantation procedure, and (III) post-transplant kidney injury (7,8). Hepatorenal syndrome, glomerulonephritis (chronic viral hepatitis B/C related) and episodes of sepsis significantly contribute to a deteriorating kidney function in candidates for liver transplantation with higher MELD-scores (9,10). Additional patient-related predictors for the development of post-transplant CKD include age, female gender, and history of hypertension or DM (4). Blood loss and use of vasopressors during the transplant procedure and postoperative complications, e.g. infections, bleeding or biliary problems impact further on kidney function. The increased use of DCD and other ECD grafts is associated with a higher rate of post-transplant AKI, though the effect on long-term renal function has not yet been demonstrated (11,12). Finally, the use of nephrotoxic immunosuppression and development of hypertension and DM later after transplantation have a further negative influence on long-term kidney function (7).

Considering the growing pre-transplant renal problems due to the 'sickest-first' allocation policy and the increased use of ECD grafts, the rate of CKD after liver transplantation is likely to increase. The aim of this study was therefore to assess the impact of ECD grafts and other risk factors for development of CKD after liver transplantation.

METHODS

Data collection

All consecutive adult patients who underwent orthotopic deceased-donor liver transplantation over a nine-year period (2007-2015) at our centre were included in this study and their medical records were retrospectively assessed. Recipients surviving more than three months after transplantation were included and minimal follow-up was two years. Recipients that did not have two eGFR measurements at least three months apart, were excluded. The other exclusion criteria included retransplantation, super-urgent transplantation, RRT prior to liver transplantation, combined liver-kidney transplantation, and machine perfusion preservation of the graft. Completeness, plausibility and validity of the data were independently verified (by MK, AS, PM), including objective review of all historical medical charts. This study was approved by the Institutional Review Board of the Queen Elizabeth Hospital Birmingham (CARMS-02246).

Study parameters and assessment of kidney function

Donor and recipient characteristics are highlighted in **Table 1**. The DRI, MELD-score and eGFR (using the Modification of Diet in Renal Disease Study 4-equation) were calculated according to previous studies (13–16). CKD was defined following the KDIGO 2012 Clinical Practice Guidelines (17). Serum creatinine, eGFR and Tacrolimus trough levels were collected at the following time points: pre-transplant at admission, 1-7 days, 1, 3, 4, 6, 9, 12 months and each year after transplantation until the end of follow up. Recipients were divided into four groups according to severity of CKD: no CKD (eGFR ≥60 ml/min/1.73m^2), mild CKD (eGFR 30-59 ml/min/1.73m^2), severe CKD (eGFR 15-29 ml/min/1.73m^2) and ESRD (eGFR <15 ml/min/1.73m^2, requiring RRT or listed for kidney transplantation). Only if a recipient had two or more eGFR measurements were below 60 ml/min/1.73m^2 at least three months apart, the recipient was included in the CKD group. Furthermore, some recipients experienced a period of impaired kidney function due to infection, rejection or other events, but had recovery of their renal function over time. Therefore recipients were only considered having CKD, if the last two eGFR measurements were below 60 ml/min/1.73m^2. Post-transplant AKI was defined according to the well-known KDIGO criteria (18): an increase in serum creatinine by ≥26.5 µmol/L within 48 hours or an increase in creatinine to ≥1.5 times baseline within the first 7 postoperative days. AKI was classified into 3 stages: stage 1, increase ≥26.5 µmol/L or increase of 1.5-1.9-fold from baseline; stage 2, increase of 2-2.9-fold; stage 3, increase >3-fold or increase in serum creatinine to ≥354 µmol/L or initiation of RRT. The peak serum

Table 1: Donor, recipient and surgical characteristics according to graft type in liver transplantation

Graft type	Standard (n=531)		Marginal DBD (n=136)		DCD (n=259)		p-value (Standard vs. Marginal DBD)	p-value (Standard vs. DCD)
Donor & Graft								
Age (years)	50	(40-60)	65	48-73)	51	(35-62)	<0.001	0.860
Body mass index	26	(23.1-28.5)	27.5	(24.7-35.3)	24.7	(22.6-27.7)	<0.001	0.005
Donor risk index	1.6	(1.4-1.9)	2.0	(1.6-2.2)	2.3	(1.9-2.8)	<0.001	<0.001
Cold ischemia time (hrs)	7.9	(6.5-9.5)	9.2	(7.4-12.4)	7.1	(6.0-8.1)	<0.001	<0.001
Implantation time (min)	38	(32-43)	38	(33-42)	38	(32-43)	0.321	0.519
Recipient								
Age (years)	54	(45-61)	56	(48-71)	57	(51-62)	0.403	0.001
Female gender	194	(37%)	50	(37%)	86	(33%)	0.960	0.358
Body mass index	26.6	(23.5-30.5)	28.6	(24.2-31.8)	26.2	(23.6-29.4)	0.010	0.258
Laboratory MELD-score	13	(10-18)	13	(9-17)	11	(7-14)	0.074	<0.001
eGFR (mL/min/1.73 m2)	86	(67-111)	90	(71-116)	89	(71-112)	0.258	0.214
Race							0.423	0.281
Caucasian	459	(86%)	123	(90%)	217	(84%)		
Black / Negroid	15	(3%)	2	(2%)	5	(2%)		
Other	57	(11%)	11	(8%)	37	(14%)		
Liver disease							0.235	0.027
Alcohol related disease	135	(25%)	44	(32%)	66	(26%)		
Hepatitis C	112	21%)	31	(23%)	67	(26%)		
Hepatitis B	21	(4%)	5	(4%)	13	(5%)		
NASH	47	(9%)	17	(13%)	24	(9%)		
PSC	73	(14%)	12	(9%)	26	(10%)		
PBC	64	(12%)	15	(11%)	44	(17%)		
AIH	17	(3%)	4	(3%)	6	(2%)		
Other	79	(15%)	12	(9%)	19	(7%)		
Hepatocellular carcinoma	115	(22%)	29	(21%)	94	(36%)	0.933	<0.001
Previous medical history								
Hypertension	74	(14%)	26	(19%)	60	(23%)	0.131	0.001
Diabetes mellitus	138	(26%)	37	(27%)	69	(27%)	0.773	0.845
Ischemic heart disease	31	(6%)	9	(7%)	7	(3%)	0.733	0.053
Follow up (years)	4.8	(3.0-7.0)	4.1	(2.7-6.5)	4.3	(2.6-6.3)	0.182	0.004
Transplant procedure								
Operation time (hrs)	4.9	(4.1-5.8)	4.7	(4.1-5.9)	4.8	(4.0-5.6)	0.648	0.130
Red blood cells (units)	2	(0-4)	2	(0-5)	2	(0-4)	0.444	0.528
Fresh frozen plasma (units)	6	(2-10)	6	(3-10)	6	(2-11)	0.231	0.222

Auto-immune hepatitis; AKI, acute kidney injury; DBD, donation after brain death; DCD, donation after circulatory death; MELD, model for end stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis. Continuous variables are displayed as median (interquartile range).

alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level in the first 48 hours were used as surrogate marker for hepatic ischemia/reperfusion injury (19). Early allograft dysfunction (EAD) was defined according to the Olthoff criteria (20). Based on the Clavien-Dindo Classification, all postoperative complications requiring treatment during initial hospital admission were collected and used to calculate the CCI to assess postoperative morbidity (21,22). A major postoperative complication was defined as a Clavien-Dindo Classification grade ≥3B complication.

Centre practice and assessment of risk factors

In the United Kingdom, there is an increasing use of ECD grafts (marginal DBD and DCD grafts) over the last years. Two-third of the livers qualify as ECD grafts, according to the guidelines of British Transplantation Society and the European Association for Study of the Liver (23,24). To identify the impact of marginal grafts we defined ECD grafts with more strict criteria: donor age >70 years, donor BMI >35 kg/m^2, cold storage >12h, and DCD grafts. At our centre, the following surgical techniques are used for organ procurement and implantation: all grafts are procured following dual cold flush through the aorta and portal vein with subsequent removal from the donor. DCD grafts are retrieved using the super-rapid donor cannulation technique as previously described (25). Heparinized University of Wisconsin solution (5 litres) is used for pressurized aortic donor perfusion. Additional flush is performed for all livers during the bench procedure at the donor hospital through the portal vein, hepatic artery and bile duct. The standard implantation technique includes classic or modified piggyback cava-anastomosis without use of veno-venous bypass. A temporary portocaval shunt is used in selected cases. The immunosuppressive regimen consisted of prednisolone, tacrolimus and azathioprine or mycophenolate mofetil, all introduced at day 0. Prednisolone is constantly reduced throughout the first three months and stopped thereafter. Recipients with pre-transplant renal impairment received a tacrolimus regimen with lower target trough levels or had a delayed introduction of tacrolimus on day 5 and induction therapy with mycophenolate mofetil from day 0 in combination with basiliximab on day 0 and 4. Target peak trough levels for tacrolimus were 6-8 µg/L during the first month.

Statistical analysis

Data were analysed with IBM SPSS Statistics version 24 (IBM Corporation, Armonk, New York, USA). Median and interquartile range were used to analyse continuous variables and comparisons were made using the Mann-Whitney U test. Categorical variables were expressed in quantities and percentages. To compare categorical variables, the Chi-square test or the Fisher's exact test were used. P-values < 0.05

were considered statistically significant. A Cox proportional hazard model was used to identify donor-, recipient-, surgical and postoperative factors to the development of CKD.

RESULTS

Development of CKD after liver transplantation

Between 2007 and 2015, 1009 adult patients underwent primary liver transplantation for end-stage liver disease at our centre. Forty-eight recipients (5%) died within the first three months and 35 (3%) did not have at least two eGFR measurements at least three months apart and were therefore excluded. Overall, 926 recipients were included in our analysis with a median follow-up of 4.6 years (IQR 3.0-6.7 years). Already within the first week after liver transplantation 41% of the recipients experienced a significant reduction in kidney function, including 19% with an eGFR of less than 15 mL/min/1.73 m^2, representing recipients in need of temporary RRT. After the first week, majority of recipients had a quick recovery (61%), but a slow decline in kidney function was observed thereafter (**Figure 1**). In the first five years, 35% of the recipients had developed CKD and the majority (33%) had a mild form of CKD (eGFR 30-59 mL/min/1.73 m^2). Severe CKD (eGFR 15-29 mL/min/1.73 m^2) was observed in 2% of the recipients and four of them (1%) presented with ESRD (eGFR <15 mL/min/1.73 m^2 or long-term RRT). The results of this calculation were

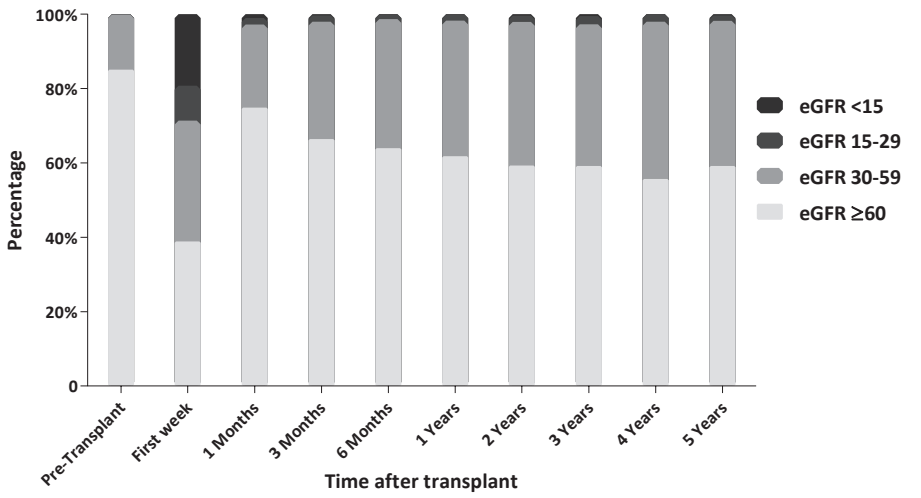

Figure 1: Long-term renal function for patients undergoing liver transplantation.

corrected for those who died in the first five years before they had developed CKD (n=71; 8%). Only one patient was listed for a kidney transplantation, 7.5 years after liver transplantation.

Quality of liver grafts

The recipients were divided into three groups according to the graft they received: a standard graft, marginal DBD graft (donor age >70 years, donor BMI >35 kg/m^2 or cold ischemia time >12 hours) or DCD graft. According to our criteria, 43% of the recipients received an ECD graft, including 259 (28%) DCD grafts and 136 (15%) marginal DBD grafts (78 (9%) donor age >70 years, 48 (5%) donor BMI >35 kg/m^2, and 45 (5%) cold storage >12h). Most recipients had one ECD criterion (39%), while only 4% had two criteria. Baseline characteristics are displayed in **Table 1**. All displayed p-values are either a comparison of marginal DBD grafts or DCD grafts with standard grafts. As expected, the DRI was higher in the marginal DBD and DCD group. The median recipient age was 55 years and 33% of the recipients were females. This was a relatively low MELD-cohort with a median laboratory MELD-score of 13 (IQR 9-17) and the lowest in the DCD recipients (11 vs. 13; p<0.001). DCD recipients also had more frequently an HCC (standard 22%; DCD 36%; p<0.001). The preoperative kidney function was equally distributed between groups (standard 86; marginal DBD 90 [p=0.258]; DCD 89 mL/min/1.73 m^2 [p=0.214]). The duration of the transplant procedure and the amount of required blood transfusion were also comparable between the three groups. After the transplant procedure, recipients of both marginal DBD and DCD grafts experienced more frequently a major complication (Clavien-Dindo grade 3B or higher) (**Table 2**). More complications requiring a relaparotomy were only

observed in the DCD group (12% vs. 6%; p=0.009) and these recipients also had a significantly higher CCI compared to the recipients of standard grafts (20.9 vs. 8.7; p=0.008). EAD was observed in 31% and 27% of the standard and marginal DBD group, respectively (p=0.358). In contrast, more than half of the recipients in the DCD group (54%) had EAD (p<0.001). Postoperative AKI in the first week after liver transplantation was common and observed in 58% of the recipients. Around one fifth of the recipients had mild AKI and distributed equally between graft groups (standard 20%; marginal DBD 20% [p=0.902]; DCD 21% [p=0.677]). Thirty-three percent in the standard group had severe AKI and 40% in the marginal DBD group (p=0.120), but significantly more recipients of DCD grafts had severe AKI (46%; p=0.001).

Table 2: Early post-transplant events according to graft type in liver transplantation.

First 90 days	Standard (n=531)		Marginal DBD (n=136)		DCD (n=259)		p-value (Standard vs. Marginal DBD)	p-value (Standard vs. DCD)
Length of stay - ICU	3	(2-5)	3	(2-5)	3	(2-5)	0.008	0.179
Length of stay - hospital	10	(8-15)	10	(8-15)	10	(7-15)	0.756	0.279
Major postoperative events								
Overall major complication*	104	(20%)	40	(29%)	77	(30%)	0.013	0.001
Severe infection (ICU readmission)	20	(4%)	3	(2%)	7	(3%)	0.597	0.440
Relaparotomy	33	(6%)	12	(9%)	30	(12%)	0.279	0.009
Early retransplantation	14	(3%)	3	(2%)	12	(5%)	0.776	0.140
Biliary complication**	31	(6%)	9	(7%)	19	(7%)	0.733	0.417
Comprehensive Complication Index	8.7	(8.7-28.9)	12.2	(0-12.2)	20.9	(8.7-42.4)	0.283	0.008
Allograft function								
Early allograft dysfunction	162	(31%)	36	(27%)	140	(54%)	0.358	<0.001
Peak transaminases in first week	1060	(648-1782)	1095	(673-1757)	1981	(1218-3197)	0.705	<0.001
Postoperative acute kidney injury								
*Form of AKI****								
No AKI	250	(47%)	55	(40%)	86	(33%)		
Mild AKI	104	(20%)	26	(20%)	54	(21%)	0.902	0.677
Severe AKI	177	(33%)	55	(40%)	119	(46%)	0.120	0.001

* Clavien Dindo stage ≥3B. Continuous variables are displayed as median and interquartile range.
** requiring endoscopic/radiologic or surgical intervention.
*** Following KDIGO criteria for AKI: mild AKI is stage 1 and severe AKI stage 2 and 3.
AKI, acute kidney injury; CKD, chronic kidney disease; DBD, donation after brain death; DCD, donation after circulatory death; ICU, intensive care unit.

Graft quality and CKD after liver transplantation

The incidence of CKD was evaluated after five years (**Figure 2**) and there was no significant difference between the three graft quality groups (standard 36%; marginal DBD 29% [p=0.164]; DCD 35% [p=0.809]). A similar pattern was observed in the incidence rates of severe CKD after five years. **Figure 3** gives an overview of the

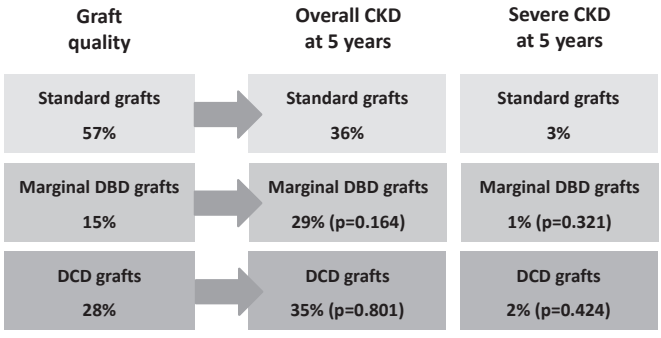

Figure 2: Graft quality and development of chronic kidney disease in the first 5 years after liver transplantation.

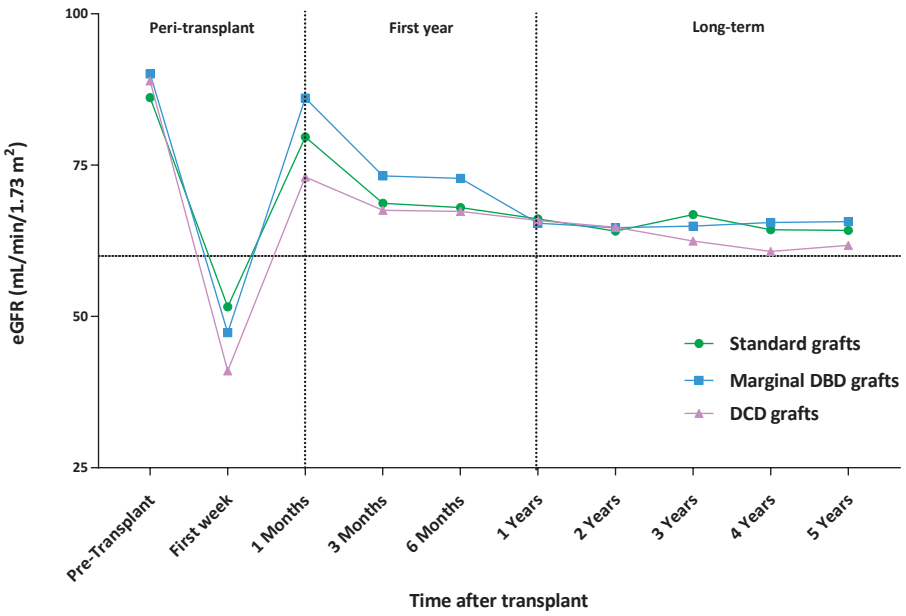

Figure 3: Graft type and long-term renal function after liver transplantation.

course of kidney function in the five years after liver transplantation according to graft type. After five years, the eGFR was 64 mL/min/1.73 m^2 in the standard group, compared to 66 mL/min/1.73 m^2 in the marginal DBD group (p=0.814) and 62 mL/min/1.73 m^2 in the DCD group (p=0.259). Furthermore, the change in kidney function from before liver transplantation until five years after liver transplantation did not differ between the groups (eGFR standard -17; marginal DBD -15 [p=0.984]; DCD -20 mL/min/1.73 m^2 [p=0.288]). A multivariable cox-regression model (**Table 3**) with all clinically relevant donor, recipient and peri-transplant factors was conducted to identify risk factors for CKD after liver transplantation. None of the ECD criteria were identified as independent risk factors for the development of CKD.

Other factors associated with the development of CKD

This multivariable cox-regression model (**Table 3**) did identify several recipient risk factors associated with post-transplant CKD: age (HR 1.059 for every year; p<0.001), female gender (HR 1.351; p=0.029), primary biliary cirrhosis as indication for liver transplantation (HR 1.548; p=0.025) and preoperative kidney function (eGFR <60 mL/min/1.73 m^2 HR 2.994; p<0.001). Additionally, postoperative renal injury was also associated with long-term renal dysfunction, but only recipients with severe AKI had an increased risk to developed CKD (mild AKI HR 1.100; p=0.581; severe AKI HR

Table 3: Multivariable cox proportional hazard model for CKD after liver transplantation.

Factor	HR	95% CI	P-value
Donor / Graft			
ECD: donor age >70 years	0.755	0.494-1.154	0.194
ECD: donor body mass index >35 kg/m^2	0.698	0.367-1.327	0.273
ECD: cold ischemia time >7 h	1.005	0.575-1.758	0.986
ECD: DCD graft	0.816	0.617-1.078	0.152
Recipient			
Age (years)	1.059	1.044-1.074	<0.001
Female gender (%)	1.351	1.031-1.772	0.029
Body mass index (kg/m2)	0.991	0.966-1.016	0.466
Diabetes mellitus (%)	0.966	0.739-1.263	0.802
Liver disease (%)			
Alcohol related disease	1.000		
Hepatitis C	1.095	0.768-1.561	0.617
Hepatitis B	0.723	0.346-1.510	0.388
NASH	1.233	0.828-1.838	0.302
PSC	1.198	0.762-1.882	0.435
PBC	1.548	1.058-2.265	0.025
AIH	0.486	0.174-1.354	0.168
Other	0.965	0.586-1.591	0.890
Biological MELD-score	0.998	0.976-1.021	0.859
Preoperative eGFR (mL/min/1.73 m2)			
>90	1.000		
60 - 90	1.598	1.192-2.144	0.002
<60	2.994	2.086-4.296	<0.001
Peri-transplant			
Red blood cell transfusions*	1.010	0.999-1.021	0.083
Early allograft dysfunction	1.102	0.852-1.426	0.459
Relaparotomy**	1.655	1.106-2.475	0.014
*Acute kidney injury****			
No AKI	1.000		
Mild AKI	1.100	0.785-1.541	0.581
Severe AKI	1.757	1.320-2.339	<0.001

* Transfusions during the transplant procedure.
** Complication requiring relaparotomy.
*** Following KDIGO criteria: mild AKI is stage 1 and severe AKI stage 2 and 3.
Auto-immune hepatitis; AKI, acute kidney injury; CI, confidence interval; DCD, donation after circulatory death; ECD, extendend donor criteria; HR, hazard ratio; MELD, model for end-stage liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

1.757; p<0.001). The impact of nephrotoxic immunosuppression was investigated as well and the median peak trough Tacrolimus levels in the five year period were evaluated. Interestingly, this level was lower in recipients who developed CKD (CKD 5.5, IQR 4.7-6.3 µg/L; no CKD 6.3, IQR 5.5-7.3 µg/L; p<0.001).

Renal recovery of recipients with severe AKI

Overall, 351 recipients (38%) experienced severe AKI in the first week after liver transplantation. Therefore, we investigated the course of kidney function in this specific group. According to the multivariable cox regression model, this group had a significant increased risk to develop CKD. In this group, 42% of the recipients developed CKD, of whom 3% severe CKD and 1% ESRD. In comparison, 32% of the recipients without severe AKI developed CKD, of whom 2% severe CKD and 1% ESRD. The recipients with severe AKI were divided into two groups: those who had a full renal recovery (eGFR >60 mL/min/1.73 m^2 on the long-term) and those who did not. **Figure 4** displays the course of long-term kidney function for these two groups and this figure highlights two factors. First, the pre-transplant kidney function was higher in recipients with a full recovery (eGFR 98 vs. 77 mL/min/1.73 m2; p<0.001). Second, recipients who did not recover their kidney function from the initial severe

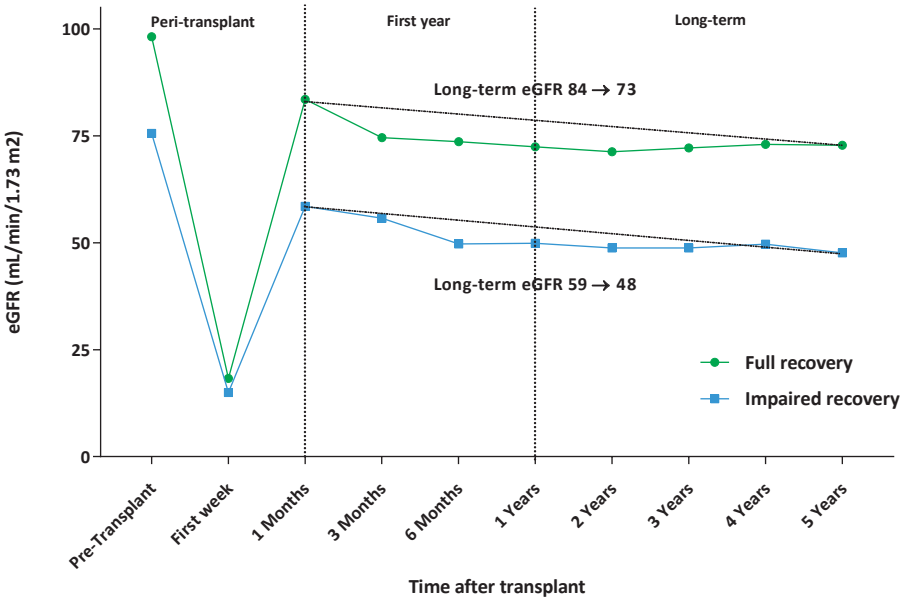

Figure 4: Recovery of kidney function in recipients with severe acute kidney injury after liver transplantation.

hit of AKI, had a similar steady decline in long-term kidney function just on a lower level when compared to recipients who demonstrated good initial kidney recovery (eGFR 84 à 73 vs. 59 à 48 mL/min/1.73 m2; p=0.541).

DISCUSSION

This study provides new insight in the course of kidney function after liver transplantation and identifies risk factors for the development of CKD. Long-term renal dysfunction was common with one third of the recipients having CKD after five years, but only a very small proportion had severe CKD or ESRD. Importantly, the use of ECD grafts was not associated with development of CKD.

In the United Kingdom, the use of marginal DBD and DCD grafts has significantly increased throughout the last decade (26). Due to the centre-based allocation of liver grafts, transplant surgeons have been able to assign high-risk organs to lower risk recipients and excellent outcomes have been achieved (27,28). With this study we confirm previous reports from our centre, where Leithead *et al* have demonstrated that the development of AKI after liver transplantation is related to DCD grafts (12,29). We created more strict criteria for marginal DBD grafts, as the standard for graft quality has been shifting from standard towards marginal grafts since these studies have been published. Even with this more strict criteria, we did not identify a direct relation between the use of ECD grafts and the development of long-term renal dysfunction and CKD was observed in around one third of recipients of standard, marginal DBD, and DCD grafts. Furthermore, none of the ECD criteria were associated with CKD in the cox proportional hazard model.

Post-transplant AKI has been linked with later CKD in previous studies. However, several definitions of AKI, CKD and ESRD were used and direct comparison of results is therefore difficult. Although the large nationwide US study from Ojo *et al* has shown that AKI, including the need for RRT, is associated with later-onset severe CKD and ESRD, this study was performed prior to the implementation of the MELD-allocation and renal sparing immunosuppression regimens (4). Several more recent studies confirmed the relation between postoperative AKI and CKD (30,31). Wadei *et al* showed that recipients who developed EAD after liver transplantation more frequently experienced AKI and later progressed to ESRD one year after the procedure (32). However, almost half of the recipients in this study with ESRD already required RRT prior to liver transplantation, a group which we have excluded in our studied cohort.

Although there is a link between the use of ECD grafts and the post-transplant AKI and AKI is a risk factor for the development of CKD after liver transplantation, no direct relation between the use of these grafts and CKD has been observed in either this study or previous studies. As shown in our previous studies, severe AKI (KDIGO stage 2 and 3) has the most significant impact on long-term graft and patient survival rates (19,29). In the present study, we highlighted the importance of the severity of AKI and development of CKD. Recipients with only mild AKI (KDIGO stage 1) did not have an increased risk to CKD, while recipients with severe AKI had a 1.8-fold increased risk. We therefore suggest that ECD grafts are not an independent risk factor for CKD, but doctors should be cautious in recipients who develop severe post-transplant AKI, as these recipients are more likely to have renal dysfunction on the long-term. Therefore, it is important to identify recipients who will not recover from the initial renal hit. Recovery of kidney function after AKI with RRT has been previously investigated by Souza *et al* (1). However, in this study more than one third of recipients required RRT prior to liver transplantation and the authors did not assess long-term kidney function. Our study is the first investigating the recovery of kidney function after severe post-transplant AKI, in a population without pre-transplant RRT. As expected, the preoperative kidney function was better in recipients who had a full recovery of kidney function after liver transplantation. Interestingly, recipients who did not have a full kidney function recovery from the severe hit of AKI, had a similar gradual decline in kidney function, just on a lower level when compared to recipients who demonstrated good initial kidney recovery. These results suggest that recovery of kidney function after severe AKI depends on the preoperative kidney function and the initial recovery in the first weeks after liver transplantation.

The following recipient risk factors were associated with CKD after liver transplantation according to the multivariable cox regression model: older and female recipients and those with an impaired preoperative kidney function. Such parameters are known risk factors for CKD in general and have also been identified previously in liver transplant recipients (4,7,33). Primary biliary cirrhosis as indication was also linked to development of CKD in this model, which has not been shown in previous studies. The majority of our recipients had long-term immunosuppression with Tacrolimus and interestingly, the Tacrolimus levels were significantly lower in recipients with CKD. However, similar results were observed in previous studies, where authors did not identify a correlation between Tacrolimus levels and long-term renal outcomes (32,34). Corman *et al* studied the course of kidney function in recipients treated with Tacrolimus-based immunosuppression and parallel our findings, describing only a modest yet constant decline in eGFR over time (33). This is presumably explained by

the adjustments made to Tacrolimus dosages in recipients with known renal impairment.

Despite the large cohort of transplant recipients, this single-centre study has several limitations. First, the retrospective design, which limits the analysis of treatment changes over time by the physicians, including modifications in the immunosuppression. Second, recipients with RRT prior to liver transplantation were excluded from the analysis. Importantly, such exclusion of recipients with impaired kidney function lead to a transparent assessment of the solitary impact of graft quality on kidney function, which appears important because ECD grafts are more frequently used today. (28). Third, the median follow-up of recipients was 4.5 years. Further studies may therefore analyse the long-term renal function more than ten years after liver transplantation.

In conclusion, there is no direct relation between the use of ECD grafts and the development of CKD after liver transplantation. The development of CKD is the result of multiple recipient risk factors and the occurrence of severe post-transplant AKI, regardless of graft type. Importantly, kidney function after severe AKI depends on the ability to recover within the first months after liver transplantation and there is only a very slight decline in renal function on the long-term. It is therefore important to engage in renal protective strategies in the first postoperative weeks to prevent further renal injury on the long-term.

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Chapter 11

Summary, discussion and future perspectives

SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

The number of liver transplantations performed globally keeps increasing every year (1). However, due to expansion of the donor pool with grafts from older donors, steatotic grafts, and DCD grafts, the quality of the average liver allograft is at risk. Therefore, transplant surgeons worldwide are trying to find thresholds to use these marginal grafts depending on their risk of postoperative complications, need for retransplantation and recipient mortality. In this thesis we have focused (I) on the additional morbidity and risk assessment in DCD liver transplantation and (II) the increasing development of renal complications due to the use of such DCD and other marginal grafts.

Comparing outcomes & risk assessment in DCD liver transplantation

Chapter 2 provides a summary on the evolving use of (Maastricht type III) DCD liver grafts. Due to the additional warm ischemia in the donor, these grafts experience more hepatic IRI (2). Hence, DCD grafts are retrieved with a 'super rapid' surgical technique, speeding up the process to get the liver into the ice box for transport to the recipient (3). Nonetheless, the use of DCD grafts is associated with an increased incidence of specific complications, such as PNF, ITBL and AKI (4–6). This leads to impaired graft and patient survival rates, with previous studies showing mixed results in comparison to DBD grafts (7–11). To limit the risk, most centres have adopted selection criteria for the donors and recipients of DCD grafts, such as a maximum for age and BMI for the donor and relatively low recipient MELD-scores. Several centres have reported their DCD experience using propensity score matching. When DCD grafts were matched to their DBD counterparts by the donor and recipient risk factors, the outcomes of the DCD grafts proved worse compared to the unmatched studies (12–15). The results of these studies reveals the 'real' additional risk of DCD grafts and highlights the importance of careful donor and recipient selection when DCD grafts are used.

Previous studies evaluating the outcomes in DCD liver transplantation mainly assessed patient and graft survival or the development of specific complications, such as ITBL. However, the experience of the patient depends on the sum of all postoperative complications. Until recently, there has not been a method to comprehend all these events and therefore Clavien and colleagues from the Zurich University Hospital have developed the *Comprehensive Complication Index* (16,17). This novel tool combines all postoperative complications according to their Clavien-Dindo into one number, which can be used for comparison of outcomes between groups

(18). In **Chapter 3** we present our comparison study of recipient outcomes after liver transplantation with DCD and DBD grafts using the CCI. Interestingly, the CCI was comparable for recipients in both groups during hospital admission. Yet, after six months DCD recipients had a significantly higher CCI. Recipients of DCD grafts also required more retransplantations in the first six months, but long-term patient survival was comparable for both groups. Recipient BMI, duration of recipient WIT and DCD grafts were identified as risk factors for a complicated postoperative course (CCI >60 after six months). Our CCI >60 threshold has recently been tested in a Canadian transplant cohort of DCD, DBD and living donor grafts (19). Similar to our results, recipients of DCD grafts did not have a higher CCI after hospital discharge, but unfortunately the comparison was not repeated after six months in their study. Benchmarking is the next attempt to improve risk assessment in liver transplantation by the Zurich team. In a multicentre outcome analysis with >2000 low-risk cases, they defined a benchmark for liver transplantation recipients (20). The authors set the Benchmark cut-off for the CCI after six months at 37.2 points, clearly lower than in our DCD cohort (53.4 points). Bearing in mind, this benchmark study consists of recipients with the lowest estimated risks and we feel that it is essential to develop such a benchmark for DCD liver transplantation separately, to determine the best achievable results in this particular group of recipients.

In this context, we aimed to identify the specific period of DWIT that is responsible for the additional hepatic IRI in DCD grafts. Therefore, we analysed the course of the agonal phase during DWIT in **Chapter 4**. There is a wide variance in the duration of the hypoxic and hypotensive agonal phase between donors. In most countries, hypotension (SBP <50 mm Hg) is considered as the start of functional DWIT (21,22). However, based on clinical experience, we hypothesized hypoxia (SpO2 <80%) plays a more important role in the onset of warm ischemia in DCD donors. Our results showed that SpO2 dropped below the threshold after two minutes, compared to nine minutes for blood pressure, resulting in a longer hypoxic agonal phase. Only the length of this hypoxic phase was associated with severity of hepatic IRI, displayed by the peak transaminase levels after the transplant. Furthermore, recipients receiving a DCD graft with a hypoxic agonal phase of more than 13 minutes had more complications assessed with the CCI and 90-day and long-term graft loss. With this study, we are the first highlighting the importance of the early-onset hypoxia during DWIT. There is not much known about the exact pathophysiology of the hepatocyte injury during the agonal phase, but the pathophysiology of hypoxic hepatitis has a similar pattern. Up to 10% of the critically ill patients in ICU with cardiac/respiratory failure or septic shock have clinical signs of hypoxic hepatitis (23). Previously, hypotension was

considered the major contributor to the hepatic injury, but recent studies suggest that hemodynamic mechanisms of hypoxia, such as hypoxemia, dysoxia and hepatic congestion play a more important role, while shock is only present in half of the patients with hypoxic hepatitis (24). It will likely require intensive animal studies with a specific hypoxic and hypotensive agonal phase models to fully understand the pathogenesis of ischemia during this period in DCD donation.

To assist the transplant surgeons in matching the appropriate DCD donor and recipient, we have developed a new prediction model for graft loss in **Chapter 5**: the UK DCD Risk Score. Using the UK national database with more than 1000 DCD liver transplants we calculated this score that consists of the seven strongest predictors (functional DWIT, cold ischemia time, MELD-score, recipient and donor age, donor BMI and retransplantation). The UK DCD risk score had a better predictive value (C-statistic of 0.79) than the known DCD prediction scores from UCLA and King's College Hospital and the new score was validated in a large UNOS-database cohort and the local DCD population in of the Queen Elizabeth Hospital in Birmingham (25,26). The score was divided into three classes: low risk (0-5 points), high risk (6-10 points), and futile (11-27 points) and the new score significantly predicted graft loss caused by PNF or ITBL. The UK DCD Risk Score is easily calculated at the time of liver acceptance and therefore has a great potential to improve the decision making in DCD liver transplantation. Furthermore, by stratifying the risk into three groups, we suggest which donor/recipient combinations will not require additional graft treatment with machine perfusion (low risk), when graft treatment is recommended (high risk), and when the graft should be declined (futile), if no adequate machine perfusion is available.

Balance of Risk & machine perfusion for DCD grafts

There is a substantial difference in the approach to DCD liver transplantation between the US and European countries, like the UK and the Netherlands. The percentage DCD grafts of the total deceased donor liver transplants increased significantly to 24% and 30% in 2016 in the UK and the Netherlands, respectively (27,28). In contrast, the total DCD liver transplants in US only increased from 5% in 2006 to 6% in 2016 (29). This is likely the result of the use of more extended criteria DCD donors (older / higher BMI) in the two European countries (21). Furthermore, the UK has a centre allocation for DCD grafts, which enables the transplant surgeon to match DCD grafts to the appropriate recipient (30). Recently, there have been more efforts in US to balance the risk between donor and recipient, so more DCD grafts can be considered for transplantation (31).

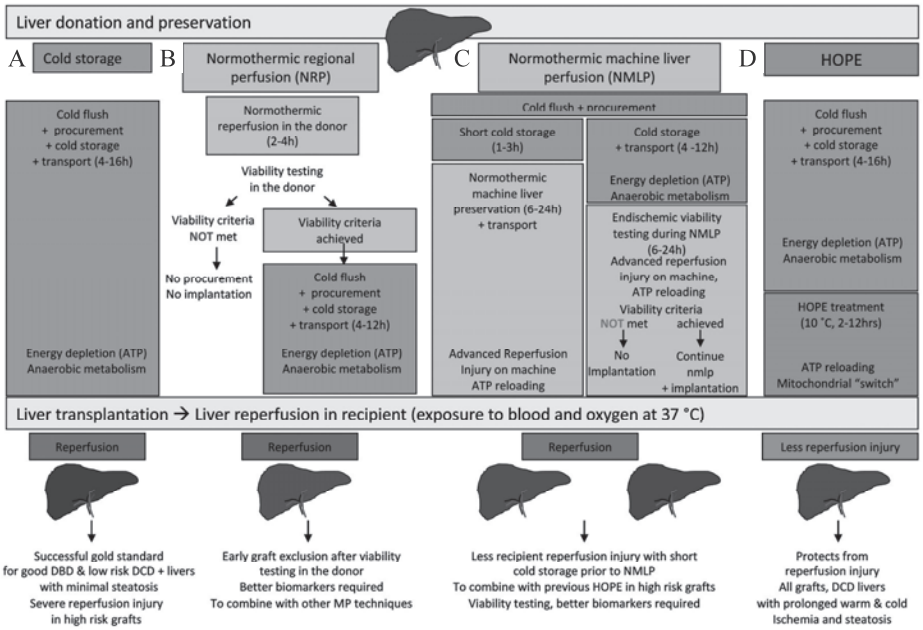

Figure 1 – Mechanism of protection and injury through the three machine perfusion techniques
From Schlegel et al, Minerva Anestesiologica, 2018 (32).

Due to the marginality of DCD grafts, these organs are one of the main targets of machine perfusion in liver transplantation. Several machine perfusion techniques have been developed over the last years and three are currently tested in the clinical setting (**Figure 1**): (I) normothermic regional perfusion (NRP) in the donor, (II) and the ex-vivo techniques normothermic machine perfusion (NMP), and (III) hypothermic oxygenated machine perfusion (HOPE) (32). During NRP in Maastricht type III donors, an abdominal regional circuit is set up for perfusion through cannulation of the aorta/iliac artery and cross-clamp of the thoracic aorta with the regular super-rapid technique. An additional venous catheter for return of the fluid is inserted in the inferior vena cava. In-situ perfusion follows with donor blood, approximately for 2-3 hours (33). NRP was first introduced in Spain in uncontrolled (Maastricht type II) DCD donors to resuscitate the liver after the cardiac arrest prior to procurement (34). In the series from Barcelona, the one-year graft survival was satisfactory with 73%, but the utilisation of these grafts was only 12% (35). The first reports of NRP in Maastricht type III donors from Spain and the UK showed better results with 80-90% one-year graft survival rates (33,36). However, only 40-50% of the NRP grafts in these series were transplanted, so the assessment of graft function during NRP needs refinement to improve utilisation of these grafts without increasing the risk. In the first

report of NMP in humans (n=20), the grafts were connected to the machine directly after procurement, reducing the period of cold storage to a minimum, where after the grafts are perfused for 10-15 hours (37). The perfused grafts (of whom 4 DCDs) showed lower peak serum transaminase levels compared to matched cold-stored grafts, but no comparable graft survival rates after one-year. A similar Canadian study in a combined DBD/DCD cohort confirmed safety and feasibility of this technique, but did no clear benefit compared to static cold storage (38). Recently, the Consortium for Organ Preservation in Europe presented the results of their randomized controlled trial (39). The most noteworthy outcome of this trial is the lower discard rate of the machine perfused grafts, probably due to surgeon bias of a working organ on a machine, compared to simple cold storage, making a more confident call to use the organ. Also in this study, the peak serum transaminase levels were lower in the perfused group with subsequent lower EAD rates. However, the incidence of the more clinically significant biliary complications and graft and patient survival were similar in the machine perfused and non-machine perfused grafts. To date, there are no reports evaluating the outcomes of NMP-perfused DCD grafts separately.

Both NRP and NMP preservation techniques are relatively labour intensive and logistically challenging, as they require transport of additional equipment and staff to join the organ retrieval team. The end-ischemic HOPE technique is less demanding as the organ can be retrieved in the regular way and will be connected to the machine in the recipient transplant centre and perfused with oxygenated perfusion fluid for 2-3 hours. The Zurich University hospital has the largest experience with DCD grafts using HOPE, as a 10-minute 'no touch' period in DCD donation is imposed by the Swiss national law. This group has shown that the early outcomes of liver function of HOPE-perfused DCD grafts were comparable or better than their matched DBD counterparts from the same centre (40). Furthermore, HOPE has shown to be effective in reducing ITBL, compared to a matched cohort of unperfused DCD grafts from two other European centres, including Erasmus MC (41). The transplant group from Groningen published their initial experience with HOPE last year and the first results from this centre also indicate that HOPE is successful in reducing biliary strictures in DCD grafts (42,43). A multicentre trial comparing the outcomes of HOPE perfused DCD grafts with static cold storage has therefore been initiated in the Netherlands and is currently recruiting patients (NCT02584283).

The kidneys at risk after liver transplantation

Renal complications after liver transplantation have become a more serious issue over the last years due to (I) the 'sickest first' allocation of patients on the waiting list,

favouring patients with renal failure and (II) the expanding use of marginal grafts that have more severe hepatic IRI causing additional kidney damage in the early postoperative period (44–46). In **Chapter 6** we summarized the donor, recipient and surgical risk factors for AKI with a focus on the pathogenesis of the impact of graft quality on this complication. Postoperative AKI is associated with increased use of hospital resources and costs, graft loss and mortality (47–50) and recipients with AKI are at risk for developing CKD, especially when they require temporary RRT (51). Similar to AKI, post-transplant CKD has a multifactorial origin and is explained by the three-hit model (52). The first hit for the kidney is subsequent to the liver disease in patients with cirrhosis. Peri-operative events, such as extensive blood loss and reperfusion during the transplant procedure and postoperative complications are responsible for the second hit and finally, the third hit is a chronic process after liver transplant, due to the use of nephrotoxic immunosuppression and new-onset diseases, such as DM.

In **Chapter 7** we analysed the impact of the postreperfusion syndrome on development and severity of AKI after liver transplantation. PRS is the first manifestation of severe hepatic IRI after reperfusion and has previously been linked to severe renal failure with RRT and an increased mortality risk (53). Our findings show that the decrease in blood pressure after reperfusion has a linear relation with both the severity of hepatic IRI and postoperative AKI. Furthermore, if recipients experienced PRS, the odds of developing AKI showed a more than two-fold increase and long-term patient survival decreased significantly with the severity of AKI. Our results were recently confirmed in a large living-donor liver transplantation cohort from Korea (54,55). PRS can be used as an early warning sign for other problems in the early postoperative course and it is being used in the assessment of early graft function in machine perfusion as well (33,39). In addition, biomarkers that are expressed in case of severe reperfusion injury have been linked to postoperative AKI, highlighting the significance of PRS in the development of renal problems after liver transplantation (56,57).

To further explore the comprehensive impact of all the warm ischemia periods in DCD liver transplantation, we introduced a new period of warm ischemia time in **Chapter 8**; the combined WIT. This is the sum of the agonal phase and asystolic phase during DWIT and the recipient WIT. We evaluated the length of this combined WIT and the incidence of severity of AKI after DCD liver transplantation in the two cohort of the Erasmus MC, Rotterdam and the Queen Elizabeth Hospital in Birmingham. In both centres, the duration of combined WIT was associated with severity of AKI and recipients receiving a graft with more than one hour of combined WIT had

a more than two-fold increase in the risk of developing severe AKI. In addition, our results confirmed that recipients with the most severe form of AKI had the worst postoperative outcomes with longer hospital admission and higher retransplantation and mortality rates. The newly defined period of combined WIT could be useful in assessing the risk for other postoperative complications with the use of DCD grafts, such as PNF and ITBL.

Early identification of recipients at risk for post-transplant AKI is required for prevention of this serious complication and we aimed to develop a new prediction model for severe AKI. We choose severe AKI as the endpoint for this score, as this has the strongest relation with other recipient outcomes, described in our previous studies. We feel that the score is of most clinical use if it is available directly at the end of the transplant, so that preventive strategies can be undertaken to minimize further renal damage. The new AKI Prediction Score is presented in **Chapter 9**. Using the well-known Framingham Risk Scheme, we developed an easy-to-use prediction model, consisting of five donor, graft and recipient factors: Donor and recipient BMI, use of a DCD graft, FFP transfusion requirements during the transplant and the duration graft implantation. We identified three risk groups, to stratify the recipients with a low, intermediate and high risk for postoperative severe AKI. In case of an intermediate risk, we suggest a renal sparing immunosuppression protocol is considered (58,59). Early initiation of RRT has been proven to be effective in critically ill patients and therefore we would consider early RRT in high-risk recipients, according to the AKI Prediction Score (60). These potential helpful clinical applications make the new score unique. This is not the first prediction model for post-transplant AKI, but the previous scores did all use postoperative risk factors, including the use of nephrotoxic immunosuppression and postoperative inotrope requirements (61,62).

A logical consequence of the pre-transplant kidney problems in patients with ESLD and AKI in the peri-operative period is development of long-term renal impairment. On average, after 5 years 2% of the recipients is either RRT dependent or has received a kidney transplant. This number can increase up to 11% of the recipients who are still alive after 25 years (63). The evolving use of marginal grafts over the last years and the increasing burden of post-transplant AKI encouraged us to evaluate the impact of marginal grafts and development of CKD (46). In **Chapter 10** we show that recipients of marginal grafts (DCD grafts and marginal DBD grafts [long cold storage, higher BMI, older donors]) do not have an increased risk to develop CKD per se and the incidence of CKD is around 40% for recipients of standard, marginal DBD, and DCD grafts. However, those who experience severe AKI after the trans-

plant and require RRT have a significantly impaired long-term kidney function. Our results showed that recipients with RRT that had an additional complication requiring a reoperation in the early postoperative period were less like to have a full recovery of kidney function on the long-term.

General conclusion

In **Part I** of this thesis we present the extra morbidity for recipients of DCD grafts with the novel CCI, identify prolonged hypoxia during DCD donation as the driving force of hepatic ischemia and present a new risk score to assist the transplant surgeon in making the decision for the best DCD graft and recipient combination and to help in deciding which graft will require additional therapy, e.g. machine perfusion. The results presented in **Part II** of this thesis highlight the impact of DCD grafts and other marginal grafts on postoperative AKI. Although no direct correlation was observed between these grafts and long-term kidney function, development of severe AKI with RRT-requirement should be avoided at all costs, as those recipients have an increased risk for CKD. Our new AKI Prediction Score could be useful to limit the risk for severe AKI and subsequent development of chronic renal impairment.

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Chapter 12

Dutch summary /
Nederlandse samenvatting

AFKORTINGEN

ANF	Acuut nierfalen
BMI	Body mass index
CCI	Comprehensive Complication Index
CNF	Chronisch nierfalen
DBD	Donation after brain death; donatie na hersendood
DCD	Donation after circulatory death; donatie na circulatoire dood
DWIT	Donor warme ischemietijd
HOPE	Hypotherme geoxygeneerde machineperfusie
IC	Ischemische cholangiopathy
IRS	Ischemie / reperfusie schade
MELD	Model for end-stage liver disease; model voor eind stadium leverfalen
NMP	Normotherme machineperfusie
NRP	Normotherme regionale perfusie
PNF	Primaire non-functie
PRS	Postreperfusiesyndroom
UNOS	United network for organ sharing
WIT	Warme ischemietijd

SAMENVATTING, DISCUSSIE EN TOEKOMSTPERSPECTIEVEN

Wereldwijd worden er jaarlijks meer levertransplantaties verricht (1). Desalniettemin, door de uitbreiding van de donor pool met veelal grafts van oudere donoren, steatotische grafts en "donation after circulatory death" (DCD) grafts, is de kwaliteit van de gemiddelde donorlever niet gegarandeerd. Daarom zoeken transplantatiechirurgen en hepatologen wereldwijd naar de limieten om deze organen te gebruiken, aan de hand van hun risico op postoperatieve complicaties, retransplantatie en mortaliteit. In dit proefschrift hebben wij de focus gelegd op (I) de extra morbiditeit en risicoinschatting in DCD levertransplantatie en (II) de toename in renale complicaties door het gebruik van deze DCD en andere marginale donorlevers.

Risicoanalyse in DCD levertransplantatie

Hoofdstuk 2 dienst als samenvatting over het toenemende gebruik van (Maastricht type III) DCD donorlevers. Door de extra warme ischemietijd in de donor, hebben deze organen meer last van lever ischemie/reperfusie schade (IRS) (2). DCD grafts worden daarom uitgenomen met de 'super-rapid' chirurgische techniek, om de lever sneller naar de ontvanger te kunnen transporteren (3). Desondanks is het gebruik van DCD levers geassocieerd met een hogere incidentie van specifieke complicaties, zoals primaire non-functie (PNF), ischemische cholangiopathie (IC) en acuut nierfalen (ANF) (4–6). Dit leidt tot een slechtere overleving van de graft en de patiënt. Eerdere studies die de resultaten van DCD en 'donation after brain death' (DBD) grafts onderzochten hebben uiteenlopende resultaten laten zien (7–11). Om het risico te minimaliseren, hanteren de meeste centra selectiecriteria voor de donoren en ontvangers van DCD grafts, zoals een maximum voor de leeftijd en body mass index (BMI) voor de donor en relatief lage Model for End-stage Liver Disease (MELD)-scores van de ontvanger. Enkele centra hebben hun uitkomsten gerapporteerd door middel van 'propensity score matching'. Na het matchen van DCD grafts met hun DBD tegenhangers aan de hand van donor en ontvanger risicofactoren, lijken de uitkomsten van DCD grafts slechter dan in studies zonder matching (12–15). Deze studies brengen echter wel het 'echte' additionele risico van deze grafts aan het licht en benadrukken het belang van selectie van donor en ontvanger met het gebruik van deze organen.

Eerdere studies naar de uitkomsten van DCD levertransplantatie hebben met name overleving en specifieke complicaties zoals IC, geanalyseerd. Voor de patiënt zijn echter alle postoperatieve problemen en complicaties van belang. Daarom hebben Clavien *et al* van het Zurich Universiteitsziekenhuis een nieuwe methode ontwikkeld

om alle postoperatieve complicaties samen te voegen: De Comprehensive Complication Index (CCI) (16,17). Dit nieuwe instrument combineert alle complicaties (aan de hand van de Clavien-Dindo classification) tot één getal, wat gemakkelijk gebruikt kan worden om de uitkomsten tussen groepen te vergelijken (18). In **Hoofdstuk 3** presenteren wij een vergelijke studie naar de uitkomsten van de patiënten na levertransplantatie met DCD en DBD organen, aan de hand van de CCI. De eerste meting aan het einde van de ziekenhuisopname liet vergelijkbare resultaten zien in beide groepen. Echter, tegenovergestelde uitkomsten werden geobserveerd na een half jaar, waar DCD ontvangers een significant hogere CCI hadden. Zij hadden ook een vaker behoefte aan een retransplantatie, maar de lange termijn overleving voor deze patiënten was gelijk aan DBD ontvangers. Daarnaast waren de BMI van de ontvanger, de duur van de warme ischemietijd én het gebruik van een DCD graft geassocieerd met een gecompliceerd postoperatief beloop (CCI >60 na 6 maanden). Deze CCI >60 grens is recentelijk ook getest in een groot Canadees cohort van DCD, DBD en levende-donor levertransplantatie, waarbij de resultaten gedurende ziekenhuisopname ook geen verschillen liet zien (19). Helaas is dezelfde analyse in deze studie niet herhaald na zes maanden. De volgende stap die het team in Zürich om risico-inschatting in lever transplantatie te verbeteren is “benchmarking”. Door middel van een multicenter-uitkomstanalyse van meer dan 2.000 laag-risico patiënten, hebben zij een nieuwe benchmark geformuleerd voor patiënten die een levertransplantatie hebben ondergaan (20). De auteurs hebben de benchmark cut-off na zes maanden gezet op een CCI van 37.2, wat aanzienlijk lager is dan in ons DCD cohort (53.4 punten). Daarbij moet worden benadrukt dat deze benchmark studie bestaat uit patiënten met het laagst ingeschatte risico. Wij denken dat het essentieel is om zo’n benchmark te ontwikkelen voor DCD levertransplantatie, om de best mogelijke resultaten te voorspellen in deze specifieke groep patiënten.

De extra periode van warme ischemie; de donor warme ischemietijd (DWIT) is verantwoordelijk voor de additionele lever IRS in DCD grafts. Daarom hebben wij de duur en het verloop van de eerste periode van DWIT onderzocht in **Hoofdstuk 4**. Deze agonale fase is de periode tussen het stoppen van de behandeling van de donor en zijn of haar overlijden. Er is een wijde variantie in de duur van hypoxie en hypotensie gedurende deze periode tussen donoren. In de meeste landen wordt hypotensie (systolische bloeddruk <50 mm Hg) gezien als het begin van de functionele DWIT (de periode waarin de ischemie daadwerkelijk aanwezig is) (21,22). Echter, op basis van klinische ervaring, is onze hypothese dat hypoxie (SpO2 <80%) een belangrijkere rol speelt in de aanvang van warme ischemie in DCD donoren. Onze resultaten laten zien dat SpO2 passeerde de grens van 80% al twee minuten na het

stoppen van de behandeling, in vergelijking met negen minuten voor de grens van hypotensie, resulterend in een langere hypoxische agonale fase. Alleen de duur van deze hypoxische periode was geassocieerd met de ernst van de lever IRS, gemeten aan de hand van de peak transaminasen na levertransplantatie. Daarnaast hadden ontvangers van een DCD grafts waarbij de agonale fase langer was dan 13 minuten meer postoperatieve complicaties en een hogere kans op verlies van de graft binnen 90-dagen en op de lange termijn. Dit is de eerste studie waarin het belang van vroege en langdurige hypoxie gedurende de agonale fase wordt aangetoond. Er is maar weinig kennis over de exacte pathofysiologie van de hepatocytenschade gedurende de agonale fase, maar de pathofysiologie van hypoxische hepatitis kent eenzelfde patroon. Tot wel 10% van de ernstig zieke patiënten opgenomen op de intensive care met cardiogene/respiratoir falen of septische shock hebben klinische tekenen van hypoxische hepatitis (23). Tot op heden werd gedacht dat hypotensie de voornaamste factor is in hepatische schade, maar recente studies laten zien dat mechanismen van hypoxie, zoals hypoxemie, dysoxie en congestie van de lever een nog belangrijkere rol spelen, terwijl shock maar werd geobserveerd in de helft van de patiënten (24). Waarschijnlijk zijn intensieve observationele proefdier studies met specifieke hypoxische and hypotensieve agonale fase modellen nodig om de pathogenese van ischemie gedurende deze periode in DCD donatie te begrijpen.

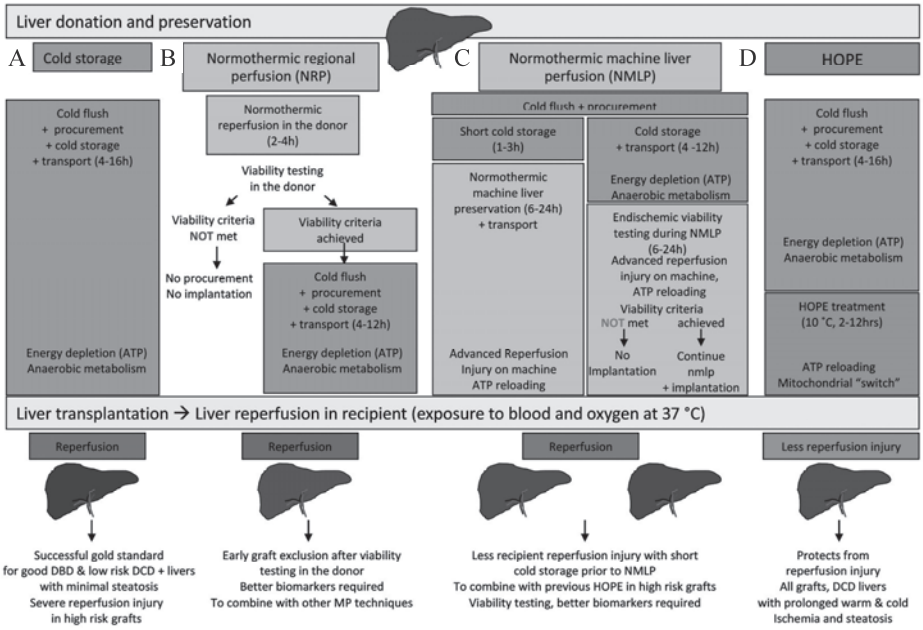
In **Hoofdstuk 5** wordt een nieuw predictiemodel gepresenteerd dat is ontwikkeld om de transplantatiechirurgen te assisteren in het matchen van DCD donoren en ontvangers: de *UK DCD Risk Score*. De nationale database van het Verenigd Koninkrijk met meer dan 1000 DCD levertransplantaties is gebruikt om dit model te ontwikkelen, dat bestaat uit de zeven sterkste voorspellers voor het verlies van de graft (duur van functionele DWIT en koude ischemietijd, MELD-score, leeftijd van de donor en ontvanger, donor BMI en retransplantatie). Onze nieuwe score heeft een betere predicatieve waarde (C-statistiek van 0.79) dan de eerder gepubliceerde scores van de University of California in Los Angeles en King's College Hospital in Londen (25,26). De score is ook gevalideerd in een groot nationaal cohort uit de Verenigde Staten (UNOS) en de lokale DCD populatie in Birmingham. Mogelijke donor/ontvanger combinaties worden ingedeeld in drie categorieën: laag risico (0-5 punten), hoog risico (6-10 punten) en futiel (>10 punten) en het aantal punten in de score is ook voorspellend voor het verlies van de graft door PNF of IC. De score is snel en gemakkelijk te berekenen tijdens de beoordeling van een orgaanaanbod en heeft daarbij een grote potentie om het beslissingsproces in DCD levertransplantatie te verbeteren. Daarnaast kan met behulp van de drie risicogroepen worden bepaald of de graft waarschijnlijk geen behandeling met machineperfusie nodig heeft (laag

risico), machineperfusie wordt aangeraden (hoog risico) of de graft moet worden afgewezen als er geen adequate machineperfusie beschikbaar is (futiel).

Risicoverdeling en machineperfusie voor DCD grafts

Er is een substantieel verschil in de benadering van DCD levertransplantatie tussen de Verenigde Staten en de Europese landen, zoals het Verenigd Koninkrijk en Nederland. Het percentage DCD grafts van de totale levertransplantaties met postmortale donoren is de laatste jaren toegenomen tot 24% in het Verenigd Koninkrijk en 30% in Nederland in 2016 (27,28). Daarentegen, in de Verenigde Staten is het aantal DCD levertransplantaties gelijk gebleven op een laag niveau van 5% in 2006 tot 6% in 2016 (29). Dit is waarschijnlijk het resultaat van het gebruik van meer *extended criteria* DCD donoren (hogere leeftijd / hoger BMI) in de Europese landen (21). In het Verenigd Koninkrijk heeft men daarnaast ook nog de zogenoemde centrumallocatie voor DCD grafts, wat de chirurgen de mogelijkheid geeft om DCD grafts te matchen met een geschikte ontvanger (30). Recentelijk zijn er ook in de Verenigde Staten inspanningen gedaan om het risico tussen de donor en ontvanger te verdelen, zodat meer DCD grafts gebruikt kunnen worden voor transplantatie (31).

DCD grafts zijn één van de belangrijkste doelwitten voor machineperfusie in levertransplantatie en verschillende technieken zijn over de laatste jaren ontwikkeld, waarvan er momenteel drie in de klinische praktijk worden gebruikt (**Figuur 1**): (I) normotherme regionale perfusie (NRP) in de donor en de ex-vivo technieken (II) normotherme machine perfusie (NMP) en hypotherme geoxygeneerde machineperfusie (HOPE) (32). Gedurende NRP in Maastricht type III donoren wordt een abdominaal regionaal circuit aangelegd voor perfusie door cannulatie van de aorta/arteria iliaca communis en cross-clamping van de thoracale aorta met de reguliere super-rapid uitnametechniek. Daarnaast wordt een extra katheter ingebracht in de vena cava inferior voor de teruggave van bloed, gevolgd door 2-3 uur in-situ perfusie (33). NRP werd als eerste geïntroduceerd in Spanje in ongecontroleerde (Maastricht type II) DCD donoren om de organen te resusciteren na de hartstilstand voor de uitname van de organen (34). De uitkomsten van de eerste serie uit Barcelona waren bevredigend met een één jaars-overleving van de DCD grafts van 73%. Dit was wel met een zéér strenge selectie, want maar 12% van de geperfundeerde grafts werd gebruikt (35). De eerste resultaten van NRP in Maastricht type III donoren vanuit Spanje en het Verenigd Koninkrijk lieten betere resultaten zien met 80-90% één jaars-graftoverleving (33,36). Maar ook hier werd maar 40-50% van de grafts gebruikt, dus het is noodzakelijk dat de beoordeling van graftfunctie tijdens NRP wordt verfijnd om de utilisatie van DCD grafts te uit te breiden zonder het risico voor



Figuur 1 – Schade en protectiemechanismen in de drie machine perfusietechnieken
Uit Schlegel et al, Minerva Anestesiologica, 2018 (32).

de patiënt te vergroten. In de eerste studie met NMP in mensen (n=20) werden de grafts direct na de uitname aan de machine verbonden, zodat de koude ischemietijd wordt gereduceerd tot een minimum, waarna de grafts voor 10-15 uur worden geperfundeerd (37). De grafts die machineperfusie ondergingen (waarvan 4 DCD's) hadden een lagere piek serum transaminase, in vergelijking met grafts die op ijs werden getransporteerd. Er werd geen verschil geobserveerd in graftsoverleving na één jaar. Een vergelijkbare Canadese studie in een gecombineerd DCD/DBD cohort bevestigde de haalbaarheid en veiligheid van deze techniek, maar wederom werd geen duidelijk voordeel gevonden ten opzichte van opslag van de lever op ijs (38). Het *Consortium for Organ Preservation in Europe* heeft onlangs de resultaten van zijn gerandomiseerde studie gepubliceerd (39). De meest opmerkelijke uitkomst van deze studie was het lagere afwijzingspercentage in de machine groep, wat mogelijk het resultaat is van chirurg-gerelateerde bias van een 'werkend' orgaan op de machine, in vergelijking met simpele opslag op ijs, om een meer zekere beslissing te nemen het orgaan te gebruiken. Ook in deze studie was de postoperatieve transaminase piek lager in de machineperfusie groep, net als de incidentie van *early allograft dysfunction*, maar ook hier werd geen verschil gezien in de overleving van

graft en patiënt op de lange termijn. Tot op heden zijn er geen studies die het effect van NMP specifiek in DCD grafts hebben geanalyseerd.

NRP en NMP zijn beiden arbeidsintensieve en logistiek veeleisende preservatietechnieken, omdat extra spullen en personeel nodig zijn voor de uitname. De eind-ischemische HOPE techniek is minder veeleisend, omdat de graft volgens de normale procedure kan worden uitgenomen en getransporteerd en pas bij aankomst in het transplantatiecentrum wordt aangesloten op de machine voor 2-3 uur perfusie. Het Zürich Universiteitsziekenhuis heeft de meeste ervaring met HOPE voor DCD grafts, omdat er een langere *no-touch* periode is van 10 minuten, opgelegd bij door de Zwitserse wet. De eerste HOPE-DCD grafts hadden vergelijkbare of zelfs betere graftfunctie als vergelijkbare DBD grafts van hun centrum (40). Daarnaast zijn er ook aanwijzingen dat HOPE effectief is tegen de ontwikkeling van IC in een studie met vergelijkbare niet geperfundeerde DCD grafts van twee andere Europese centra, waaronder het Erasmus MC (41). De transplantatiegroep uit Groningen heeft hun eerste ervaring met HOPE (specifiek D-HOPE, waarbij de lever wordt geperfundeerde door de vena portae en de arteria hepatica) en hun eerste resultaten zijn ook positief over de potentie van deze perfusietechniek om biliaire stricturen te reduceren (42,43). Daaropvolgend is een multi-centrum gerandomiseerde studie geïnitieerd in Nederland, waarin de uitkomsten van HOPE worden vergeleken met standaard opslag op ijs voor DCD grafts en de *D-HOPE* studie is momenteel patiënten aan het rekruteren (NCT02584283).

Het risico voor de nieren na levertransplantatie

Renale complicaties na levertransplantatie komen steeds meer voor door (I) de *zieksteeerst* allocatie van patiënten op de wachtlijst voor een levertransplantatie, waarbij patiënten met nierfalen prioriteit krijgen en (II) het de uitbreiding van het gebruik van marginale grafts met meer lever IRS leidend tot extra nier schade in de vroege postoperatieve fase (44,45). **Hoofdstuk 6** dienst als samenvatting van de donor, ontvanger en chirurgische risicofactoren voor ANF na levertransplantatie met een focus op de impact van graftkwaliteit op het ontstaan van deze complicatie. Postoperatief ANF is geassocieerd met een toename in het gebruik van ziekenhuismiddelen en kosten, retransplantatie en mortaliteit (46–49). Patiënten met ANF hebben daarnaast ook een ook verhoogd risico op het ontwikkelen van chronisch nierfalen (CNF), met name als zij dialyse-behoeftig zijn in de eerste periode na levertransplantatie (50). CNF na levertransplantatie is het resultaat van verschillende risicofactoren en wordt vaak geanalyseerd volgens het *drie-factoren* model (51). De eerste schade aan de nieren vindt al plaats voor de transplantatie, aangezien veel patiënten met

gedecompenseerde cirrose ook nierfalen hebben. Peri-operatieve gebeurtenissen, zoals extensief bloedverlies en reperfusieschade gedurende de transplantatie en postoperatieve complicaties zijn verantwoordelijk voor de tweede *hit* aan de nieren. De laatste schade komt door langdurige blootstelling aan nefrotoxische immunosuppressie en het ontstaan van nieuwe chronische ziektes, zoals diabetes mellitus.

In **Hoofdstuk 7** hebben wij de impact geanalyseerd van het postreperfusiesyndroom (PRS) op de ontwikkeling en ernst van ANF na levertransplantatie. PRS is het eerste teken van ernstige lever IRS na reperfusie en is in het verleden al geassocieerd met postoperatieve dialysebehoefte en een verhoogd mortaliteitsrisico (52). Onze bevindingen laten zien dat de mate van bloeddrukdaling na reperfusie een lineaire relatie heeft met de ernst van lever IRS en postoperatief ANF. Bovendien, patiënten met PRS hadden meer dan twee keer zoveel kans om ANF te ontwikkelen en de patiënt overleving was ook slechter voor patiënten met ernstig ANF. Onze resultaten zijn recentelijk bevestigd in een groot levende donor cohort uit Zuid-Korea (53,54). PRS kan worden gebruikt als vroeg waarschuwingssignaal voor andere potentiele problemen na de transplantatie en PRS wordt ook regelmatig gebruikt in de beoordeling van vroege graftfunctie in machineperfusie (33,39). In aanvulling hierop, biomarkers die tot uiting komen bij ernstige lever IRS zijn geassocieerd met postoperatief ANF en benadrukken hiermee het belang van PRS in de ontwikkeling van renale complicaties na levertransplantatie (55,56).

In **Hoofdstuk 8** wordt een nieuwe periode van warme ischemietijd in DCD levertransplantatie gepresenteerd om de invloed van alle warme ischemieperiodes te analyseren: de gecombineerde warme ischemietijd (gecombineerde WIT). Dit is de som van de agonale and asystolische fase gedurende DWIT en de implantatie warme ischemietijd. We hebben de duur van deze gecombineerde WIT en de ernst van ANF na DCD levertransplantatie geëvalueerd in de twee cohorten van het Erasmus MC in Rotterdam en het Queen Elizabeth ziekenhuis in Birmingham. In beide centra was de duur van gecombineerde WIT gerelateerd aan de ernst van ANF en patiënten waarbij deze periode langer dan 60 minuten duurde hadden vaker ANF. Daarnaast bevestigden onze resultaten ook dat patiënten met ernstig ANF (KDIGO criteria graad 2/3) slechtere postoperatieve uitkomsten hadden, zoals een langere opnameduur en een hoger retransplantatie- en mortaliteitsrisico. De nieuwe gecombineerde WIT zou ook gebruikt kunnen worden om het risico voor andere complicaties van DCD grafts te evalueren, zoals PNF en IC.

Vroege identificatie van patiënten met een verhoogd risico op post-transplantatie ANF is essentieel om deze complicatie te voorkomen. Dit motiveerde ons om een predictiemodel te ontwikkelen om het risico voor ANF voor iedere patiënt te voorspellen. We hebben ernstig ANF gekozen als uitkomstmaat, omdat deze de sterkste relatie heeft met andere postoperatieve uitkomsten in eerdere studies. De score heeft de meeste klinische waarde als hij direct beschikbaar is aan het einde van de transplantatieprocedure, zodat preventieve maatregelen kunnen worden genomen om verdere schade aan de nieren te voorkomen. De ontwikkeling van deze nieuwe *AKI Prediction Score* wordt gepresenteerd in **Hoofdstuk 9**. Met gebruik van de welbekende *Framingham* risicomodellen hebben we een gemakkelijk te gebruiken model ontwikkeld met vijf risicofactoren: BMI van de donor en ontvanger, transplantatie met een DCD graft, transfusie met plasma gedurende de procedure en de duur van de implantatie. Drie risicogroepen zijn geïdentificeerd om patiënten in te delen met een laag, gemiddeld en hoog risico op ANF. Bij een gemiddeld risico adviseren wij een niersparend immunosuppressie protocol waarbij calcineurin inhibitoren in de eerste dagen na levertransplantatie wordt gemeden (57,58). Daarbij is vroege dialyse effectief gebleken in het verminderen van de mortaliteit in ernstig zieke patiënten op de IC en daarom zou vroege dialyse overwogen kunnen worden in patiënten met een hoog risico, aan de hand van de *AKI Prediction Score* (59). Deze potentieel nuttige toepassingen van de score maken de *AKI Prediction Score* uniek. Het is niet de eerste predictiemodel voor AKI, maar eerdere scores maakten allen gebruik van postoperatieve risicofactoren, waaronder het gebruik van immunosuppressie (60,61).

Een logische consequentie van pre-transplantatie nierproblemen en ANF in de perioperatieve periode is de ontwikkeling van CNF. Vijf jaar na de levertransplantatie zijn gemiddeld 2% van de patiënten dialysebehoeftig of hebben een niertransplantatie ondergaan. Dit getal loopt op tot 11% van de patiënten die nog in leven zijn 25 jaar na transplantatie (62). Het toenemende gebruik van marginale grafts gedurende de laatste jaren met meer postoperatief ANF als gevolg, was voor ons de reden om de invloed van deze grafts op CNF te onderzoeken (63). In **Hoofdstuk 10** tonen wij dat patiënten die een marginale graft ontvangen (DCD grafts en DBD grafts van oudere of obese donoren of een lange koude ischemietijd), niet direct een verhoogd risico hebben op CNF en de incidentie van CNF na vijf jaar was 40% voor ontvangers van standaard, marginale DBD en DCD grafts. Echter, patiënten die ernstig ANF met dialysebehoefte hadden na de transplantatie hadden wel een significant verminderde nierfunctie op de lange termijn. Met name patiënten met dialyse na de transplantatie die nog een andere complicatie krijgen, hebben een groter risico om geen volledig herstel van hun nierfunctie te krijgen op de lange termijn.

Algemene conclusie

In **Deel I** van dit manuscript wordt de extra morbiditeit voor patiënten die een DCD graft ontvangen geanalyseerd met de nieuwe CCI, langdurige hypoxie gedurende DCD donatie geïdentificeerd als de drijvende kracht voor lever ischemie en introduceren wij een nieuwe score om de transplantatiechirurg te assisteren in het beslissingsproces voor de beste combinatie van DCD donor en ontvanger. De bevindingen gepresenteerd in **Deel II** benadrukken de invloed van DCD en andere marginale grafts op de ontwikkeling van postoperatief ANF. Ondanks dat er geen directe relatie werd geobserveerd tussen deze levers en lange termijn nierfunctie, ernstig ANF met dialysebehoefte zou ten allen tijden voorkomen moeten worden, omdat deze patiënten een verhoogd risico hebben op nierfunctiestoornissen op de lange termijn. Onze nieuwe AKI Prediction Score kan nuttig zijn in het identificeren en limiteren van ernstig ANF en bijkomende ontwikkeling van CNF.

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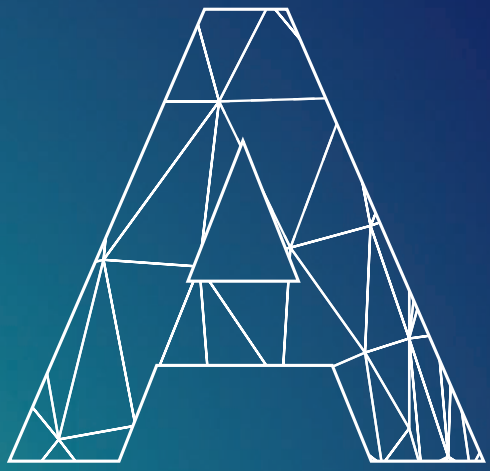

Appendices

List of Publications
PhD Portfolio
Dankwoord / Acknowledgements
About the Author

LIST OF PUBLICATIONS

In this thesis

Kalisvaart M*, Haan J de*, Polak W, Gommers D, Metselaar H, IJzermans J, Jonge de J. Onset of donor warm ischemia time in DCD liver transplantation: hypotension or hypoxia?
Liver Transpl. 2018;24(8):1001-1010.
*Authors contributed equally.

Schlegel A, Kalisvaart M, Scalera I, Laing RW, Mergental H, Mirza DF, et al. The UK DCD Risk Score: A new proposal to define futility in donation-after-circulatory-death liver transplantation. J Hepatol. 2018;68(3):456–64.

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Kalisvaart M, Schlegel A, Trivedi P, Roberts K, Mirza D, Perera T, et al. Chronic kidney disease after liver transplantation: can we safely use extended criteria grafts? Submitted.

Kalisvaart M, Schlegel A, Umbro I, De Haan J, Polak W, IJzermans J, et al. The AKI Prediction Score: A new prediction model for acute kidney injury after liver transplantation. Submitted.

Other publications

Originals

Marcon F, Schlegel AA, Bartlett D, Kalisvaart M, Bishop D, Mergental H, et al. Utilisation of declined liver grafts yields comparable transplant outcomes and previous decline should not be a deterrent to graft use. Transplantation. 2018;102(5):1.

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PHD PORTFOLIO

Name PhD Student	Marit Kalisvaart
Department	Erasmus MC: Department of Surgery
	Queen Elizabeth Hospital, Birmingham, UK: Liver Unit
PhD Period	January 2015 – November 2018
Promotor	Professor JNM IJzermans
Supervisors	Dr J de Jonge
	Professor P Muiesan

PhD training	Year	ECTS
Courses		
Basic course in liver transplantation (ELITA)	2016	2
(Inter)national oral presentations & attending conferences		
Joined congress of the British and Dutch Transplantation Society	2015	2
Biannual congress of the European Society of Organ Transplantation	2015	4
Annual congress of the Dutch Transplantation Society	2016	4
Annual congress of the International Liver Transplantation Society	2016	2
Annual congress of the British Transplantation Society	2017	2
Annual congress of the International Liver Transplantation Society	2017	2
Biannual congress of the European Society of Organ Transplantation	2017	6
Annual congress of the International Liver Transplantation Society	2018	2
Biannual congress of the Transplantation Society	2018	4
Biannual congress of the International HepatoPancreatoBiliary Assocation	2018	4
8th International Meeting on Transplantation from DCD	2018	2
Teaching		
Anaesthetic governance meeting (Liver Unit, Queen Elizabeth Hospital)	2017	2
Supervising medical student Virginia Aijtink (Erasmus MC)	2017	2
Supervising medical student Damian Broadhurst (Queen Elizabeth Hospital)	2017-18	2
Other		
Reviewer activities: • Abstract reviewer for annual congress of the International Liver Transplantation Society • Scientific journals: American Journal of Transplantation, Liver Transplantation, Transplantation, Transplant International, HPB, Clinical Transplantation	2017-18	4
Trainee member of the Vanguard Committee of the International Liver Transplantation Society	2018	2
Total		48

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ABOUT THE AUTHOR

Marit Kalisvaart was born in 1988 and spent most of her childhood in the village of Prinsenbeek, in the south of the Netherlands. She started her medical studies in 2008 and graduated in 2015 at the Erasmus University Medical Center in Rotterdam. Marit then combined a senior house office job in General Surgery at the Maasstad Hospital (supervision Mr RA Klaassen) with a PhD Project in Liver Transplantation at the Erasmus University Medical Center in Rotterdam (supervision Prof JNM IJzermans / Dr J de Jonge). She presented the results of the study in Chapter 3 in this thesis on the annual meeting of the European Surgical Association in Bucharest in May 2017. In September 2016 she moved to Birmingham, UK to expand her PhD research under the supervision of Prof P Muiesan at the Liver Unit of the Queen Elizabeth Hospital and gain clinical experience in HPB Surgery & Liver Transplantation. The research presented in this thesis is the result of the collaboration between the Department of Surgery of the Erasmus University Medical Center and the Liver Unit of the Queen Elizabeth Hospital. In January 2019 she will continue her surgical career as a registrar (oder Arztassistentin) in General Surgery in the University Hospital Zurich in Switzerland under the supervision of Prof P.-A. Clavien and Dr S. Käser.