The challenge of quality assessment and regional perfusion to increase donor organ utilisation

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Chapter 1

General introduction and outline of the thesis

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Organ Donation

Organ transplantation has been one of the most impressive achievements in modern clinical medicine. Due to its success, an increasing number of patients is waitlisted for transplantation whilst the gap between the demand and the number of available donor organs is widening.[1,2] As a result of this donor shortage, many transplant centres are accepting organs from older and higher risk donors for transplantation, including those obtained from extended criteria donors (ECD) and donation after circulatory death (DCD) donors.

Extended Criteria Donors

Although organs that are considered to be extended criteria donor organs may seem a well-defined entity, in reality, the definition and criteria vary between the type of donor organ. As regards the kidney, ECDs are brain-dead donors and defined according to the UNOS (United Network for Organ Sharing) to include donors either over the age of 60 years, or older than 50 years but with at least two of the following donor medical risk factors: a history of hypertension, a cerebrovascular accident as the cause of death, a serum creatinine (SCr) >1.5 mg/dL (132 μmol/L) prior to organ retrieval.[3,4] In donation after brain death (DBD) donors - including higher risk ECDs - following the diagnosis of brain death, donor management will continue and support haemodynamic and metabolic stability. The perfusion of all donor organs remains optimised until the circulation stops and the organs are retrieved. As a consequence, in DBD donors, in the presence of perfusion and absence of hypoxia, warm ischaemic time of donor organs does not occur.

ECDs are widely used in kidney transplantation. Transplantation of kidneys from these donors is often associated with delayed graft function (DGF) posttransplant and a decrease in graft survival when compared with grafts from standard criteria donors.[5-8] In liver transplantation several risk models have been introduced, predominantly based on donor and recipient factors, to predict the outcome after transplantation.[9] To identify a liver graft as higher risk or ECD, a variety of donor factors including demographic characteristics, aspects of donor management, liver function tests, and the presence of steatosis are used.[10-13] Transplantation of these higher risk liver grafts is associated with early allograft dysfunction (EAD), primary non (and never) function (PNF), and an increased complication rate.[11,14-18]

Donation after circulatory death donors

Prior to the brain death laws in de mid 70s all organ transplants using deceased donors were obtained from the so-called non-heart-beating donors (NHBD).[19] NHBD was renamed as donation after cardiac death and then modified to donation after

circulatory death (DCD) as the circulation has stopped but the heart is not 'dead'.[20] DCD donation was classified for the first time during an international workshop in Maastricht in the Netherlands in 1995 by Kootstra et al.[21] establishing the four categories of the 'Non Heart Beating Donor (NHBD) Maastricht Classification'. On several occasions modifications of this classification of DCD donors have been described.[22] During the 6th International DCD Conference in Paris in 2013, consensus about definitions and terminology was reached as regards DCD and published.[23] **Table 1** illustrates the main five categories. In the Netherlands, all types of DCD donors are permitted, although there is currently no active protocol for types I, II and IV DCD donation.

DCD donors are an important resource to effectively increase the numbers of donor organs. Ideally, this donor type will add extra organs for transplantation to the pool of donor organs. In 2010, in the Netherlands, 32% of deceased donors were DCD donors and this increased to 60% in 2020.[2,24] In the United Kingdom, the number of DCD donors increased over the last decade from 336 donors in 2009-2010 to 634 in 2019-2020, representing 40% of all deceased donors.[25,26]

In DCD donation a patient's neurological condition and prognosis is considered to be irreversible and further medical treatment futile. Therefore, following consent from the family or legal guardian, life sustaining therapy is withdrawn, and the patient is extubated. This will result in a respiratory arrest followed by a circulatory arrest and the death of the patient. The time from extubation, initiating a period of hypoxia in the donor organs, to circulatory arrest can vary per patient. Therefore, times of warm ischaemia may vary between DCD donors. Furthermore, the duration of the no-touch period, defined as the time between circulatory arrest and the determination of (neurological) death, varies widely across countries (ranging from 5 to 30 minutes) contributing to the warm ischaemia time.[27] Despite that consent has been given for organ donation, in most countries any activities towards the retrieval and preservation of the donor organs (e.g., pre-mortem administration of substances or cannulation) are prohibited and only legally permitted after the declaration of death.[27]

In DCD donation the period of donor total warm ischaemia time, defined as the time between withdrawal of life sustaining support and the start of cold perfusion during retrieval, is inevitable. During this warm ischaemia time, hypoxia and hypotension followed by the circulatory arrest in the donor will result in ischaemic injury of the organs. However, the exact amount of additional damage to the organs is unknown and this makes the donor a 'black box' since the extent of the injury will only become apparent after transplantation in the recipient. Results after transplantation of higher risk DCD donor organs using strict selection criteria are acceptable but remain

associated with poorer initial graft function[17,18,28,29] and organ specific complications when compared to standard criteria donors.[30-32] Due to the concerns regarding the quality of the DCD donor organs, the decision whether to accept or decline a donor organ at time of offering is more difficult for the clinician and probably many DCD donor organs are discarded as they are perceived as "not transplantable". Registry data reveal that DCD procedures are associated with significantly lower organ recovery rates per donor than in DBD donors.[27,33,34] To reduce uncertainty and increase utilisation, improved assessment of organ viability and optimisation of preservation strategies are required.

Table 1. Modified Maastricht Classification for DCD donors.[23]

Category I		
Uncontrolled	IA. Out-of-hospital IB. In-hospital	Found dead due to a sudden unexpected circulatory arrest without any attempt of resuscitation in the out-of-hospital or in-hospital setting
Category II		
Uncontrolled	IIA. Out-of-hospital IB. In-hospital	Witnessed circulatory arrest with unsuccessful resuscitation, including the addition of the location
Category III		
Controlled	III	Ventilated patients awaiting circulatory arrest where the withdrawal of life sustaining therapy is planned
Category IV		
Uncontrolled Controlled	IV	Sudden (or unexpected) circulatory arrest after declaration of brain death (uDCD IV) or when the law does not permit the declaration of brain death (e.g., Japan or China) a donation after brain death donor is followed by controlled circulatory arrest (cDCD IV)
Category V		
Controlled	V	Euthanasia or medically assisted cardiocirculatory death

Donor Organ Assessment

To date, the decision to accept or decline a donor organ is based on organ function prior to admission and during donor management as well as considering known risk factors in donor and/or recipient and the macroscopic appearance of the graft. Clinical expertise and the more subjective perception of risk also play an important role. A more cautious attitude of clinicians towards accepting higher risk organs may reduce the incidence of poor graft survival in recipients on one hand, however, on the other hand it will further compromise the donor pool and as such contribute to donor organ shortage. As clinical decisions about suitability and perceived risk of donor organs carry a high degree of subjectivity, there is a need for evidence and clinically relevant markers to help the decision to accept the organ at time of offering.

The National Institutes of Health Biomarkers Definitions Working Group has defined a biomarker as follows: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". During the different phases of transplantation, from donor management to donor organ retrieval and eventually transplantation in the recipient, an ideal biomarker should function as a useful tool to rapidly, accurately, and objectively assess the viability and quality of the organ.

Over the past years, multi-analyte 'omics' technologies including proteomics and metabolomics have been increasingly used to identify novel biomarkers in transplantation, and this field of research is constantly evolving.[35-41] In proteomics research, the complete set of proteins (proteome) in a biological sample is analysed. Individual proteins are separated from complex mixtures, quantified, characterised, and finally identified. Metabolomic research involves the high-throughput measurement and dynamic analysis of low molecular mass cellular products (i.e., metabolites) in the metabolome. Proteomics and metabolomics allow for the identification of molecular signatures and can be used to generate a diagnostic fingerprint. As such, prognostic biomarkers are of interest as they may predict posttransplant outcomes. These biomarkers can be analysed during management in the donor but also when a graft is perfused after retrieval on a dedicated device prior to transplantation (see Machine Preservation of Abdominal Organs below). In both situations, it allows for assessment of the organ and to quantify the amount of sustained injury present in the organ.

Another exciting and emerging field of research is based on DNA methylation. The epigenetic process of the methylation of cytosine adjacent to guanine (CpG sites) results in cell type specific gene regulation and as such functions as a fundamental mark of cell identity.[42] As DNA methylation patterns can be used to identify cell death in specific tissues, it may be considered as an highly interesting method to unravel mechanisms of injury and repair in organ donation and transplantation.[43-45]

Machine Preservation of Abdominal Organs

In the past 50 years the predominant method of organ preservation when bridging from the donor hospital to the recipient transplant centre has been a simple form of Static Cold Storage (SCS).[46] To maintain viability, donor organs are flushed after retrieval with a cold preservation solution, subsequently submerged in the same preservation solution in a plastic bag and stored on melting ice in a box. Hypothermia has been one of the basic principles in organ preservation. Aiming at a core temperature of the donor organ of 2-5°C, the cellular metabolism is decreased and this in turn slows

down the deterioration of the organ significantly. SCS is a simple way to preserve, store, and transport transplantable organs and it sufficed for many years whilst the average donor was young and without too much co-morbidity prior to admission.

Whilst ECD and DCD organs are accepted due to a shortage of better quality organs, the transplant community realises that optimising organ preservation strategies has become an absolute necessity aiming to better protect vulnerable grafts and enhance organ quality and function.[47,48]

The historic concept of dynamic organ preservation, was first developed in the 1930's by Carrel and Lindbergh.[49,50] The group of Belzer and Starzl further established hypothermic dynamic preservation in clinical kidney transplantation in the late 1960's.[46,51] During the past decade and in the context of developments in miniaturised pump technologies, novel portable and more user friendly devices were developed. Dynamic preservation techniques are increasingly used in clinical practice [52-54] and described in more detail below. **Figure 1** demonstrates the different organ preservation strategies used in clinical practice from donor to recipient.

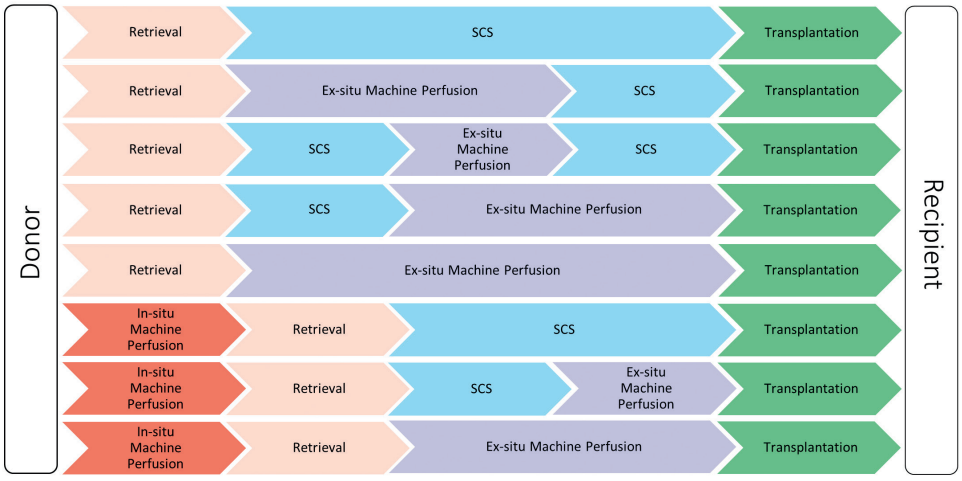

Figure 1. An overview of the several possibilities in organ preservation from donor to recipient currently used in clinical practice. In-Situ Machine Perfusion is performed in the donor on hypo- or normothermic conditions. Ex-situ Machine Perfusion might be performed at hypothermic (0-4˚C), subnormothermic (20-25˚C) or normothermic (37˚C) temperatures.

When machine perfusion is used, in most techniques after retrieval, the donor organ is connected to a pump device (ex-situ; outside of the donor or recipient) and perfused with preservation solution. Machine perfusion can be applied in a continuous fashion

(from retrieval in the donor hospital until implantation in the recipient centre) or just for a fixed period of time (e.g., following SCS and for a few hours immediately prior to implantation in the recipient, as an end-ischaemic hypo- or normothermic perfusion).

Hypothermic (oxygenated) Machine Perfusion (HMP/HMPO$_2$)

During HMP, continuous circulation with acellular preservation solution is used at a temperature ranging between 5-10˚C. Superior function and better survival in kidney transplantation using HMP compared with SCS has been reported.[55-58] Due to the hypothermic condition, the cellular metabolic activity is decreased but not completely ceased. As such, the addition of oxygen in HMP (HMPO$_2$) to restore ATP content and reduce oxidative stress may support the organ during the aerobic activity.[59-61] The beneficial effect of the addition of oxygen in HMPO$_2$ leads to improvement of graft function when compared with standard HMP.[62] As regards the liver, HMP was found to be feasible in animal models and first tested by Guarrera et al.[63] in a clinical pilot case-controlled series in 2010. More recently, a multicentre randomised controlled trial showed that hypothermic oxygenated machine perfusion of DCD livers led to a lower risk of non-anastomotic biliary strictures (NAS) when compared to SCS.[64] Successful preservation of the human pancreas using HMP was described as feasible and safe and a clinically relevant number of viable islets could be isolated after 6 hrs of HMPO$_2$.[65-67]

Normothermic Machine Perfusion (NMP)

In NMP, the organ is either continuously or during a fixed period perfused with oxygenated blood at 37˚C. Nutrients and metabolic compounds are administered during NMP to aim for a more physiological state. During NMP, normal cellular activity is resumed which allows the organ to recover after a period of cold flush and cold storage or HMP. Normal temperature allows for better assessment and testing the viability of the organ. Another advantage of NMP is the possibility to provide targeted interventions aiming to optimise the quality of the injured organ. Using pharmacological and biological agents, or mesenchymal stem cells, repair or even regeneration in the donor organ is stimulated. Continuous NMP of the liver and NMP of the kidney have been found to be safe and feasible.[68,69] As regards the pancreas, NMP is not yet ready for clinical application and needs more refinement.[70]

Normothermic Regional perfusion (NRP)

The technique of in-situ organ perfusion during donor surgery is known as regional perfusion (RP).[71] In normothermic regional perfusion (NRP), cannulas are introduced via the iliac artery or distal aorta and connected to an extracorporeal membrane oxygenator (ECMO) to ensure the continuous perfusion of multiple organs simultaneously in the donor (in-situ) (**Figure 2**).

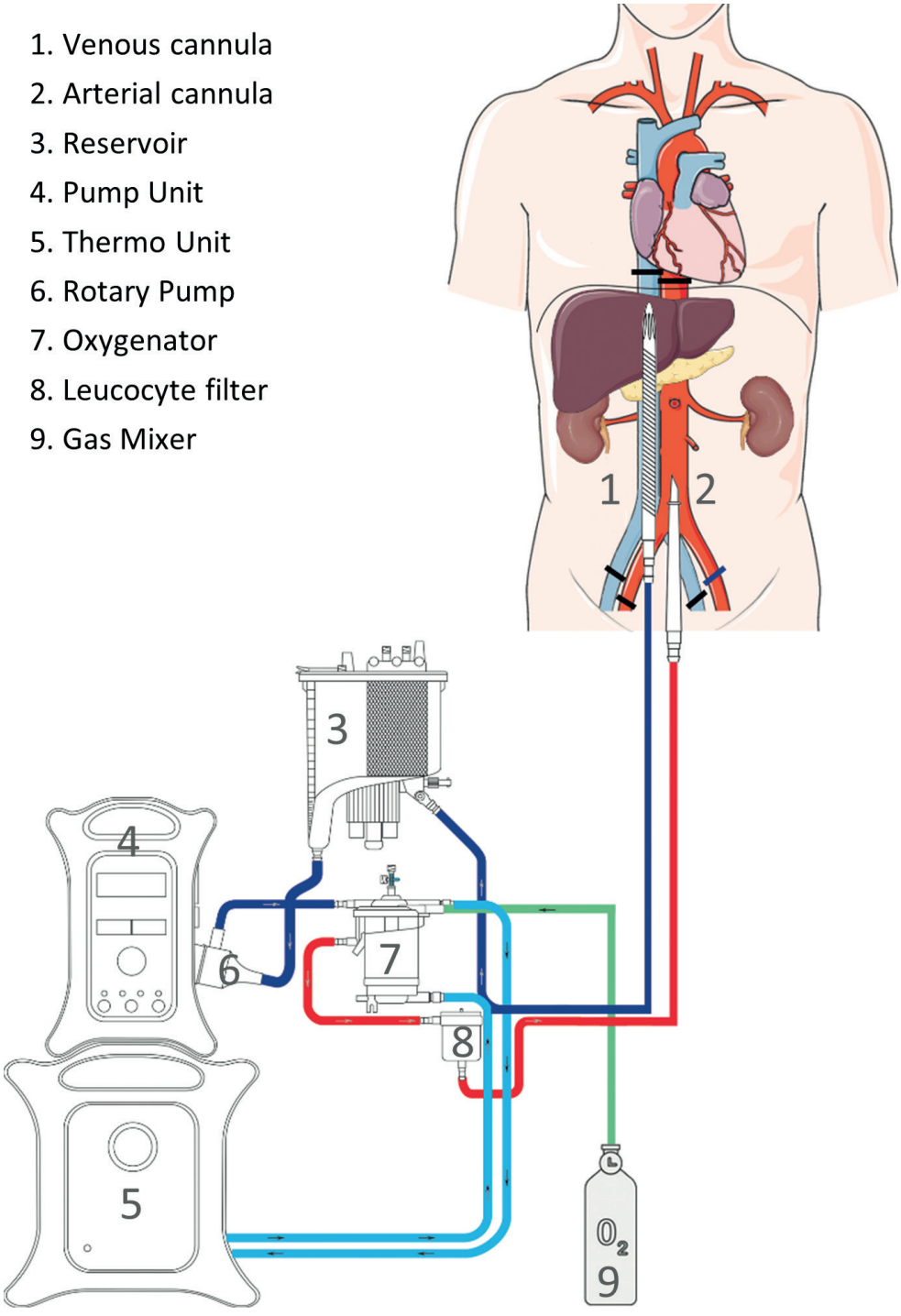

Figure 2. The technique of abdominal Normothermic Regional Perfusion (aNRP)

NRP was first pioneered in Spain in 1989. Preliminary experiments in a porcine DCD model showed that warm perfusion, using the aorta in the donor animal, was feasible and improved liver graft viability.[72,73] It was introduced in clinical practice in Belgium, China, France, Italy, the Netherlands, Norway, Portugal, Russia, Spain, Switzerland, the United Kingdom and the United States of America.

During hypothermic regional perfusion, also called 'core-cooling technique', the donor is actively cooled, and a continuous gas exchange can take place during the organ recovery rate. The oxygen consumption decreases as normal metabolic processes are reduced due to the efficient cooling.[74-76] Despite the theoretical benefits for its use, the literature for this technique in clinical setting is scarce and therefore not described in further detail here.[77,78]

NRP can be applied for abdominal organs (i.e., liver, pancreas, and kidneys) only (aNRP), but also in combination with heart and lungs in the thoracic compartment (TA-NRP). During NRP, a normothermic oxygenated circulation is restored in the donor resulting in recommencing cellular metabolism. This allows for actual assessment of the donor organ viability as biochemical analysis of blood samples can be obtained in a non-invasive way from the circuit. As such, the 'black box' of DCD becomes more transparent, helping the clinician to decide whether to accept or decline the abdominal organs for transplantation. Initiation of NRP as soon as possible after circulatory arrest will reduce the extent of ischaemic injury, incurred during the dying process of the DCD donor. It will also affect in a positive way the inevitable vasoconstriction.[79] Using NRP will allow to convert a 'hasty' surgical DCD procedure, where time is of essence, into a less rushed and more stable 'DBD-like' operation potentially resulting in less organ damage and increasing organ utilisation.[80-82] In addition, aNRP of liver, pancreas and kidneys in DCD donors may be more cost-effective when compared to the application of ex-situ HMP or NMP for each individual organ. However, a cost-effectiveness analysis of this technique remains to be investigated.

Thesis Outline and Rationale

The ongoing shortage of donor organs has led to acceptance by most transplant centres of older and higher risk organs to increase the donor pool in solid organ transplantation. In the General Introduction in **Chapter 1** the necessity is described to improve assessment of donor organs by identifying clinically relevant markers and establish a method of machine preservation in higher risk donors that will promote a more rapid resuscitation of organ function and better organ quality in transplantation.

In the **first part of this thesis**, we evaluate the results achieved with the current use of older and higher risk donor kidneys. **Chapter 2** focuses on the transplantation of kidneys from donors with Acute Kidney Injury (AKI) which is perceived by many transplant professionals as a relative contraindication for transplantation. As the kidney has an intrinsic capacity to recover from this abrupt deterioration of kidney function, we hypothesise that using kidneys from donors with an AKI could reduce an unnecessary discard and expand the donor pool whilst not impacting on clinical outcomes. To determine whether discarding AKI donor kidneys is justified, we investigate the effect of donor AKI on outcomes following kidney transplantation analysing large cohorts of donor-recipient combinations from the National Transplant Database in the United Kingdom hosted by NHS Blood and Transplant.

Objective criteria are lacking how to assess donor organ quality. The goal of the studies in the following chapters describes the attempt to identify clinically relevant biomarkers as well as develop an assay that can give clinicians sufficient guidance during the decision whether to accept or decline an organ for transplantation.

In **Chapter 3** we analyse blood and urine from donation after circulatory death (DCD) donors before retrieval. The donor kidneys retrieved from these donors developed either immediate function (IF), delayed graft function (DGF) or primary non-function (PNF) after transplantation. The goal in this chapter is to determine whether a molecular signature can be identified in the donor associated with each of these different clinically early outcomes using a proteomic approach.

Following the publication that Flavin Mononucleotide (FMN) release into the perfusate, during Hypothermic Machine Perfusion (HMP) of livers, predicts success or failure after liver transplantation, we engaged in a study to validate this role of FMN in kidney perfusion. In **Chapter 4** we report our analysis and results as regards the use of this marker in kidney transplantation using a large cohort of samples obtained during Hypothermic (oxygenated) Machine Perfusion ($HMPO_2$ or HMP). Fluorescence spectroscopy is used to assess fluorescence intensity in the FMN region and subsequently correlated with early and late post transplantation outcomes for the kidney. Targeted liquid chromatography mass spectrometry is applied to validate the results.

In **Chapter 5** we aim to develop a novel method to quantify the relative amount of unmethylated Insulin (*INS*)-DNA, reflecting β-cell death. Using a methylation sensitive restriction enzyme digital droplet PCR, the goal is to validate this assay on two cell lines and further extend the use of this assay by measuring the fraction of unmethylated Insulin (*INS*)-DNA, reflecting β-cell death, in different purities of islets obtained after pancreas isolation.

In the **second part of this thesis**, we focus on organ preservation strategies for abdominal organs to assess the quality of the graft.

Therefore, in **Chapters 6** and **7** we evaluate the novel organ preservation technique of abdominal normothermic regional perfusion (aNRP) that has been used in a few other countries in the past years and is now being introduced in clinical practice in the Netherlands. This method was developed to improve immediate function and outcomes from organs obtained from donation after circulatory death (DCD) donors. aNRP promises to allow a rapid resuscitation after circulatory arrest of the donor and better reconditioning prior transplantation when compared to standard preservation techniques of DCD donor organs. In **Chapter 6** we report the results of a systematic review of aNRP in DCD donors. We explore the literature focussing on the added value of aNRP when compared to standard organ perfusion techniques. In **Chapter 7** we investigate the current donor organ assessment used for the donor liver during aNRP.

Finally, in **Chapter 8**, we summarise the results of the studies in this thesis and provide a brief outlook and future perspectives.

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