

# **The physiological changes of circulatory death with respect to organ donation**

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This dissertation is submitted for the degree of Doctor of Philosophy



## **Declaration**

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text

It does not exceed the prescribed word limit for the relevant Degree Committee.

Poppy Sarah Aldam

Cambridge, January 2019

This work is dedicated to Stuart and our beautiful girls

Donation of organs after circulatory death (DCD) is re-emerging as an important source of organs for transplantation worldwide, and in the United Kingdom DCD donors comprise 39% of all deceased organ donors. However, organs from DCD organ donors work less well after transplantation than those from brainstem dead organ donors. This increased prevalence of initial poor function, despite good performance in the donor prior to death, suggests that changes in donor physiology during the agonal phase, together with the subsequent period of warm ischaemia, may be responsible for the differences seen in organ function. Although donated organs and warm ischaemia have been extensively studied, the physiological changes occurring in the DCD organ donor during the dying process remain poorly understood and ill-defined mechanistically. In this thesis, the physiology of the DCD donor between withdrawal of life supporting treatment and death is examined in detail for the first time in human donors. Extensive public and patient engagement work demonstrate public support for research in the potential organ donor, and this finding is borne out by focus group work.

Examination of a cohort of DCD donors demonstrated previously undocumented patterns of physiology, which have significant implications for the function of transplanted organs. A key finding is the lack of concordance between arterial oxygen saturations when measured by pulse oximetry and by arterial blood gas (ABG) analysis. This has demonstrated that saturation assessment by ABG analysis document oxygen saturation being above generally accepted minimal levels for up to 40 minutes longer in donors during the maximum accepted agonal period of 240 minutes. I also present evidence of cardiothoracic organ retrieval decisions based on saturations which have led to potentially transplantable organs being declined.

An investigation of markers of anaerobic metabolism in the potential donors who do proceed to DCD revealed correlations between hypotension, oxygen delivery and oxygen extraction ratio, and elevated lactate levels. Further examination of the relationship between oxygen delivery and systolic blood pressure in this cohort demonstrate that blood pressure is conserved in many patients beyond the point at which oxygen delivery falls to critical levels. This finding suggests current organ retrieval decisions based on systolic blood pressure may not be best practice or evidence based.

These physiological changes during the agonal period of circulatory death are accompanied by cognate changes in human donor biology that have not previously been documented in DCD donors. These include evidence of sympathetic stimulation (elevated catecholamine levels), activation of the hypothalamic-pituitary-adrenal axis (with cortisol levels elevated in a subgroup surviving over 30 minutes after withdrawal of life support), and immune activation (changes in IL-6 and TNF- $\alpha$  that mirror those seen in animal models of DCD donation).

In conclusion, this thesis demonstrates physiological changes not previously recorded in human subjects in a cohort of DCD organ donors undergoing circulatory death. These changes have implications for the management of potential organ donors undergoing circulatory death, and impact on the organs they donate. Modulation of these changes represent a therapeutic target, successful modulation of which could translate to improved donation rates and organ transplantation outcomes.



## Abbreviations

ABG	Arterial blood gas
ADH	Anti-diuretic hormone
APACHE	Acute Physiology and Chronic Health Evaluation
BMI	Body mass index
BTS	British Transplantation Society
CaO <sub>2</sub>	Venous oxygen content
CJD	Creutzfeldt-Jakob disease
CKD	Chronic kidney disease
CNS	Central nervous system
CVC	Central venous catheter
CvO <sub>2</sub>	Arterial oxygen content
DBD	Donation after brainstem death
DCD	Donation after circulatory death
DGF	Delayed graft function
DIC	Disseminated intravascular coagulation
DO <sub>2</sub>	Oxygen delivery
ECMO	Extra corporeal membrane oxygenation
FiO <sub>2</sub>	Fraction of inspired oxygen
GFR	Glomerular filtration rate
GMC	General Medical Council
HIV	Human immunodeficiency virus
HMGB-1	High mobility group box 1 protein
HPA	Hypothalamic pituitary axis
HRA	Health Research Authority
HTA	Human Tissue Authority
IABP	Intra-aortic balloon pump
ICNARC	Intensive care national audit and research centre
ICP	Intracranial pressure
ICU	Intensive care unit
IFN- $\gamma$	Interferon gamma
IL-1 $\beta$	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-12p70	Interleukin 12
IL-2	Interleukin 2
IL-4	Interleukin 4
MCA	Mental Capacity Act
NCCU	Neurosciences critical care unit
NHSBT	NHS Blood and Transplant
NICE	The National Institute for Health and Care Excellent
NIHR	National Institute for Health Research
NORS	National organ retrieval service
NRP	Normothermic Regional Perfusion
ODR	Organ donor register
OER	Oxygen extraction ratio

PaCO <sub>2</sub>	Arterial partial pressure of carbon dioxide
PAFC	Pulmonary artery flotation catheter
PaO <sub>2</sub>	Arterial partial pressure of oxygen
PEEP	Positive end expiratory pressure
PICC	Peripherally inserted central catheter
QUOD	Quality in organ donation initiative
REC	Research Ethics Committee
RINTAG	Research, Innovation and Novel Technology Advisory Group
SaO <sub>2</sub>	Arterial oxygen saturation
SNOD	Specialist nurse in organ donation
SOFA	Sequential organ failure assessment
SpO <sub>2</sub>	Pulse oximetry measurement of oxygen saturation
TB	Tuberculosis
TNF- $\alpha$	Tumour necrosis factor alpha
TSH	Thyroid stimulating hormone
VAD	Ventricular assist device
VO <sub>2</sub>	Oxygen consumption
WLST	Withdrawal of life supporting treatment

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## Table of Contents

<b>Title Page</b> .....	<b>1</b>
<b>Declaration</b> .....	<b>3</b>
<b>Abbreviations</b> .....	<b>7</b>
<b>Acknowledgements</b> .....	<b>9</b>
<b>Chapter 1: Introduction and literature review</b> .....	<b>15</b>
<b>1.1 Chapter overview</b> .....	<b>15</b>
<b>1.2 Organ donation in 2018</b> .....	<b>15</b>
1.2.1 Types of organ donation .....	15
1.2.2 DCD Donor statistics and demographics .....	18
<b>1.3 The history of organ donation</b> .....	<b>20</b>
<b>1.4 Organ outcomes after DCD donation</b> .....	<b>22</b>
<b>1.5 Identification and Management of the potential DCD organ donor</b> .....	<b>25</b>
1.5.1 Donor Identification .....	25
1.5.2 Consent for donation .....	28
1.5.3 Withdrawal of life supporting treatment .....	29
<b>1.6 Predicting time to asystole in the DCD donor.</b> .....	<b>30</b>
<b>1.7 Warm ischaemic time and its implications for organ donation</b> .....	<b>32</b>
1.7.1 Definitions of warm ischaemic timeframes.....	32
1.7.2 Metabolic process during warm ischaemia.....	35
<b>1.8 Physiological changes occurring in circulatory death</b> .....	<b>37</b>
1.8.1 Human studies.....	37
1.8.2 Animal models of circulatory death .....	38
1.8.3 Pathophysiological response to brainstem death .....	40
1.8.4 The deleterious effects of catecholamine excess.....	45
<b>1.9 Opportunities to modulate transplanted organ outcomes</b> .....	<b>46</b>
1.9.1 Donor optimisation .....	46
1.9.2 Targeted donor interventions .....	49
<b>1.10 Directions of travel of DCD donation</b> .....	<b>50</b>
1.10.1 Improving consent to donation rates .....	50
1.10.2 Improving optimisation of DCD donor organs .....	50
1.10.3 Development of programmes to use uncontrolled DCD donors .....	52
<b>1.11 Chapter summary and hypothesis</b> .....	<b>52</b>
<b>1.12 Thesis overview</b> .....	<b>53</b>
<b>Chapter 2: The ethical and legal considerations of research consent in the potential DCD organ donor</b> .....	<b>55</b>
<b>2.1 Introduction and chapter overview</b> .....	<b>55</b>
<b>2.2 Assessment of capacity to consent in research</b> .....	<b>56</b>
<b>2.3 Consultation of deputies regarding involvement in research.</b> .....	<b>58</b>
<b>2.4 Consideration of what constitutes best interests in the potential donor?</b> .....	<b>58</b>
<b>2.5 The organ donor register and research decisions</b> .....	<b>59</b>
2.5.1 The Organ Donor Register .....	59
2.5.2 Application of the ODR to the question of research: .....	60

2.6	Ensuring that organ donor research meets the Mental Capacity Act's requirements.	61
2.7	Research involving human tissue - The Human Tissue Act 2004	64
2.8	Chapter Summary	65
<b>Chapter 3: Public and Patient Involvement and Engagement work</b>		<b>67</b>
3.1	Chapter overview and Introduction	67
3.2	Precedent studies	68
3.3	Survey	69
3.3.1	Methods	69
3.3.2	Survey Results	71
3.4	Focus group meeting	74
3.4.1	Donor Family Network meeting	74
3.4.2	NIHR Blood and Transplant Research Unit Public and Patient engagement event	75
3.5	Chapter summary and discussion	76
<b>Chapter 4: Methods and Materials.</b>		<b>79</b>
4.1	Introduction and chapter overview	79
4.2	Patient identification	79
4.2.1	Setting	79
4.2.2	Patient identification by SNOD team	80
4.3	Inclusion, exclusion and withdrawal criteria	81
4.4	Consent process	82
4.5	Withdrawal of life supporting treatment	82
4.6	Demographic and longitudinal information collection	83
4.7	Blood sampling setup	84
4.7.1	Methods	84
4.7.2	Results	85
4.8	Blood sampling	86
4.8.1	Blood samples taken	86
4.8.2	Blood sampling schedules	86
4.9	Study flowchart	88
4.10	Analyses undertaken	88
4.10.1	Arterial and venous blood gas analysis	88
4.10.2	Cytokines profiles	89
4.10.3	Adrenaline and noradrenaline levels	89
4.10.4	Cortisol levels	90
4.10.5	Acknowledgements	90
4.11	Statistical analyses	90
4.12	Ethical approval and institutional review	91
<b>Chapter 5: Donor Demographics and physiological changes</b>		<b>93</b>
5.1	Chapter overview and introduction	93
5.2	Patient recruitment results	94
5.3	Patient Demographic Results	95
5.4	Physiological changes in proceeding donors.	100
5.4.1	Systolic Blood pressure	101
5.4.2	Oxygen saturation	102
5.4.3	Arterial and venous blood gas data	104
5.4.4	PaO <sub>2</sub>	104
5.4.5	PaCO <sub>2</sub>	105
5.4.6	Lactate	107
5.4.7	pH	108
5.4.8	Venous oxygen saturation	109

5.5	Chapter summary and discussion .....	110
<b>Chapter 6: Measurements of arterial oxygen saturation in the potential donor 113</b>		
6.1	Introduction and chapter overview .....	113
6.2	Patients.....	114
6.3	Measurement of oxygen saturations:.....	114
6.3.1	Pulse oximetry measurement of oxygen saturations (SpO <sub>2</sub> ).....	114
6.3.2	Arterial blood gas analysis measurement of oxygen saturations (SaO <sub>2</sub> ).....	115
6.4	Comparisons of Pulse oximetry saturations and arterial oxygen saturation measurements .....	116
6.5	Clinical implications of inaccuracy in saturation measurements .....	120
6.6	Chapter summary and discussion .....	123
6.6.1	Chapter summary.....	123
6.6.2	Discussion of sources of pulse oximeter inaccuracy .....	123
6.6.3	Chapter Summary and Conclusions.....	126
<b>Chapter 7: Assessing the onset of tissue hypoxia in the DCD donor..... 129</b>		
7.1	Chapter Introduction and overview .....	129
7.2	Methodology of lactate assessment .....	131
7.3	The role of hypotension in the elevation of lactate.....	132
7.4	The role of arterial and venous oxygenation.....	133
7.5	Calculation of Oxygen Extraction ratio.....	138
7.6	Chapter Summary and Discussion.....	140
<b>Chapter 8: The stress and HPA axis response to circulatory death..... 147</b>		
8.1	Chapter introduction and overview.....	147
8.2	Patient selection and sample collection.....	148
8.3	Adrenaline release during the withdrawal period in DCD organ donors .....	149
8.4	Peak adrenaline levels during the withdrawal period .....	150
8.5	Changes in adrenaline level during the withdrawal period in DCD donors .....	151
8.6	Relationship between plasma adrenaline level and lactate level .....	152
8.7	Noradrenaline release during withdrawal of life supporting treatment .....	153
8.8	Peak noradrenaline levels during the withdrawal period.....	154
8.9	Changes in noradrenaline level during the withdrawal period in DCD donors.....	155
8.10	Relationship between plasma noradrenaline level and lactate level.....	156
8.11	Cortisol release during withdrawal of life supporting treatment in the proceeding DCD donor.....	158
8.12	Chapter summary and Discussion.....	161
<b>Chapter 9: The immune response to circulatory death in the DCD donor ..... 165</b>		
9.1	Chapter Overview and Introduction .....	165
9.2	Initial Immune function in patient cohort.....	166
9.3	Temporal changes during the withdrawal period .....	169
9.4	Impact of time to death on immune system activation.....	172
9.5	Donor six – a case of immediately post brainstem death? .....	174
9.6	Chapter Summary and Discussion .....	175
<b>Chapter 10: Discussion Conclusion and Further work chapter ..... 179</b>		
10.1	Introduction.....	179
10.2	Public engagement work and consent rates .....	179
10.3	Demographic data.....	182
10.4	Physiological data .....	184
10.5	Measurement of arterial oxygen saturation. ....	187
10.6	Measurements of cardiovascular physiology during circulatory death.....	189

10.7	The stress response to cardiorespiratory death .....	195
10.8	The immune response to cardiorespiratory death .....	199
10.9	Future work .....	203
10.10	Conclusions.....	207
References.....		209

# **Chapter 1: Introduction and literature review**

## **1.1 Chapter overview**

This chapter will present the background to the thesis, setting out the hypotheses which will be investigated in subsequent chapters. The first section of the chapter provides a discussion of the current state of organ donation in 2018, with a description of Donation after Circulatory Death (DCD) and the processes it entails. This is followed by a discussion of current models used to understand the process and progress to asystole in DCD donation. I next consider the physiological changes that are known to occur in models of circulatory death and discuss the process of brainstem death as a comparator. The subsequent chapters will then focus on physiological processes of interest during circulatory death, considering the assessment of oxygenation, perfusion, activation of the autonomic nervous system and hypothalamic-pituitary axis, and activation of the immune system. This is followed by an assessment of the current knowledge base regarding predictors of transplanted organ outcomes. Finally, I will set out my principal hypotheses which will be tested in subsequent chapters.

## **1.2 Organ donation in 2018**

### **1.2.1 Types of organ donation**

Organ donation is defined as the process of surgically removing an organ or tissue from an individual, with the intention of integrating it into another individual. Organs can be donated either from patients who have died (deceased organ donation) or, in the case of selected organs, from living donors. This thesis will focus solely on organs from deceased donors.

Deceased organ donation may occur in one of two ways: Firstly, a patient may be examined clinically and declared brainstem dead by neurological criteria (Academy of Medical Royal Colleges 2008). This is known as donation after Brainstem Death (DBD), although has previously been referred to as 'Heart-beating donation' in the literature. Alternatively, a patient can undergo withdrawal of life supporting treatment and subsequently donate their

organs once their heart has stopped beating and they are certified dead by the absence of a circulation. This is known as Donation after Circulatory Death (DCD), although was previously referred to as 'non-heart-beating donation' and 'donation after cardiac death', the latter being changed when heart transplants were performed from DCD donors.

The diagnosis of brainstem death in a patient requires certain prerequisites to be met, to ensure metabolic and circulatory derangements are not contributing to the patient's comatose condition. In the United States, the Harvard code of practice sets a standard for 'whole brain' death (Wijdicks 2001) which requires ancillary testing in the form of cerebral angiography or similar alongside clinical criteria to confirm death. The United Kingdom criteria are based upon the principle that the key elements required for life – the capacity for consciousness and the loss of brainstem function, including the ability to breathe (Academy of the Medical Royal Colleges 2008) – can be satisfied through clinical examination of brainstem reflexes alone. Upon completion of brainstem death criteria and consent for organ donation, a process of donor optimisation can be undertaken to try to maximise the quality and number of organs donated (NHSBT Donor optimisation extended care bundle 2014). This process aims to minimise the effects the hormonal and metabolic derangements commonly seen after brainstem death. Retrieval of organs for transplantation can proceed once the criteria for brainstem death have been fulfilled and appropriate consent for organ donation undertaken.

There are two types of DCD donation – controlled and uncontrolled. These categories and the clinical circumstances in which they arise were first summarised in the Maastricht classification of DCD donors (Kootstra et al 1995), which was revised in 2012 (Detry et al 2012) and is summarised in table 1 below:

Maastricht Category	DCD type	Context	Subcategories
1	Uncontrolled	Dead upon arrival	1a. Circulatory death outside hospital with no witness. 1b. Circulatory death outside hospital with witnesses and rapid resuscitation attempt
2	Uncontrolled	Unsuccessful resuscitation attempt	2a. Unexpected circulatory death in Intensive care patient 2b. Unexpected circulatory death in hospital Emergency Department or ward with witnesses and rapid resuscitation attempt
3	Controlled	Circulatory arrest following planned withdrawal of life supporting care	3a. Expected circulatory death in Intensive Care Unit 3b. Expected circulatory death after withdrawal of care. Withdrawal phase >30mins 3c. Expected circulatory death after withdrawal of care. Withdrawal phase <30mins
4	Either	Circulatory arrest in a patient declared brainstem dead	4a. Unexpected circulatory arrest in a brainstem dead donor in Intensive Care Unit. 4b. Expected circulatory arrest in a brainstem dead donor
5	Controlled	Euthanasia	5a. Medically assisted circulatory death in hospital setting 5b. Medically assisted circulatory death in operating theatre

*Table 1.1: The Maastricht classification of donation after circulatory death. Adapted from Detry et al 2012.*

In the United Kingdom today only controlled DCD donation (Maastricht 3 and 4 donors) are considered for donation. Attempts to re-establish programmes to retrieve organs from Maastricht 2 category donors have been unsuccessful due to logistic issues, although there was a successful programme in Leicester before the millennium. Successful programmes do exist in Spain which operates a presumed consent for donation system (Sanchez-Fructuoso et al 2006) and in France (Fieux et al 2006). The French system described by Fieux et al reported a 90% one year graft success rate for kidneys from Maastricht 2 donors, although acknowledges a yield of only 31 transplantable kidneys from 122 emergency mobilisations of surgical teams. Only Belgium and The Netherlands have an established programme for Maastricht 5 donors (Bollen et al 2016). These countries with well-established DCD donation programmes could be considered the exception rather than the rule – worldwide many

nations have little or no DCD donation and have the potential to benefit hugely from this source of donated organs (Matesanz et al 2016).

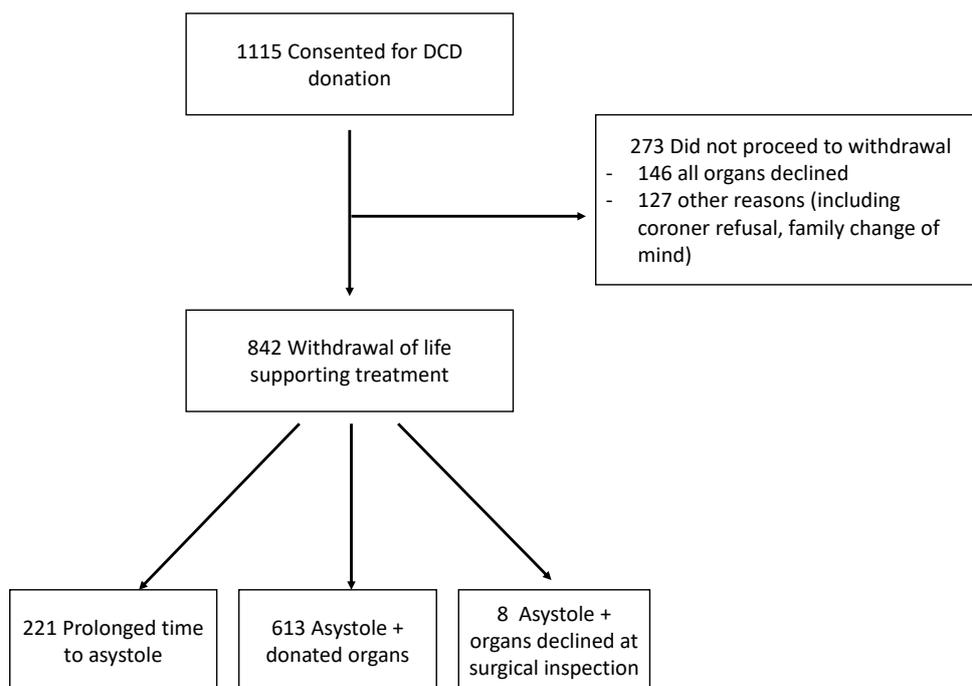
In the United Kingdom, a typical DCD organ donor is a patient who has suffered a devastating intracranial injury from which there is no chance of recovery to a quality of life which the patient would find acceptable. They are reliant on invasive treatment provided by the intensive care unit, but their injury falls short of rendering them brainstem dead. A joint decision is made between the treating clinician and the next of kin that ongoing treatment is not in the patient's best interest, and a plan is made for life-supporting treatment to be withdrawn and death to be allowed. This is a Maastricht 3 controlled DCD donation. Consideration should be given to the patient's prior expressed wishes regarding organ donation as part of end of life planning (GMC guidance 2010). Should the patient have expressed a prior desire to donate their organs in the event of their death this can then be facilitated. Treatment is withdrawn in a monitored location with a surgical team scrubbed and waiting in a nearby operating theatre. Should asystole occur within 3 or 4 hours, organs can subsequently be retrieved for transplantation; beyond that time the surgeons stand down and return to base. Guidance from the Academy of Medical Royal Colleges states that death can be diagnosed based upon the permanent absence of respiration and circulation. In practice, this is confirmed after 5 minutes of continuous mechanical asystole, as judged by lack of a pulsatile output on arterial pressure monitoring.

### **1.2.2 DCD Donor statistics and demographics**

In the year 2017/2018 there were 619 DCD donors within the United Kingdom, each donating an average of 2.7 organs per donor. This represents a 6% overall increase in DCD donors from the 2016/17 year. There was a 13% increase in kidney transplants from DCD donors, DCD heart transplants increased 92% from 13 grafts in 2016/17 to 25 in 2017/18. DCD lung transplant activity also increased from 27 to 36 transplants from DCD donors between 2016/17 and 2017/18. There was a marginal decrease in DCD liver transplant activity to 200 grafts in 2017/18 from 208 grafts the previous year (NHSBT Annual activity report 2018).

In the same period, consent was gained for DCD donation from 1115 adult potential donors, of which 613 proceeded to donate their organs, representing a 53.3% donation rate for DCD donors after consent. Of the 502 donors who did not go on to donate their organs, the main reasons were a prolonged time to asystole in 221 donors (44.0% of non-proceeding donors). For 146 potential donors (29.0% of non-proceeding donors) all the organs were declined before treatment was withdrawn (see figure 1 below).

Of those 842 potential donors who underwent withdrawal of life supporting treatment, 221 donors did not donate due to prolonged time to asystole, representing 26.2% of this group and 19.8% of consent donors (NHSBT potential donor audit 2017/18). This information is represented in a flowchart below.



*Figure 1.1: Flowchart to represent outcomes for 1115 donors consented to organ donation in 2017/2018.*

As part of the national and international drive to expand the organ donor pool, DCD donor demographics have changed substantially over the last 10 years. NHS Blood and Transplant (NHSBT) reports donation and transplantation activity annually, the results of which demonstrate that DCD donors are becoming older, with an increasing BMI and more medical co-morbidities. The 2017/18 Annual Activity Report stated that DCD donors had a mean age

of 53 years and a mean BMI 27kg/m<sup>2</sup>. Data for 2008/9 demonstrated that 20% of DCD donors were aged over 60 years and 28% had a BMI above 30kg/m<sup>2</sup>. In contrast, in 2017/18, 36% of DCD donors were over 60 and 30% had a BMI over 30kg/m<sup>2</sup> (NHSBT 2017/18 Annual report). This expansion in the donor pool by considering DCD donors who would previously have been thought of as unsuitable has, without doubt, led to increased numbers of transplanted organs. Early results DCD renal transplants suggest that these organs may function as well, certainly in the short to medium term, as organs from brain dead donors (Palkoci et al 2018, Summers 2010) which provides validation for the consideration and utilisation of this cohort of donors.

### **1.3 The history of organ donation**

The surgical technique of vascular anastomosis, for which Carrel was awarded the 1912 Nobel Prize (Sade 2005) underpins the ability to technically perform an organ transplantation. In the early 1900s, interest arose in the concept of treating renal failure with transplantation of porcine kidneys (Hume et al 1955). This was first attempted by Jaboulay in 1906, and while the surgical technique was successful, the patient died (Morris 2004). Renal transplantation remained a focus of interest, with renal failure being a common and untreatable pathology. The alternative treatment of haemodialysis would not be pioneered for another 40 years. Early attempts at renal transplants from deceased human donors were made in the 1930s, but involved organs retrieved from donors long after death, and were unsuccessful (Watson et al 2012). It was not until the 1950s, when the concept of avoidance of prolonged warm ischaemia in retrieved organs was developed, that the first successful renal transplant occurred. Furthermore, the difficulty of the immune response to transplantation was being understood for the first time (Gibson). Successful renal transplantation between identical twins was achieved in 1956 - overcoming both the problem of the immune response (Starzl 1993) to transplantation and the deleterious effect of protracted warm ischaemia (Merril et al 1956).

It was the discovery by Sir Roy Calne in 1960 that the chemotherapy agent 6-mercaptopurine could be used as an experimental immunosuppressive agent (Calne 1960), and the later use

of ciclosporin in 1978, which allowed adequate immunosuppression to facilitate long term graft and recipient survival. The first case of successful kidney transplant from a deceased donor was in 1962 (Starzl 1994) and success for other organs followed rapidly, with the first successful lung transplant in 1964 (Hardy et al 1963), liver transplant in 1967 (Starzl 1968), and the heart in 1967 (Barnard 1968).

These early organ transplant successes were contemporaneously described as being from 'non-heart beating' donors, with DCD remaining the sole source of organs for transplantation until formalisation of the concept of brainstem death (Harvard medical school 1984, Conference of the royal medical colleges 1976, Barber et al 1981). With the advent of a consensus opinion regarding the formalisation of brainstem death, organs from brainstem dead donors became exclusively used for deceased donor transplants. This preference is based on the ability to optimise organ condition prior to retrieval and minimize warm ischaemic times, and this remained the case for 30 years. Improvements in public safety legislation and neurological critical care in the late 1990s led to a decrease in numbers of patients declared brainstem dead (Kompajne et al 2011). At the same time, waiting lists for patients in need of organ transplants continued to rise.

These factors combined to trigger a renewed interest in donation after circulatory death in the United Kingdom. DCD donation has increased substantially over the last decade, from 37 DCD donors in 2000-2001 to 619 in 2017-2018 – which represents 39% of deceased organ donation (NHSBT Annual Activity Report 2017-18) and a DCD donation rate of 14.7 per million of the UK population (Matesanz et al 2016). This substantial increase in donor numbers is multifactorial but corresponds with national implementation of recommendations made by the UK Organ donation taskforce in 2008, which was charged in 2006 with identifying obstacles to organ donation (Poyntz et al 2008). Potential solutions recommended by the Taskforce included the creation of a network of specialist nurses in organ donation (SNOD) trained specifically to identify and recruit potential donors, seek consent from donor families and coordinate the donation process. At the same time the National Kidney Allocation Scheme was revised to improve the equity of DBD organ allocation across the UK, allocating both kidneys from a DBD donor nationally but allowing centres to keep DCD kidneys for their own patients; this proved to be a major driving force

to increasing local DCD donation in some centres. The Taskforce also drew attention to the need for a consistent UK wide approach to the DCD donor, which resulted in the publication of clear guidance on the ethical and legal standards to be applied to DCD donation (UK Donation Ethics Committee 2011, NHS Blood and Transplant National standards 2012). These publications have been included into Professional conduct standards for the care of dying patients by the General Medical Council and Intensive Care Society, which encourages exploration of patient wishes regarding organ donation as part of good routine end of life care (GMC guidance 2010) and has led to widespread support for DCD donation from the Intensive Care community across the United Kingdom.

## **1.4 Organ outcomes after DCD donation**

In the UK in 2017-18, an average of 2.8 organs were transplanted from each proceeding DCD donor, compared to 3.7 from each DBD donor (NHSBT transplant activity report 2017/18). Factors associated with this lower number of transplantable organs per donor in the case of DCD donation are complex and multifactorial. Physical damage to the organs is related to the physiological changes that occur during the period of warm ischaemia between treatment withdrawal and death, with increased warm ischaemic times predictive of worse function (Port et al 2002). These changes have been poorly characterised and are the subject of study throughout this thesis. However, other factors are implicated in the lower rates of acceptance of organs from DCD donors, including transplanting clinician concern about the function of such organs (Callaghan et al 2014) and the cumulative burden of medical comorbidity that is typical of the DCD donor (McDonald et al 2013). In addition, hearts are still rarely used from such donors.

Renal transplantation remains the treatment modality of choice for patients with end stage renal failure (Wolfe et al 1999). UK Transplant Registry analyses demonstrate that DCD kidneys have a higher incidence of delayed graft function (DGF) (Summers 2015) which is usually defined as the requirement for dialysis in the first week post transplantation (Mallon et al 2013), however numerous different definitions are used internationally, making direct comparisons of incidence challenging (Yarlagadda et al 2008). Kidneys from DCD donors have

twice the risk of delayed graft function as those from DBD donors, reported by Summers et al to be 49% (Summers et al 2015). Early DGF is associated with increased length of hospital stay and increased treatment costs for the recipient (Nagaraja et al 2012). Despite this increase in DGF, analysis by Summers et al (Summers et al 2015) demonstrated no significant difference in renal graft survival or function at 5 years between DCD and DBD kidneys when adjusted for the age of the recipient. The mean age of recipients of DBD and DCD kidneys was significantly different in this analysis (47 vs 54 years) suggestive of possible unconscious bias regarding allocation of organs perceived to be 'suboptimal' to younger recipients.

Transplanted livers from DCD donors are noted to have inferior graft survival when compared to DBD donors (Merion et al 2006, Selck et al 2008, Detry et al 2010, Abt et al 2004) with one meta-analysis suggesting DCD liver recipients had a 60% increased one year mortality compared to DBD graft recipients (Jay et al 2011). This increased mortality represents a combination of primary non-function (Abt et al 2004), hepatic artery thrombosis (Foley et al 2005) and biliary complications (Calne 1977, Chan et al 2008). Jay et al demonstrated that DCD liver recipients had a 10.8 times increased odds ratio for ischaemic biliary complications (Jay et al 2011, Chan et al 2008). This translated into an increased rate of graft loss and need for re-transplantation (Nguyen et al 2009). The net result of ischaemic biliary complications is increased cost (Jay et al 2011) increased inpatient hospital stay (Doshi et al 2007) and increased patient mortality (Jay et al 2010). The mechanisms underlying this increased rate of ischaemic biliary complications are not fully understood, but one suggestion is that it relates to the sensitivity of biliary epithelium to ischaemia (Noack). Analysis of factors related to poor outcomes in DCD liver transplants demonstrated a correlation between warm ischaemic time of >30mins with primary graft non function (De Vera et al 2009). Spain and France are the only countries that currently have an active DCD liver transplant programme from uncontrolled DCD donors, and to do this with minimal biliary complications, both employ a novel preservation technique in the donor called normothermic regional perfusion. Countries who prescribe a longer 'stand-off period' between the onset of asystole and organ retrieval (Geraci et al 2011) have encountered higher rates of primary non-function and ischaemic complications (Monbaliu et al 2012). There is a strong evidence that period of warm ischaemia endured by the DCD donor liver prior to its retrieval is responsible for its inferior function in the recipient.

Multiple case series and institutional reports suggest that recipient outcomes for lung transplantation from DCD donors are comparable with those from DBD donors (Mason et al 2008, De Oliveria et al 2010, Levvey et al 2012, De Vleeschauwer et al 2011). An International Society for Heart and Lung Transplantation DCD Registry Report by Cypal et al comparing survival of lung transplantation from DCD and DBD donors (306 DCD, 3992 DBD donors) found no significant difference in graft survival at 30 days (96% DCD vs 97% DBD) or one year (89% DCD vs 88% DBD,  $p=0.59$ ) (Cypal et al 2015). Five-year survival was identical in both groups at 61% ( $p=0.87$ ) and there was no significant difference in median hospital stay. There are multiple studies reporting no significant difference between rates of primary graft dysfunction reported between recipients of controlled DCD lungs and DBD lungs (Van de Wauwer et al 2011, Levvey et al 2012, Romano et al 2016). However, a recent study from Sabashnikov et al demonstrates that high grade primary non function is significantly higher in DCD lung transplant recipients, with a lower  $\text{PaO}_2/\text{FiO}_2$  ratio during the immediate postoperative 24 hours ( $p=0.018$ ) (Sabashnikov et al 2016). Cypel et al further demonstrated that 30-day survival rates were significantly different depending upon donor mechanism of death, with worse outcomes reported for recipients receiving lung transplants from donors with traumatic brain injuries when compared with recipients of transplants from donors with hypoxic brain injury or cerebrovascular accidents (Cypel et al 2015). Uncontrolled DCD lung donation is reported only from the Madrid group (Gomez-de-Antonio et al 2012). They demonstrate that with strict selection criteria recipients of lung transplants from uncontrolled DCD donors have similar one year survival to DBD lung transplant recipients, although have a higher incidence of primary graft dysfunction (Erasmus et al 2016). The equivalence in function between DCD and DBD lungs is suggested to be related to the insult that DBD lungs suffer during the process of brainstem death, where haemodynamic, neuroendocrine and metabolic disturbances result in the development of neurogenic pulmonary oedema (Egan et al 2004). These processes will be considered further in section 8.3.

Although the first successful heart transplant was from a DCD donor (Barnard 1968), DCD heart donation is only recently re-emerging as a technique and remains in its infancy. A retrospective review of the UK Transplant Registry data suggests less than 5% of referred DCD donors would be suitable for DCD heart donation (Messer et al 2015). There are

currently four centres worldwide with established DCD heart transplant programmes, three of which are UK based. With 39 DCD heart transplants to date, Papworth Hospital has performed the greatest number of DCD heart transplants. In their programme, recipient survival to discharge following DCD heart transplantation is 93%, with only 13% requiring ECMO support in the post-operative period. Recipients required a median stay of 5 days in intensive care with a median stay of 20 days in hospital (Page et al 2018). This group published their initial 28 DCD cardiac transplants as a case series, suggesting survival of DCD hearts at both 90 days and 1 year was comparable to DBD organs (Messer et al 2017). There are as yet no published five year survival data. Expansion of cardiac transplantation to utilize the DCD pool of donors has clear benefits to patients on the heart transplant waiting list, with the last published data suggesting a substantial number of patients die or becoming too unwell for transplantation while awaiting a suitable organ (NHSBT 2017-18 Activity report)

## **1.5 Identification and Management of the potential DCD organ donor**

### **1.5.1 Donor Identification**

Potential DCD organ donors are usually identified either in the Emergency Department or the Intensive Care Unit. A team of Specialist Nurses in Organ Donation (SNOD), established based upon the recommendations of UK Organ donation taskforce in 2008, are embedded within each hospital and are responsible for identification and characterisation of potential donors. Identification begins with referral of the patient to the SNOD team—this referral may be nurse or doctor led and can occur 24 hours a day. Consideration of the dying patient as a potential donor is considered part of good end of life planning (GMC 2010), and referrals should be made even of patients that staff may consider unsuitable. In the case of an intensive care patient, the decision to withdraw life supporting treatments should already have been made by the treating clinical team and family separate to any decision regarding organ donation (NHSBT guidelines 2012).

The SNOD will then interrogate the Organ Donor Register (ODR) to ascertain whether the patient had previously registered a wish to donate their organs in the event of their death. Of note, the ODR in England and Scotland operates on an 'opt-in' basis – meaning that signing the ODR is an active decision made by a patient. In December 2015, Wales became the first part of the UK to move to an 'opt-out' system of deemed consent to organ donation. This means if a person has not registered that they do not wish to become an organ donor they will be presumed to have no objection to donation in the event of their death. A three year review of this change to the 'opt-out' system in Wales has demonstrated a statistically significant increase in donation rates since the introduction of deemed consent compared to the rest of the UK. Wales achieves a rate of 23.8 donors per million of the population (NHSBT 2017-18 annual activity report), which exceeds the donation rate of England (23.0 pmp), Scotland (18.0 pmp) and Northern Ireland (19.9 pmp).

Within the UK there are strict criteria which may exclude patients from becoming DCD organ donor. Absolute contraindications to DCD donation of any organ include:

- Aged 86 and above
- Any cancer with evidence of spread outside affected organ (including lymph nodes) within 3 years of donation (however, localised prostate, thyroid, in situ cervical cancer and non-melanotic skin cancer are acceptable)
- Melanoma (except completely excised Stage 1 cancers)
- Choriocarcinoma
- Active haematological malignancy (myeloma, lymphoma, leukaemia)
- Definite, probable or possible case of human Transmissible Spongiform Encephalitis, including Creutzfeldt-Jakob disease (CJD) and variant CJD, individuals whose blood relatives have had familial CJD, other neurodegenerative diseases associated with infectious agents
- TB: active and untreated
- HIV disease (but not HIV infection) HIV infection means people who have infection with HIV but none of the associated complications. Organs from such donors would be transplanted into individuals who are infected with HIV.

Furthermore, there are absolute contraindications to the transplantation of specific organs from a DCD donor:

1. Liver

- Known cirrhosis
- Known portal vein thrombosis

2. Kidney

- Chronic renal failure on dialysis or with GFR under 30 ml/min (CKD Stage 4) – this does not include acute renal impairment, even if this had necessitated acute renal replacement therapy during the current ICU admission
- Acute cortical necrosis on current kidney biopsy

3. Pancreas

- Diabetes mellitus (type 1 or 2) although this does not include the insulin resistance and glucose intolerance associated with critical illness.
- BMI > 35kg/m<sup>2</sup>
- Age >65 years

4. Lung

- Age >65 years
- Previous thoracic surgery or empyema (does NOT include cardiac surgery or simple thoracocentesis)
- Existing lung disease, e.g. COPD, pulmonary emboli, asthma (but only if on systemic steroids; does NOT include occasional inhaler)
- Bilateral lung collapse
- Grossly abnormal chest X-ray
- Known, proven pulmonary infection
- Prolonged (>7 days) ventilation
- Need for ventilation with >60% oxygen or PEEP > 5 cm water.
- Patients found on evaluation to be at high risk of being difficult to re-intubate.

(Adapted from NHSBT guidelines: Organ Donation after Circulatory Death  
Report of a consensus meeting 2010)

Advice on donation from patients with intra-cerebral tumours is outlined by Warren et al (Warren et al 2012), which advises that organs from donors with primary Central Nervous

System tumours should be used unless the tumour is a lymphoma (even if the lymphoma is considered a primary intra-cerebral lymphoma). The SNOD will assess the patients' prior medical history to ascertain if there are any absolute contraindications. Should the patient have substantial medical co-morbidities which fall short of an absolute contraindication to donation, a discussion of the nature of the co-morbidity with transplant surgical teams is considered appropriate.

### **1.5.2 Consent for donation**

After exploration of absolute contraindications to organ donation and the patient's status on the Organ Donor Register (ODR), the SNOD approaches the family to seek consent for organ donation. A collaborative approach for donation (SNOD in attendance with treating clinician) is preferred in the UK and believed to confer improved consent to donation rates (Gortmaker et al 1998). The UK ACRE trial (Assessment of Collaborative Requesting) sought to determine any benefit of collaborative consent for organ donation after brainstem death (Young et al 2009). This study found no statistical difference between the two groups but remains contentious as it contradicts previous work in the field, which is all observational. The authors concluded that 'collaborative requesting confers little or no advantage in request for organ donation' and cite the trial as an example of a 'topic where observational data send a strong signal, which is subsequently negated in a randomized trial' (Vincent et al 2012). Evidence does support the fact that consent rates for donation are higher if the approach is made by an individual with expertise and training (Riker). NHSBT data from 2017/18 demonstrate that a SNOD was present for 85.6% of approaches. The current consent rate for DCD donation is 60% nationwide, with a consent rate of 88.8% if the patient is known to be on the ODR at the time of approach to the family (NHSBT transplant activity report 2017/18).

The consent taken by the SNOD team involves consent for donation of specific organs, including tissues, and the necessary testing of the donor to facilitate safe organ donation. It also includes consent for NHSBT approved research studies, which involve donor blood samples, biopsies of retrieved organs and the use for research of organs which are deemed to be non-transplantable at the time of retrieval. The study that forms the basis of the results

chapters in this thesis is the first research project to intensively study the DCD donor prior to death, a process for which no current model for seeking consent from next of kin exists.

### **1.5.3 Withdrawal of life supporting treatment**

Upon consent for organ donation, the SNOD team will then undertake the necessary donor testing and subsequent offering of organs to the transplant centre, the details of which are beyond the scope of this thesis. Once organs have been placed with receiving centres, arrangements are made for attendance of the National Organ Retrieval Service (NORS) surgical teams and a time is set for withdrawal of life supporting treatment.

Substantial inter-individual variability exists between unit practices for withdrawal of life supporting treatments (Sprung 2003). Many professional bodies provide guidance in relation to the practice of withdrawal of life supporting treatment, with guidance issued from the GMC, BMA and Intensive Care Society. In contrast to practice elsewhere, such as Spain, France and Italy, no intervention on the donor is permitted before death is verified.

Evidence is limited to suggest definitive best practice for treatment withdrawal, but the consensus meeting guidelines would suggest airway management at treatment withdrawal should include tracheal extubation or tracheostomy decannulation (BTS Consensus meeting 2010). The same guidance suggests that withdrawal of care that involved disconnection from mechanical ventilation but left the airway maintained by a device is associated with a longer agonal period and a lower likelihood of donation. While no one practice is specified, it is strongly encouraged that every unit should have a clear and consistently applied protocol for the withdrawal of treatment for potential DCD donors. This should include readily available pharmacological management for any subsequent respiratory distress after treatment withdrawal.

Treatment withdrawal location should be guided by the position of the proposed location relative to the operating theatre complex. Guidance from the Intensive Care Society suggests withdrawal within the theatre complex only if transfer from the intensive care unit would be prohibitively lengthy or complex. However, withdrawal in the theatre complex reduces warm

ischaemic time and thus has the potential to influence organ outcomes, thus could be considered in the best interests of the patient who had expressed a wish to become an organ donor. Whether the decision is to withdraw care in the ICU or theatre complex, the chosen location environment should ensure comfort, dignity and privacy for the patient, and adequate space with ready access for next of kin. There should be a pre-formulated plan for location of subsequent care should death not occur within the 3 to 4 hours while the surgical team are in attendance. Throughout the process of withdrawal of treatment and the subsequent agonal period there should be an appropriately experienced clinician readily available to manage symptoms should they arise and certify death when/if it occurs.

Certification of death should be performed by an appropriately qualified doctor based upon the continuous observation of 5 minutes of mechanical asystole observed on an arterial line trace (Oram et al 2011). In the absence of an arterial line trace, certification of death should take place after 5 minutes of continuous electrical asystole on an ECG trace. Any return of cardiac activity during this period of observation should prompt commencement of a further five minute observation period after asystole develops. After this five minute period of observation, the certifying doctor should undertake their standard clinical examination to confirm death, which is suggested by the Academy of Medical Royal Colleges to include confirmation of the absence of pupillary responses, absence of a corneal reflex and absence of response to painful stimulation. Death is confirmed upon the completion of these tests (Academy of Royal Medical Colleges 2008).

## **1.6 Predicting time to asystole in the DCD donor.**

Reasons for donation not proceeding are varied, but in the majority of cases are due to organs being deemed unsuitable by transplant centres or NORS retrieval teams (32.7%), or due to the time to asystole exceeding the threshold for donation (41.6%) (NHSBT potential donor audit). This threshold is organ specific, up to a maximum of four hours, and discussed in section 1.7.1. At present, there is no predictive tool in use in the United Kingdom that accurately predicts which potential DCD organ donors undergoing withdrawal of treatment will die within a timeframe that allows donation.

Accurate prediction of time to death is necessary for optimization of the logistics of the organ retrieval process and for effective counselling of next of kin (Wiegand 2008, Bradley et al 2013, Suntharalingam et al 2009). The complexity of the logistics involved in organising DCD donation represent a substantial difficulty in organ procurement (Murphy et al 2016).

Multiple predictive tools have been developed which attempt to address the identification of donors who will die within the permitted timeframe for DCD donation (Rabinstein et al 2012, Fulton et al 2017). Factors associated with early death after treatment withdrawal include a younger age, mandatory mode of ventilation, high FIO<sub>2</sub>, the use of inotropes, and a low arterial pH (Suntharalingam et al 2009). Two predictive tools, the University of Wisconsin (Lewis et al 2003) and the UNOS scoring systems (UNOS) are available from North America, but neither has been validated for UK practice. In the USA, more than 50% of patients meeting more than one of the 14 UNOS criteria died within an hour of withdrawing life support treatment (DeVita et al 2008). It is notable that few of the UNOS criteria refer to baseline physiology, with the majority of criteria referring to the presence of mechanical organ support (ECMO, VAD, IABP), making them less applicable to the vast majority of DCD donors in UK practice.

Furthermore, the majority of previous work on prediction of time to death has used tests not widely adopted in routine UK clinical practice (Dhanani et al 2014, Fulton et al 2017), and hence apply poorly to a UK donor population and are not validated in the UK donor cohort (Guo et al 2017). Indeed, there is evidence that the opinion of the treating intensive care physician regarding whether death will occur in a time frame that permits donation may be as accurate as any of the current predictive tools.

As discussed later, donor characteristics are changing: mean donor age and BMI are increasing, and donors are less likely to have suffered a trauma related death (NHSBT potential donor audit 2017/18). These changes have been demonstrated to have an adverse effect on transplantation outcomes (Summers et al 2015) and the changing demographics of the donor population make it likely that pre-existing attempts to predict timeframe for donor demise will fail to translate to the current donor populations (Lewis et al 2003, Brieva et al 2013). Meta-analyses in the area of prediction of time to death are difficult to undertake, due to differing national and international practices and inconsistent degrees of data collections (Munshi et al 2015). Improved standardisation of data collection prior to and

during the withdrawal of life supporting treatment in the DCD donor would allow better characterization of features predictive of death within a timeframe.

## 1.7 Warm ischaemic time and its implications for organ donation

### 1.7.1 Definitions of warm ischaemic timeframes

Inherent in DCD organ donation is a variable period of warm ischaemia before circulatory arrest, often termed functional warm ischaemia. This period starts when organ perfusion becomes inadequate and ends when organs are cold-perfused with preservation solution *in situ*.

The definitions given to timeframes after withdrawal of treatment are fundamental to understanding the processes occurring during the withdrawal period and are used extensively throughout this thesis. The accepted names for these periods are given below in figure 1.2:

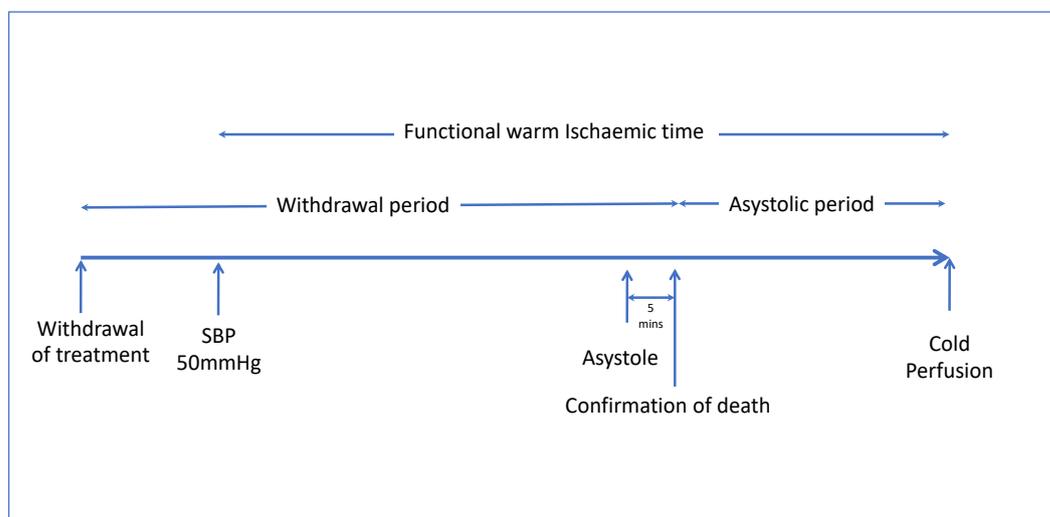


Figure 1.2: Pictorial representation of definitions of timeframes after withdrawal of life supporting treatment.

The withdrawal period is the time from the withdrawal of life supporting treatments to the point of asystole. This is also referred to as the agonal period in some texts. The Functional

Warm Ischaemic time commences when the systolic blood pressure has a sustained fall below 50mmHg (Ho et al 2008) (sustained is considered to be for a period of two minutes or longer) and extends up to the onset of cold perfusion of the organs *in situ*. Cardiothoracic retrieval teams may also use oxygen saturations of below 50% or 70% to signify the onset of the warm ischaemic period (NHSBT National standards for organ retrieval 2012), but this is variable between cardiothoracic retrieval centres.

The asystolic period (referred to in some articles as the primary warm ischaemic time) is the time from asystole to the *in situ* perfusion of the organs with cold preservation solution. The use of the term functional warm ischaemia reflects the growing evidence that warm ischaemic injury occurs prior to asystole in the donor. The use of a systolic blood pressure of 50mmHg to signify the onset of the warm ischaemic period is based on consensus opinion, and although recommended in the British Transplantation Society (BTS) guidelines for management of the DCD donor, the document acknowledges that 'there is little published evidence to support this' (BTS guidelines July 2015). The guidelines accept the empirical element to the utilisation of 50mmHg systolic blood pressure to define the onset of functional warm ischaemia, pointing out that organs from young fit donors may tolerate longer periods of profound hypotension well. By contrast, organs from older, hypertensive patients are likely to have different autoregulatory thresholds and may suffer substantial ischaemic at systolic blood pressure well in excess of 50mmHg (Bernat et al 2010). A threshold systolic blood pressure of 50mmHg is not internationally agreed, with other national programmes using different values which vary between MAP 50mmHg and Systolic Blood pressure of 80mmHg (Singh et al 2017).

BTS guidelines discuss the use of oxygen saturation levels of below 70% as a marker for the onset of the functional warm ischaemic period. The current guidance states that there is not enough information to support the use of an oxygen saturation target as an indicator of poor outcome, and hence a reason for non-retrieval of the organ (BTS guidelines July 2015). The guideline does encourage accurate recording of the oxygen saturation in order that future work can be undertaken to correlate saturations with graft outcomes. Threshold oxygen saturations of 70% are not universally agreed, with centres reporting the use of threshold values of 80% (Kalisvaart et al 2018), 60% (Coffey et al 2017) and 50% (Harefield protocols).

The tolerable length for the functional warm ischaemic period is debatable and organ specific. Organs with higher oxygen requirements have a decreased ability to tolerate warm ischaemia. Suggested ranges are from 30 minutes of functional warm ischaemia for lung and liver transplants (Levvey et al 2008) to 240 minutes for kidney transplants (Florak et al 1986). There is experimental evidence that suggests organs may in certain cases remain viable for substantially longer periods of time (Egan et al 2004, Van Raemdonck et al 2013) but this is unpredictable and unvalidated.

Functional warm ischaemic times are used to declare as yet unretrieved organs 'non-viable' and stand NORS teams down from the retrieval process. National stand-down times for DCD organ donation vary between organs, and are from the onset of functional warm ischaemia as given below (adapted from BTS guidelines):

- Liver: 30 minutes (although 20 minutes is ideal, and age is an important factor)
- Lungs: 60 minutes (time to inflation of lungs)
- Kidney: 120 minutes - then reassess with regard to logistics; can extend to a further 120 minutes in selected donors.

For cardiac DCD, a retrieval time limit of 30 minutes for FWIT has been adopted following the criteria of the abdominal transplant surgeons. However, there have been well established small and large animal models to suggest that the heart may be tolerant of 60 minutes of functional warm ischemia (Gundry et al 1992).

The period of time between withdrawal of life supporting treatment and asystole is extremely variable. Analysis of NHSBT data by Bradley et al suggests that, within the UK DCD donor population, 40% of potential donors undergoing withdrawal of life supporting treatment die within the first hour, and 60% within 3 hours (Bradley et al 2013). NHSBT data from 2017-18 suggests only 55% of consented DCD donors went on to donate organs (NHSBT potential donor audit 2017/18), with the majority of non-proceeding donations being due to prolonged periods of functional warm ischaemia. The true duration of functional warm ischaemia for an individual donor is unknown and currently unmeasured. The physiological changes occurring during this period of incomplete, but prolonged, tissue hypoxia, on a

background of poor or absent physiological homeostasis, are unknown and unquantified. However, existing data from organ donation following brain death provide some useful directions for investigation and will be considered below.

### **1.7.2 Metabolic process during warm ischaemia.**

Warm ischaemia is mechanistically described as cell and tissue ischaemia under conditions of normothermia (Halazun et al 2007). During a period of ischaemia there is insufficient delivery of oxygen and nutrients for cells to undergo normal aerobic metabolism. Cells instead switch to anaerobic metabolism. Glucose is initially metabolised through glycolysis, which generates two molecules of pyruvate from each molecule of glucose. When oxygen is available, pyruvate enters the tricarboxylic acid cycle where it is metabolised to carbon dioxide and water in the presence of oxygen. When oxygen is unavailable, glucose metabolism is anaerobic, and the pyruvate generated by glycolysis is converted to lactate, which results in acidosis. Further, anaerobic glucose metabolism is inefficient, yielding only two molecules of ATP for each molecule of glucose (as against 38 in the presence of oxygen). Since cellular metabolic processes are heavily dependent on ATP, intracellular ATP stores deplete rapidly under anaerobic conditions. Consequently, decreases in cellular oxygen delivery will result in increased lactate production via anaerobic metabolism, and this is detectable in peripheral blood samples (Meakins et al 1927). ATP is essential for the maintenance of membrane-associated ion exchange channels and as warm ischaemia progresses membrane integrity is lost, cellular dysfunction and ultimately cell death occur. Cooling does not completely abolish metabolism, and the same processes described above occur but at a rate that is markedly decreased (Burg et al 1964). This means that in hypothermic conditions cells and organs are able to survive for longer periods without adequate delivery of oxygen and nutrients.

Each organ has a different threshold period for functional warm ischaemia during DCD donation as described in section 1.7.1. The rationale for these thresholds are multifactorial, related in part to the oxygen consumption of the organ (Szostek et al 1999) and the specific metabolic rate of the organ (Wang et al 2010) which will determine the period of ischaemia that is permissible before cell damage and cell death. The current permissible periods of

warm ischaemia allowed in the donor are outlined in 1.7.1 above. However, the thresholds are also in part pragmatic, related to the availability of techniques to support the recipient in the case of early graft dysfunction. In the case of the kidney, the recipient of a non-functioning organ can be supported by dialysis while renal recovery is anticipated, or repeat transplantation is arranged (Szabo). In the case of cardiac transplantation there are some reports of Extra Corporeal Membrane Oxygenation (ECMO) being used to support DCD transplant recipients with early graft dysfunction (Chew et al 2018), and a small study by Chew et al of 18 DCD heart recipients, showed that such support appeared to support cardiac recovery to normal cardiac function with no impact on short term graft survival.

In addition, when considering allowable functional warm ischaemic time, there is thought given to the organ's regenerative ability, and its intrinsic autoregulatory mechanisms. During hypotension, decreased renal blood flow results in afferent arteriolar vasoconstriction in an effort to preserve glomerular blood flow. This has the deleterious effect of decreasing flow to the renal tubules, causing Acute Tubular Necrosis (Rao et al 1983). However, the renal tubules can regenerate although the mechanisms by which this occurs are poorly understood (Toback et al 1993). Consequently, with appropriate interim renal support graft function can be recovered (Hall et al 2014). Hepatic regeneration is also well recognised (Michalopoulos 2013) and the liver is noted to have a high regenerative capacity (Taub 2004). Of note, the region of liver with the lowest capacity for regeneration is the bile duct (Nakanuma et al 2001) – and it is this region that is most prone to damage in the DCD liver graft, with ischaemic cholangiopathy occurring in an estimated 29% of DCD livers (Jay et al 2011) and causing increased recipient morbidity (Chan et al 2008). Consequently, despite its regenerative ability, the lack of interim options to support the liver transplant recipient during a period of poor graft function leads to caution when considering permissible warm ischaemic periods in the DCD donor. Neither lung nor cardiac grafts demonstrate significant regenerative ability.

## 1.8 Physiological changes occurring in circulatory death

### 1.8.1 Human studies

While cellular death and its pathophysiological mechanisms are well understood, the processes occurring in organism death remain poorly characterised. The physiological changes that occur in cellular death have been the subject of intensive study for many years and are well defined (Kroemer et al 2005). The Nomenclature Committee on Cell Death (Kroemer et al 2009) proposes that a cell should be considered dead when any one of the following molecular or morphological criteria is met: '(1) the cell has lost the integrity of its plasma membrane, as defined by the incorporation of vital dyes *in vitro*; (2) the cell, including its nucleus, has undergone complete fragmentation into discrete bodies (which are frequently referred to as 'apoptotic bodies'); and/or (3) its corpse (or its fragments) has been engulfed by an adjacent cell *in vivo*.'

In the case of organs donated from a deceased donor, it is clear that the cells and organs still function. In the case of the donor after circulatory death, the organ cells may have been in the process of dying, but by the above definitions cannot be considered dead – indeed they still function upon transplantation. The case of brainstem death will be considered separately in section 1.8.3 below.

Given that by definition circulatory death involves irreversible asystole and apnoea, it follows that this must be preceded by progressive hypotension and hypoxaemia. Much of the literature that has studied dying patients comes from the field of Palliative Care medicine.

A study by Bruera *et al* of the variation in vital signs during the dying period in patients with advanced cancer found that impending death within three days was associated with tachycardia ( $p=0.01$ ) hypotension ( $p=0.04$ ), and hypoxia ( $p=0.02$ ). The study comments that many dying patients had normal vital signs until the last few hours of life (Bruera et al 2014) and that there is a general paucity of studies examining how patient physiology changes in the last days of life. Those studies which have been performed by the palliative care community centre on patients with terminal cancer (Kao et al 2009) which are a significantly

different cohort to the potential DCD organ donor. Furthermore, impending death is considered to be within 3 days by the palliative care community (Hui et al 2015), a vastly different timeframe to that which is permitted for DCD donation.

In intensive care medicine, there are retrospective large data studies which analyse the presence of deleterious physiological changes and attribute a 'risk' of death related to the degree of derangement (Poole et al 2012, Patel et al 1999). Scoring systems such as The Sequential Organ Failure Assessment (SOFA score) (Shapiro et al 2006), Simplified Acute Physiology Score (SAPS score, now on revision IV) (Moreno et al 2005), the Acute Physiology And Chronic Health Evaluation (APACHE score) (Zimmerman et al 2006), and (most commonly in the UK) the Intensive Care National Audit and Research Centre (ICNARC) score (Ferrando-Vivas et al 2017) are widely adopted to predict the risk to the individual and requirement for organ support. Such studies assess population risk rather than individual risk (Afessa et al 2007) and do not include how physiology changes but use its measurement at a specific point to estimate risk.

There is no other setting apart from palliative care and organ donation where circulatory death is 'allowed' in modern medicine; in all other settings it is actively fought against. There are no described studies which undertake assessment of blood samples during the dying process in human subjects.

### **1.8.2 Animal models of circulatory death**

The lack of any meaningful opportunities to study human death in a cohort of patients that are comparable to the DCD donor have led to the development and use of animal models of cardiorespiratory death. These models have been used to test surgical techniques, physiological changes, organ viability and interventional treatments. The three main animal models are porcine (White et al 2016), canine (Roberts et al 1996) and rodent models (Kearns et al 2017).

These models generally involve anaesthesia of an animal subject to induce unconsciousness, intubation of the airway and ventilation, paralysis to abolish any respiratory activity and then

extubation or cessation of ventilation. The animal subject then undergoes progressive cardiorespiratory decline due to hypoxia and ultimately cardiac arrest, which typically occurs within 15 minutes of terminal extubation.

While the widely used porcine model of DCD donation proves to be a useful model, it has fundamental weaknesses which make its translation to the human DCD donor problematic. Firstly, the porcine model involves a healthy young adult animal - which stands in direct contrast to the average UK DCD donor age of 54 with a BMI of 27kg/m<sup>2</sup> (NHSBT activity report 2017). Furthermore, the majority of donors have one or more medical co-morbidities (NHSBT activity report 2017/18) which have implications on their likelihood of death within a timeframe (Dhanni et al 2012), in contrast to the healthy study animal used in the standard porcine model.

The use of anaesthesia for the animal models is clearly ethically appropriate (Perry et al 2007), and mandated by UK guidelines on the use of animals in scientific experimentation (Nuffield Council on Bioethics 2005, Russell et al 1959). In the absence of anaesthesia, in a paralysed animal unable to report pain or suffering, many of the described study interventions would induce unacceptable symptoms. However, the metabolic effects of anaesthesia are complex and include decreased brain and organ oxygen consumption (Kaike et al 2003). Consequently, the administration of anaesthetic agents to animal models premortem has the potential to influence the organ outcomes reported by the studies.

Finally, many of the animal models of donation after circulatory death involve the terminal extubation of an anaesthetised and paralysed animal (White et al 2016, Kato et al 2006). These animals will not breathe after extubation and will progress to die in a short time period. This contrasts directly with the majority of DCD donors who breathe with either a regular respiratory effort or with an agonal pattern of breathing (Suntharalingam et al 2009). This period of potentially prolonged tissue hypoxia adds to the burden of warm ischaemic injury suffered by the organ prior to retrieval.

The animal study that provides the most insight into DCD donor physiology is a study by White et al which examines a porcine DCD model. The model was prepared as discussed

above and, following baseline physiological assessment of the anaesthetised and paralysed animal, ventilation was discontinued, and the animal was extubated. Serial blood samples were then taken from the ascending aorta at 30 second intervals up to 20 minutes post extubation. Nineteen animals were studied; all suffered asystolic circulatory arrest between 7 and 8 minutes after extubation. This study was able to plot changes in systolic blood pressure, heart rate and oxygen delivery during the dying process. Arterial blood gas analysis showed a precipitous decline in partial pressure of oxygen in the first three minutes post-extubation, while arterial partial pressure of carbon dioxide increased with a corresponding decrease in blood pH. Blood lactate level was seen to rise slowly over the first four minutes post-extubation then rise rapidly up to the point of circulatory death at 8 minutes. Analysis of samples for catecholamine levels showed a large elevation of adrenaline and noradrenaline between 2- and 10-minutes post extubation, a phenomenon undocumented systematically in the setting of human DCD donation, although suggested by work in porcine model performed by Belzer's group in 1971 (Keaveney 1971). The study also showed decreases in levels of interleukin-6 and TNF-alpha during the dying process (White et al 2016). This study provides substantial insights into the processes of circulatory death in the potential donor but remains limited by the shortfalls of the porcine models described above.

Animal work has characterised the haemodynamic and other physiological changes occurring following ventilatory arrest in otherwise healthy animals and suggested a catecholamine response that is similar to or in excess of brainstem death, with reduction in peripheral perfusion in advance of a fall in systolic blood pressure (White). Such studies have also suggested that a cytokine "storm" is also likely to occur during the process of circulatory death (Rhee et al 2011, Guo et al 2014).

The limitations of animal models in the setting of DCD donation make a compelling case for a controlled study of human DCD donors to appreciate the physiological changes that occur in the unique set of biological parameters involved in circulatory death.

### **1.8.3 Pathophysiological response to brainstem death**

In contrast to circulatory death, the physiological processes of brainstem death have been the subject of extensive study in both animal models and critically ill patients. Understanding

of these pathophysiological processes has allowed optimisation of organs donated from brainstem dead donors to occur (NHSBT extended donor care bundle 2014). This has resulted in increased numbers of transplantable organs and improved graft function in recipients (Rosendale et al 2002).

Brainstem death is preceded by a period of raised intracranial pressure (ICP) due to an expansion in volume of intracranial contents. This period is variable in length, with slower rises in ICP allowing for some compensation to occur via the Monroe-Kellie hypothesis (Mokri 2001) and physiological consequences to be of lesser magnitude than expected for a specific ICP (Tameem et al 2013). The classic physiological response to critical elevation in ICP was described by Cushing in 1901 as a triad of hypertension, bradycardia and irregular respiratory pattern (Cushing 1902). The pathophysiological endpoints of brainstem ischaemia are complex, and affected by burden of pre-existing disease (Salim et al 2006) but can be summarised by table 1.2 below:

<b>Pathophysiological Process</b>	<b>Mechanistic basis</b>	<b>Incidence</b>
Hypothermia	Vasodilatation Hypothalamic dysfunction	100% without active management
Hypotension	Vasodilatation, myocardial dysfunction	81-97% (Smith 2004, Salim 2006)
Diabetes insipidus	Posterior pituitary dysfunction	46-78% (Salim 2006, Gramm 1992)
Disseminated Intravascular coagulation	Tissue factor release	29-55% (Hefty 1993, Salim 2006)
Arrhythmia	Catecholamine release, myocardial dysfunction	25-32% (Smith 2004, DuJardin 2001)
Pulmonary oedema	Catecholamine induced capillary endothelial damage	13-18% (Salim 2006, Smith 2004)

*Table 1.2. Pathophysiological consequences associated with brainstem death. Adapted from McKeown et al 2012 with permission*

The cardiovascular responses to brainstem death occur in three phases. Initially, as described above, the classic Cushing response to intracranial hypertension involves systemic hypertension and bradycardia as a compensatory response to maintain cerebral blood flow. This is followed by a period of catecholamine hypersecretion leading to vasoconstriction,

profound hypertension and tachycardia (Bugge et al 2009). This phase is commonly described as a 'catecholamine storm' and is associated with adrenaline and noradrenaline release grossly in excess of levels routinely measured during physiological stress (Perez-Lopez et al 2009). This supra-physiological catecholamine release has been associated with damage to donor organs, with specific evidence suggesting myocardial dysfunction (DuJardin et al 2001, Novitsky et al 1997) and the development of neurogenic pulmonary oedema (Smith 2004). A detailed account of the deleterious effects of catecholamine excess is given below. The period of catecholamine excess is rapidly followed by a period of catecholamine depletion, which is associated with loss of sympathetic tone and systemic vasodilatation. Porcine studies suggest catecholamine levels return to baseline within 30 minutes of their initial elevation (Ali et al 2011). In combination with the development of myocardial dysfunction this leads to severe hypotension, which without active management, can precipitate organ hypoperfusion and cardiac arrest. Hypotension is worsened by the co-existence of hypovolaemia due to the development of diabetes insipidus, and by hypoxia resulting from neurogenic pulmonary oedema. Thus, without rapid management, the physiological consequences of brainstem death can lead to loss of previously viable donor organs and potential loss of the donor in the event of cardiac arrest, from which it is not possible or appropriate to attempt cardiopulmonary resuscitation (Brown 2016).

Neurogenic pulmonary oedema occurs in approaching 20% of donors after brainstem death. The pathophysiology behind its development is poorly understood but is suggested to be due to a combination of elevated pulmonary hydrostatic pressure during extreme hypertension (Avlonitis et al 2005) and catecholamine induced endothelial damage (Novitsky et al 1987). Evidence for the deleterious effects of catecholamine surges in this setting comes from the fact that the development of neurogenic pulmonary oedema can be at least partially prevented by  $\alpha$ -adrenergic blockade in a rat model (Sakakibara et al 1992).

The development of endothelial injury is thought to establish an inflammatory process within the lungs that is currently poorly characterised (Fisher et al 1999) but the magnitude of which correlates with recipient graft function (Fisher et al 2001). The development of neurogenic pulmonary oedema may be minimised with good ventilatory strategies as part

of donor optimisation (NHSBT extended donor care bundle 2014) but remains a major cause of donor lungs being declined for transplantation.

The acute elevation of ICP during the process of brainstem death leads to pituitary ischaemia and loss of pituitary function. The temporal relationship between loss of pituitary function and ICP elevation is influenced by the rate of rise of ICP and follows a variable pattern, particularly in cases where treatment to attempt ICP control is instituted (Novitsky et al 2006). Loss of posterior pituitary function leads to decreased antidiuretic hormone (ADH) secretion from the supraoptic nucleus of the hypothalamus. This precipitates development of the clinical syndrome of Diabetes Insipidus, which is characterised by the production of large volumes of dilute urine and may rapidly lead to hypovolaemia, hyperosmolarity, and hyponatremia if untreated (Wood 2004). Loss of anterior pituitary function leads to decreased Thyroid Stimulating Hormone (TSH) secretion, with the result of decreased T3 and T4 hormone levels (Novitsky et al 1987, Gramm et al 1992) The resulting clinical syndromes of acute hypothyroidism are particularly evident in the brainstem dead patient maintained for some time on intensive care, but may be ameliorated by prompt T3 replacement (Donor optimisation bundle). Hypothyroidism combines with loss of hypothalamic function to precipitate the development of hypothermia, which is worsened by the co-existence of vasodilatation due to catecholamine depletion. The effect of brain death on the hypothalamic pituitary adrenal (HPA) axis is unclear, with conflicting outcomes in different studies. Levels of cortisol have been reported to be normal, low or high (Dimopoulou et al 2003, Lopau et al 2000). Hyperglycaemia is commonly seen in the brainstem dead organ donor. Its development is multifactorial; a combination of worsening of the pre-existing state of insulin resistance seen in the critically ill patient (Zauner et al 2007) and the effect of catecholamine excess.

Coagulopathy is a common development in the brainstem dead patient (Smith 2004). This is a phenomenon seen commonly in head-injuries and trauma (Talvig et al 2009), related to the general inflammatory response seen in trauma and critical illness. Furthermore, the injured brain has been demonstrated to release tissue thromboplastin, which has been shown to worsen the degree of coagulopathy in a porcine brainstem death model (Barklin 2009). Any coagulopathy present is exacerbated by the co-existence of hypothermia. The

development of disseminated intravascular coagulopathy (DIC) in the brainstem dead patient has also been shown to have a deleterious effect on transplant outcomes, with a reported increase in the incidence of delayed graft function in renal transplantations (Hefty et al 1993). The precise mechanism underpinning these phenomena remain unclear, but the development of micro-thrombi seen in DIC is felt to be contributory (Meyers et al 2015).

The development of inflammation in brainstem death has been the subject of intensive investigation (Barklin 2009) but remains poorly understood. Its mechanisms are again multifactorial, related to the development of hormonal derangement, catecholamine surge and thromboplastin release previously discussed. These changes are superimposed on the pre-existing state of systemic inflammation seen in relation to brain injuries and trauma (Yoshimoto et al 2001). Activation of both the innate and adaptive immune systems is suggested (Watts et al 2013) and elevations in inflammatory cells have been well documented (Bugge 2009). Studies by Weiss et al comparing cytokine levels in organ biopsies from DBD donors and live donors found an increase in IL-4, IL6, IFN- $\gamma$  and TNF- $\alpha$  as well as increased numbers of CD3+ and CD25+ lymphocytes in DBD liver donor biopsies (Weiss et al 2007). Other studies have suggested similar findings in kidney biopsies although substantial discrepancy in the degree of cytokine profile change have been reported – this is suggested to be related to the cold ischaemic time endured by the organs (Araki et al 2006). Bronchoalveolar lavage from DBD lung recipients have been found to contain higher IL-8 levels than from controls (Fisher et al 1999) and the degree of IL-8 elevation correlates with early graft failure after transplantation (Fisher et al 2001). Review of the available literature strongly supports innate immune system activation during brainstem death, but the contribution of confounding factors, such as cold storage of organs, requires further understanding. A detailed understanding of this inflammatory response has the potential to identify targets for intervention in the DBD donor and is considered a key strategy for advancing donor management (Watts et al 2013).

In summary, the physiology and immunology of brain death are well characterised (Watts et al 2013, Barklin 2009). Cushing's response to the cerebral hypoperfusion of brain death involves autonomic reflexes that increase systemic blood pressure, including release of catecholamines, which increase peripheral resistance by shutting down circulation to other

organs while attempting to preserve perfusion of the brain (Westendorp et al 2011). The above changes lead to activation of the innate immune system (Novitsky et al 2006) which has been demonstrated to have an effect upon organs retrieved from braindead donors for transplantation (Venkateswaran et al 2010).

#### **1.8.4 The deleterious effects of catecholamine excess**

It is well reported that surges in catecholamine release may be detrimental, with much of the evidence for this coming from the mechanistic study of brainstem death and organs retrieved from brainstem dead donors. Hearts donated after brainstem death have frequently been reported to show myocardial dysfunction related to the catecholamine release associated with brainstem ischaemia (Guglin 2014)

Studies to understand the mechanisms behind stress cardiomyopathy provide valuable insight into the deleterious effects of catecholamine surges on organs. The mechanism most commonly used to explain the phenomena is that there is diffuse disturbance of the coronary microcirculation causing microvascular endothelial damage due to excess  $\beta$ 1-adrenoceptor activation by elevated catecholamine levels (Akashi). Clinical studies of patients admitted to hospital with stress cardiomyopathy demonstrate circulating catecholamine levels over twice that expected for patients experiencing acute coronary syndromes (Wittstein et al 2005). Similar studies examining the role of noradrenaline in stress cardiomyopathy have found higher levels in subjects with stress cardiomyopathy in intensive care than in a matched cohort of critically ill patients without cardiomyopathy (Park et al 2005).

Further clinical evidence for the deleterious effects of surges in catecholamines come from examination of the myocardial function of patients undergoing surgical resection of pheochromocytoma. Tumour manipulation during surgery is associated with supra-normal secretion of adrenaline and noradrenaline (Suzuki et al 2014) and these elevations have been associated with the subsequent development of acute left ventricular dysfunction in the previously structurally normal heart. The risks of this catecholamine induced ventricular dysfunction can be ameliorated by pre-treatment of the patient with alpha-antagonists (Hariskov et al 2013), which substantially reduces the risk of hypertensive crisis during surgical manipulation of the tumour.

Experimental studies have demonstrated that exposure of cardiac tissues to supra-normal catecholamine levels for a modest time period causes depletion of ATP, accumulation of lactate in the tissue, neutrophil infiltration (Nef et al 2007) and the development of contraction band necrosis (Todd et al 1985). Contraction band necrosis is a specific form of myocyte injury that is found to follow myocyte exposure to high catecholamine levels and has been reported in both animal (Movahed et al 1994) and human (Yamanaka et al 1994) studies.

Animal work in porcine models of brainstem death and DCD donation (Ali et al 2011) gives rise to the suggestion that the magnitude of catecholamine release seen in the model of DCD donation may exceed that seen at the point of brainstem ischaemia and leading to brainstem death. Work by Ali et al demonstrated that plasma adrenaline and noradrenaline levels in their porcine DCD model of death exceeded those seen in their brainstem death model by 30-fold and 50-fold respectively. The catecholamine levels measured in human brainstem death are reported to peak at 6ng/ml and 3.8ng/ml for adrenaline and noradrenaline respectively (Perez-Lopez et al 2009) and to reach peak values within 15 minutes of brainstem death (Chen et al 2008). There are no measured values for catecholamines during the process of cardiorespiratory death.

## **1.9 Opportunities to modulate transplanted organ outcomes**

### **1.9.1 Donor optimisation**

Detailed understanding of the physiological processing occurring in brainstem death have led to the institution of treatment plans designed to limit or halt the development of these processes. This is known as donor optimisation. Donor optimisation aims to minimise organ damage and organ loss from treatable causes, and has been a key strategy that has led to the number of organs being donated per DBD donor increasing over recent years (Salim et al 2005).

Prior to donor optimisation strategies being routinely implemented, studies suggest up to 25% of organs from DBD donors were deemed unsuitable for transplantation due to the pathophysiological effects of brainstem death (Mackersie et al 1991). Standardised donor optimisation has been particularly effective in increasing the rate heart donation from DBD donors (Wheeldon et al 1995). Donor optimisation has also led to a decrease in the numbers of DBD donors who suffer cardiac arrest prior to organ retrieval (Rosendale et al 2003).

Donor optimisation is an 'active process' which often necessitates escalation of treatment if the full donation potential of the donor is to be realised. The focus of therapy switches from treatment of the patient which aims to continue and restore quality of life, to treatment of the donor which aims to restore or maintain function of transplantable organs. The institution of 'donor care bundles' has allowed standardisation of DBD donor management between sites and provides a benchmark standard of care of the purposes of internal audit and unit comparison.

The legal precedent and ethical basis for optimisation of the DBD donor was clearly set out by the UK Donation Ethics Committee. Donor optimisation after the completion of brainstem death criteria in the patient with a prior declared wish to donate their organs is considered an extension of patient best interests and is fully supported by UK law (UK Donation Ethics Committee 2011).

The UK donor optimisation strategy is known as the 'Donor Optimisation Extended Care Bundle' and its core components and targets are summarised in table 1.3 below

<b>System</b>	<b>Physiological Target</b>	<b>Components</b>
Respiratory	PaO <sub>2</sub> >10kPa pH>7.25	Lung recruitment manoeuvres Lung protective ventilatory strategy Regular chest physiotherapy 30° head of bed elevation Ensure adequate Endotracheal cuff pressure Bronchoscopy and lavage as indicated
Cardiovascular	MAP 60-80mmHg	Ensure adequate fluid balance Correct hypovolaemia with fluid boluses If requiring vasopressors commence vasopressin 0.5-4units/hr Wean catecholamine vasopressors as able Consider dopamine/dobutamine if required
Fluid/Metabolic	Electrolytes within normal range	Methylprednisolone 15mg/kg Review IV fluid composition and administration to maintain Na < 150mmol/l Target urine output 0.5-2.0ml/kg/hr Treat diabetes insipidus with DDAVP 1mcg IV Insulin infusion target BM 4-10mmol/l Continue NG feed Maintain normothermia
Thromboembolic protection		TED stockings as clinically indicated Calf compression devices as clinically indicated Continue prophylactic low molecular weight heparin
Lines/monitoring		Arterial line – left side preferable Central venous access Routine observations for level 3 patient
Investigations		12 lead ECG CXR Troponin level Echocardiogram

*Table 1.3: UK Donation after Brainstem Death Donor Optimisation Extended Care Bundle. Adapted from NHSBT guidance with permission.*

The results of donor optimisation programmes in DBD donors demonstrate that the environment in which organs are functioning in the donor prior to organ retrieval contributes to injury suffered by the donor graft, both through adding to the burden of injury but also through immune modulation. It has been proposed that this early graft injury may alter its interaction with the host's immune system in a way that accelerates rejection and hence graft failure (Libby et al 2001). Consequently, early activation of the innate immune system

(the “alarmin response”) has been tied to late graft failure in the organ recipient through endothelial cell injury predisposing to early rejection (Al-Lamki et al 2008) and sensitization of adaptive immunity to modulation of late rejection. Understanding the degree to which these processes occur in DCD organ donors prior to death may provide opportunities to modulate the donor environment, thus reducing early injury suffered by donor organs.

### **1.9.2 Targeted donor interventions**

Further evidence that specific intervention in the DBD donor prior to organ retrieval may be able to modulate transplanted organ outcomes comes from a study of temperature management in DBD donors (Niemann et al 2015). The study randomized DBD donors to targeted temperature management groups of either 34 to 35°C (hypothermia) or 36.5 to 37.5°C (normothermia) after declaration of death. Kidneys retrieved from donors randomized to the lower target group had a significantly decreased incidence of delayed graft function. Targeted temperature management in the context of intensive care management of out of hospital cardiac arrest survivors has previously been shown to be protective against renal injury (Wolfram et al 2008, Polderman 2009). Such interventions to the donor to improve transplanted organ function are acceptable in UK practice only upon confirmation of brainstem death. Despite this fact, a review of studies of donor interventions in the DBD donor by Feng et al (Feng 2010) noted that ‘there are currently substantial scientific, logistical and ethical obstacles that discourage innovation in donor management and organ preservation.’

At present in the UK there is no intervention to improve transplanted organ outcome permitted prior to circulatory arrest of the DCD donor. This is not the case in some European countries such as France and Spain where practices can include femoral cannulation and heparinisation prior to death (Rudge et al 2012). Legislation and regulation in this area are currently under review by NHSBT and the Human Tissue Authority.

## **1.10 Directions of travel of DCD donation**

The evidence explored in this chapter demonstrates the success of the DCD programme in terms of increasing the numbers of organs donated. It also demonstrates that DCD organ recipients may have similar outcomes to those in receipt of DBD organs.

There remains substantial scope to increase the numbers of donated DCD donor organs. NHSBT flagship document 'Taking organ donation to 2020' was published in 2013. This multiagency strategy document considered the direction of travel of DCD donation and looked at potential areas where donor numbers and organ quality could be improved. Application of these strategies to the DCD donation process has the potential to increase DCD donation in the following ways:

### **1.10.1 Improving consent to donation rates**

A key strategy outlined in the Taking Organ Donation to 2020 was to increase consent rate to in excess of 80%. At that time, the consent rate was 57%. According to the NHSBT 2017/18 annual activity report this figure is now 66%, leaving significant work remaining to achieve the target. Suggested focus for further increases in the consent rate hinge around early involvement of the SNOD team and utilisation of a collaborative approach. As previously described, recent review of the switch to the 'opt out' policy in Wales, which presumes consent for donation unless previously specified otherwise, has seen a large increase in consent rates (NHSBT 3 year review of Welsh system). Taking donation to 2020 also notes that consent rates could be improved if the logistics of DCD donation were streamlined. Of consented patients in 2017/18 who did not go on to donate, in 5% of cases the cause listed is 'family changed their minds'.

### **1.10.2 Improving optimisation of DCD donor organs**

The NHSBT Annual Activity Report for 2017/18 states that each DCD donor donated an average of 2.7 organs. By contrast, each DBD donor donated 3.7 organs on average. Were the DCD donation potential able to be brought up by one organ per donor, to be in line with

the DBD donation potential, this would equate to an extra 619 donated organs per year. All but 23 DCD donors in 2017/18 donated their kidneys, and of the 1179 retrieved organs 1024 were transplanted (87%). Of the 299 retrieved livers 201 were transplanted (67% of retrieved) and of 82 pairs of donated lungs 74 were transplanted (90%). Consequently, the potential for donor expansion in terms of organ optimisation lies is greatest for liver grafts, but still has the potential to influence the numbers of kidneys and lungs transplanted. No data are available for the numbers of DCD hearts not transplanted after retrieval.

A discussion regarding optimisation of donor physiology is possible using one of the two following approaches:

1. *Targeting physiological targets for optimisation, based on an understanding of terminal physiology in the DCD donor.* These approaches are the focus of this thesis. However, at present no intervention is ethically or legally permitted in the DCD donor prior to death in order to facilitate donation or improve organ quality. Exploration of the ethical and legal argument underpinning this position is beyond the scope of this thesis. This current legal position is under consideration by NHSBT and the Human Tissue Authority and may be subject to change in the future. However, any intervention would require evaluation to demonstrate it did not alter the likelihood of a patient demise. Taking Organ Donation to 2020 suggests that the responsibility lies with UK Health Departments, national ethics organisation and professional bodies to 'review what pre-mortem interventions could legally and ethically be undertaken to maximise the potential for organ donation'.
2. *Machine perfusion and assessment techniques.* While it is beyond the scope of this thesis to consider techniques that allow *in situ* normothermic regional perfusion or *ex situ* organ perfusion assessment of organs prior to transplant, these techniques are valuable platforms for organ optimisation. They allow for dynamic assessment of organs prior to their transplant, allowing for the retrieval of potentially marginally organs which can be assessed prior to their implantation. They also provide a potential platform to initiate the treatment of donor organs prior to their transplantation, thus bypassing the current ethical difficulties in treating the DCD

donor prior to death. The understanding of the physiological changes in the withdrawal period displayed later in this thesis allow the identification of potential targets to minimise or ameliorate organ damage. Taking Organ Donation to 2020 suggests 'that research is supported that will identify organs that are associated with good or poor function and lead to new pharmacological approaches to improve organ function'.

### **1.10.3 Development of programmes to use uncontrolled DCD donors**

Other EU nations have substantial success in their uncontrolled DCD programmes, most notably France and Spain. Spain operates an 'opt out' donation policy, which supports their uncontrolled DCD activity. The French programme requires next of kin consent but is supported by legislation that allows intervention prior to consent (Delsuc et al 2018), and reports good outcomes in renal grafts (Demiselle et al 2016). Early DCD transplants in the United Kingdom were from uncontrolled donors, and while uncontrolled programmes have existed in the UK until relatively recently, none exist at present. Evaluation of the more recent UK programmes found them not supported by the general public, with low team mobilisations and few retrieved organs. Were the UK to change its organ donor register policy to an 'opt out' system, it is possible that an uncontrolled DCD donor programme would have improved public support.

## **1.11 Chapter summary and hypothesis**

The DCD organ donor presents the unique opportunity to study the physiology of circulatory death and to consider its effects on organs donated for transplant.

Evidence from the study of brainstem dead donors demonstrates that the environment in which organs are functioning in the donor prior to their retrieval contributes to the burden of injury suffered by the graft. Furthermore, evidence from the donor optimisation programme and studies that have modulated the donor environment in the DBD donor

demonstrate that organ treatments instituted prior to retrieval can influence graft function in the recipient. Despite this evidence, the donor environment during the withdrawal of life supporting treatment in the DCD donor has never been the subject of intensive study. This withdrawal period, by necessity, involves an often-prolonged period of warm ischaemia, which the evidence shows has deleterious effects on how well an organ functions in the recipient.

Consideration of the evidence in this chapter produces several hypotheses that will be addressed by this thesis.

1. Physiological changes in the DCD organ donor can be measured and quantified.
2. A marker for the onset of warm ischaemia in an individual donor can be identified.
3. There is activation of the hypothalamic-pituitary axis during cardiorespiratory death in the DCD donor.
4. There is activation of the immune system in the DCD donor prior to cardiorespiratory death.
5. Targets for potential intervention in the DCD organ donor can be identified.

The studies described in this thesis are entirely novel, in being the first attempt to intensively study DCD organ donors during the withdrawal of life supporting treatment. In addition to the scientific merits of this study, it also provides the opportunity to explore the public perceptions of the appropriateness of consent and intensive study of the potential DCD organ donor.

## **1.12 Thesis overview**

Chapter 2 will deal with the ethical and legal issues of consent for research in the potential DCD organ donor. Chapter 3 will present the findings of public and patient engagement work undertaken to support this study. Chapter 4 will present the methodology used in patient recruitment, study conduct and sample assessment. Chapter 5 will present the demographic information for the recruited study participants and will outline the observed physiological

changes during the withdrawal period. Chapters 6 and 7 will examine the measurement of oxygenation and cardiovascular physiology in the proceeding DCD organ donor in order to address the second hypothesis. Chapter 8 will present evidence for hypothesis 3, the activation of the Hypothalamic-Pituitary-Adrenal axis in the DCD donor. The final results chapter, Chapter 9, will address hypothesis 4 by exploring evidence for activation of the immune system during the withdrawal period. The discussion, results and conclusions will form Chapter 10, the final chapter of this thesis. This chapter explores hypothesis 5 – the identification of targets for potential intervention in the DCD organ donor.

## **Chapter 2: The ethical and legal considerations of research consent in the potential DCD organ donor.**

### **2.1 Introduction and chapter overview**

This chapter will present the ethical and legal considerations taken into account when designing a study that recruits potential DCD organ donors prior to circulatory death. The first part of this discussion is a consideration of the legal and ethical framework that permits research in patients who lack the capacity to consent, with specific focus on the DCD donor and how the Mental Capacity Act (MCA) may be applied to their specific situation. Subsequently there is a discussion of the assessment of best interests in the potential organ donor, and how these principles may be applied to the question of research in this setting. Finally, this chapter considers the Human Tissue Act of 2004 and how it may be applied to the potential DCD organ donor.

This chapter does not consider the ethical and legal framework that underpins DCD donation in the United Kingdom in 2018. The success of the UK DCD programme over the last 10 years may be attributed to the successful resolution of the apparent legal, ethical and professional obstacles to this model of donation. DCD donation is legitimately viewed as part of the care that a person might wish to receive at the end of their lives in the United Kingdom, and a discussion of the work that has been undertaken to achieve widespread acceptance of the programme is beyond the scope of this thesis.

The study that formed the basis of this thesis was the first proposed study of potential DCD organ donors prior to death. Consequently, careful consideration of the relevant legal and ethical guidance around research consent in this patient cohort has been essential for the creation of a study which is acceptable to donor families, medical professionals and the local and national agencies required to approve research studies.

## 2.2 Assessment of capacity to consent in research

In order to consider the mechanisms for consent in the potential DCD organ donor, it is first necessary to confirm that the patient does not have capacity to themselves give consent for involvement in research. This question can be answered by consideration of the requirements for capacity as outlined by the 2005 Mental Capacity Act (MCA). The five statutory principles are outlined in the Section 1 of the Mental Capacity Act 2005 (Department of Health 2005). The act is designed to protect those who lack the capacity to make a decision, while allowing a person to participate as far as they are able to do so in the decision-making process. The act states:

- 1. A person must be assumed to have capacity unless it is established that he/she lacks capacity.*
- 2. A person is not to be treated as unable to make a decision unless all practicable steps to help him/her to do so have been taken without success.*
- 3. A person is not to be treated as unable to make a decision merely because he/she makes an unwise decision.*
- 4. An act done, or decision made, under this Act for or on behalf of a person who lacks capacity must be done, or made, in his/ her best interests.*
- 5. Before the act is done, or the decision is made, regard must be had to whether the purpose for which it is needed can be as effectively achieved in a way that is less restrictive of the person's rights and freedom of action.*

These five principles outline the requirement that capacity must be established on an individual basis and that decisions made for an individual who lacks capacity must be made in their best interests. What constitutes best interests for the potential DCD organ donor with regard to research is an important consideration and will be examined in detail in section 2.4 below.

Having outlined the requirement that capacity must be assessed upon an individual basis, it is subsequently necessary to determine the fundamental requirements for capacity. In order to make this determination, a two stage assessment is required to consider:

1. Does the patient have an impairment, or a disturbance in the functioning, of their mind or brain?
2. Does the impairment or disturbance mean that the person is unable to make a specific decision when they need to?

The potential DCD organ donor is typically a patient who has suffered a catastrophic intracranial insult, of sufficient gravity that the family and treating clinical team consider a return to a functional level that would be compatible with the patients prior expressed wishes impossible. Such a patient clearly fulfils criteria one and two outlined above – they have a disturbance in the functioning of their brain due to the pathology that has caused their ICU admission, and consequently they are unable to make a specific decision. Hence, in the majority of potential DCD organ donors, lack of capacity can be established based upon the above criteria. However, in certain, less common cases the potential donor may not be suffering from an impairment of their mind for example a patient dying of lung disease with no other organs involved. In such cases, further assessment of capacity must be made. Capacity in these circumstances is decision specific, and in order to demonstrate that they have capacity the patient must be able to:

1. Understand the decision to be made and the information provided about the decision. The consequences of making a decision must be included in the information given.
2. Retain the information given for long enough to make the decision.
3. Weigh and balance the information given to make their decision.
4. Communicate their decision – all efforts should be made to help the person communicate their decision.

Fulfilment of these criteria in an intensive care patient reliant on mechanical ventilation and other organ support may be challenging, requiring skilled personal and substantial time input to allow assessment of capacity.

Consideration of the criteria for assessment of capacity outlined in the MCA demonstrates that in the vast majority of cases the potential organ donor lacks capacity to make a decision regarding participation in research studies.

## **2.3 Consultation of deputies regarding involvement in research.**

Once it has been established that a patient lacks capacity to agree to participate in research, the researcher is required to consult with a specified individual prior to including that person in a study. This person is known as a 'Consultee' and must be an individual involved in the patient's care, and welfare but must not be a healthcare professional or paid care worker. This usually implies seeking the opinion of the next of kin, or close family member of the patient. The Consultee should provide an account of the patient's previously expressed wishes and feelings regarding involvement in research. Specifically, the Consultee should be provided with detailed information regarding the research project and should be asked:

- for advice whether the patient who lacks capacity should take part in the research, and:
- what they think the patient's feelings and wishes would be regarding the research if they had the capacity to make their own decision.

Should the Consultee indicate that the person would probably not have wanted to be involved in the study they should not be included in the project. It is recommended that the Consultee signs a declaration form regarding the patient's known wishes and the information they have used to come to their decision. This form should be held in the patient's medical records.

## **2.4 Consideration of what constitutes best interests in the potential donor?**

The MCA outlines that decisions made for a patient who lacks capacity to make their own decisions '*must be done, or made, in his/ her best interests.*' The assessment of what constitutes best interests when considering the subject of research in the potential DCD organ donor is complex. The UK Donation Ethics Committee document 'An Ethical Framework for Controlled Donation After Circulatory Death Consultation' suggests a best interests decision in this context should include consideration of the following factors (UK Donation Ethics Committee 2011)

*a) the person's known wishes and feelings, in particular any relevant written statements;*

*b) the beliefs or values that would be likely to influence the person's decision if they had the capacity to make it;*

*c) any other factors they would be likely to consider if they were able to do so;*

*d) the views of the person's family, friends and anyone involved in their care as appropriate as to what would be in the person's best interests; and*

*e) anyone named by the person to be consulted about such decisions. (UK Donation Ethics Committee 2011)*

When considering decisions, the UK courts have established that a person's best interests are wider than simply treatment of their current medical condition. Best interests decisions include the assessment of a person's social, emotional, cultural and religious interests, and the MCA Code of Practice emphasises the importance of considering all of these aspects, including past behaviours and habits, in assessing a person's best interests.

## **2.5 The organ donor register and research decisions**

### **2.5.1 The Organ Donor Register**

As outlined above, best interests decisions should include consideration of the persons 'known wishes and feelings, in particular relevant written statements'. A clear opportunity to assess a written expression of known wishes and feelings in the potential organ donor is through consultation of the Organ Donor Register (ODR). Consultation of the ODR is an early step made by the Specialist Nurse in Organ Donation when referred a potential donor, allowing them an opportunity to assess the potential donor's views on organ donation. The ODR is a confidential list of individuals who wish to donate their organs and/or tissues upon their death. It is maintained by NHS Blood and Transplant and is signed by the individual, in health, at a point of presumed capacity. Legally, this counts as an advanced directive, a decision which cannot be overturned once the patient is in a position where they are unable

to make or express their own decision (UK Donation Ethics Committee 2011). Guidance given by the UK Donation Ethics Committee states

*'In general terms, decision-making will be guided by the person's wishes and beliefs concerning donation. It is therefore important to establish these either through knowledge of the individual's wishes (for example, by registration on the NHS Organ Donor Register (ODR)) or through an assessment of what the individual would have wanted (for example through the person's family and their knowledge of them). If a person's wishes were to be a donor, then certain actions which facilitate donation may be considered to be in their best interests if they do not cause the person harm or distress or place them at a material risk of experiencing harm or distress.'*

The Organ Donor Register states, however, that no research on organs will take place without the approval of family members. It makes no mention of research which enrolls potential donors prior to death.

The emphasis of importance of the ODR when taking into account a patient's prior wishes is emphasized by NICE guideline 135 'Organ donation for transplantation: improving donor identification and consent rates for deceased organ donation' which considers the '*registration and recording their consent to donate on the NHS organ donor register*' a method of 'establishing the patient's prior consent to organ donation'. (NICE CG 135, recommendation 1.1.9)

### **2.5.2 Application of the ODR to the question of research:**

Inherent in signing of the ODR is a decision made by a competent patient that they wish to help others in the event of their death. The person signing the register is aware that they will reap no benefit from this decision. The decision is purely altruistic, it will only help other people, with no benefit to the donor. This altruistic desire to help others in the event of their own death allows inference of the individual's values at a time when they were able to express them. When considering the question of research studies, it would therefore follow that studies which aim to increase the likelihood of organ donation being successful and increase the rate of transplant graft success should be in keeping with the donor's wishes.

## **2.6 Ensuring that organ donor research meets the Mental Capacity Act's requirements.**

It is important to ensure that a research proposal has taken into account the MCA's requirements before it enrolls patients who lack capacity. This responsibility lies with 'the appropriate body' as defined in regulations made by the secretary of state and with the researchers carrying out the research. An 'appropriate body' is an organization specifically designated as appropriate to approve research projects. In England this 'appropriate body' must be a Research Ethics Committee which is recognized by the Secretary of State (Department for constitutional affairs 2007)

The appropriate body may only approve a research project in patients who lack capacity if the research is linked to:

- an impairing condition that affects the person who lacks capacity, or
- the treatment of that condition, and
- there are reasonable grounds to believe that the research would be less effective if only people with capacity are involved.

The MCA requires that a research project making an application to enroll patients who lack capacity has made arrangements to consult patient next of kin/ deputies and that it's other requirements regarding consent and capacity are followed.

Application of the above statements to the potential DCD organ donor is straightforward. The impairing condition that affects the patient is their severe brain injury, the treatment of that condition is the decision that ongoing medical treatment is futile, and that invasive treatment should be withdrawn. It is clearly not possible to undertake research in potential DCD donors exclusively in a cohort that has capacity, given the nature of the potential donor's underlying medical condition.

Research conducted in a patient who lacks capacity should also meet one of two further requirements:

1. The research must have some chance of benefiting the person who lacks capacity, and this benefit must be in proportion to any burden caused by taking part.

According to the UK Donation Ethics committee potential benefits of research for a person who lacks capacity could include:

- *development of more effective ways of treating or managing their condition*
- *improvement in the quality of healthcare and services that they have access to*
- *discovering the cause of their condition, if they would benefit from that knowledge*

Application of the above statements to the case of the potential DCD organ donor is not straightforward. The DCD donor will receive no benefit from their involvement in the study, any benefits will only be felt by future DCD organ donors or organ transplant recipients. It is however noted that benefit may be direct or indirect and may occur at a later date. Consequently, it is possible to argue that fulfilling the wishes of the organ donor is of benefit to them, as donation constituted their previously expressed wishes. Given that these wishes were to donate organs upon their death, research which aims to increase the success rates of donated organs could be argued to be beneficial to the donor.

2. The aim of the research must be to provide knowledge about the cause of, or treatment or care of people with, the same, or similar, impairing condition.

Should a study be relying upon this requirement to gain approval, there are further requirements that must additionally be met. These criteria are that:

- The risk to the person who lacks capacity must be negligible*
- There must be no significant interference with the freedom of action or privacy of the person who lacks capacity*
- Nothing must be done to the person who lacks capacity which is unduly invasive or restrictive*

The application of the above criteria to the patient undergoing withdrawal of life supporting therapy and potentially becoming a DCD donor is more straightforward. Research in the potential DCD organ donor, which does not benefit the individual taking part, would be acceptable given it aims to provide 'knowledge about the causes, treatment or care of people with the same impairing condition, or a similar condition'. A 'similar condition' is

defined as having a different cause but translatable consequences to the individual's pathology.

The underlying pathologies affecting potential DCD organ donors are wide ranging (NHSBT potential donor audit 2017/18). However, these conditions have all resulted in irreversible brain injury and a consensual decision that ongoing treatment is futile, not in the patient's best interest, and that withdrawal of life-supporting therapies should occur. As such, research in potential DCD organ donor undergoing withdrawal of life supporting therapies can be considered to be providing knowledge about the care of people suffering with a similar condition. Indeed, the Department of Health 2010 guidance 'Legal issues relevant to non-heartbeating donation' states that:

*Once it has been established that a person wanted to donate, either through direct knowledge of their wishes or as a result of discussions about what the person would have wanted, successful donation may be seen to be in the person's wider best interests in a number of ways:*

*a) by maximising the chance of fulfilling the donor's wishes about what happens to them after death;*

*b) by enhancing the donor's chances of performing an altruistic act of donation  
(Department of Health 2010 guidance)*

The patient should suffer no harm or distress by taking part in the proposed research. This should include their psychological wellbeing as well as physical wellbeing, and it would be considered good practice to include the psychological wellbeing of family in this consideration.

The MCA code of conduct suggests that actions will not usually be classed as unduly invasive if they do not go beyond the experience of 'routine medical care'. The potential DCD organ donor will be dependent on invasive treatment provided by the intensive care unit, much of which is guided by regular blood sampling taken from indwelling arterial and central venous catheters placed for that specific purpose. Given that these catheters are already in place, it may be considered that taking blood samples from these pre-existing lines would not be unduly invasive and represents an extension of routine medical care for an ICU patient.

Clinicians will therefore need to decide if taking blood and testing blood or serum samples are in the potential donor's best interests. This will include considering if the person wanted to be a donor and whether these steps contribute to fulfilling that wish (GMC guidance). Clinicians will also need to consider the risk of any harm or distress that may be caused to the person, including consideration of the information the tests may generate (DeVeaux 2006, Dare et al 2012)

There is a precedent for the study of blood from the prospective organ donor within the UK - the Quality in Organ Donation (QUOD) study. This is a study that aims to form a biobank of samples from organ donors to be used for future research projects. At present, this study has a 63% consent rate for the storage of future samples for as yet unspecified research.

## **2.7 Research involving human tissue - The Human Tissue Act 2004**

The Mental Capacity Act allows the removal of tissue from the body of a person who lacks capacity, if it is in their best interests. The act does not specify what best interests involve - as previous discussed, what constitutes best interests is a complex and situationally dependent decision. Decisions made around the use of research involving human tissues are under the remit of the Human Tissue Act 2004 (HTAct).

Individuals with capacity must give their permission for the use of tissues for research (HTA 2004). If an adult lacks the capacity to consent, the HTAct says that tissue can be stored or used for research without seeking permission if:

*-Its use meets the Mental Capacity Act's requirements, and*

*-The proposed study has ethical approval*

In some circumstances, no consent is needed to lawfully involve a person in research, regardless of whether or not they have capacity. Under the Human Tissue Act 2004, research that deals with human tissue that has been anonymised does not require consent. This applies to both those who have capacity and those who do not. However, the research must have ethical approval, and this tissue must come from a living person. Hence, a study

including samples taken from a potential DCD donor prior to death requires approval under the Mental Capacity Act but does not fall under the remit of the Human Tissue Authority. As previously discussed, in the vast majority of cases the potential DCD donor will lack capacity.

Decisions about research involving donor organs once the person has died will be governed by the Human Tissue Act. The HTA also governs the testing of existing blood samples after death and, in the case of a person who lacks capacity, such decisions also have to be made in the person's best interests (Bell 2006).

## **2.8 Chapter Summary**

This chapter has focused upon detailed discussion of the ethical and legal considerations that require assessment when undertaking a research study in the potential DCD organ donor. Consideration of these issues when enrolling patients who lack capacity to consent in research into studies is key in order to perform high quality research which sits within the accepted UK ethical and legal frameworks. This chapter has considered the contributions of the Mental Capacity Act and Human Tissue Acts towards defining what is acceptable practice in research studies involving patients who lack capacity. Detailed discussion has focused upon what constitutes a best interests decision in the potential DCD organ donor, and how the concept of best interests could be extended to include research studies in this patient cohort.



## **Chapter 3: Public and Patient Involvement and Engagement work**

### **3.1 Chapter overview and Introduction.**

In chapter 2 I examined the ethical and legal framework for undertaking research in the potential DCD donor who lacks capacity to consent. Fundamental to the guidance for undertaking research in this patient cohort is seeking the opinion of a 'Consultee' to understand the patient's pre-existing wishes. The consultee should have the proposed research study explained to them in detail and should be asked to express an opinion on whether the patient should be involved in the research.

The research that is presented in this thesis is the first study that aims to recruit potential DCD organ donors for intensive study prior to circulatory death. The proposed study involves collection of physiological data and regular blood sampling during the period between withdrawal of treatment and circulatory death.

In order to understand whether the research study that underpins this thesis would be acceptable to members of the public (the cohort who are likely to act as consultees) a programme of public and patient engagement work was undertaken. The aims of this work were

- a. To understand if the proposed research study would be acceptable to members of the public
- b. To determine public opinion regarding whether it was appropriate for a 'Personal Consultee' to give consent for research

This chapter will firstly present the precedent for 'Personal Consultee' research authorization, with a consideration of studies and settings which utilise this consent model. Then will follow a presentation of a survey of 248 members of the public to ascertain opinion of the research proposal and the appropriateness of 'Personal Consultee' consent for research in the potential DCD organ donor cohort accompanied by a discussion of the survey results. Finally, this chapter will examine the outputs generated from a small focus group meeting involving representatives of the Donor Family Network, Kidney Patients Association and LIVER North.

## 3.2 Precedent studies

As detailed in chapter 2, mental capacity legislation, the Mental Capacity Act 2005, exists which allows for proxy decision making by a next of kin, known as a 'Consultee', for a patient lacking the capacity to consent (Department of Health 2005). Under this Act, should a person lack capacity to make their own medical or social decisions, and in the circumstance where no valid advanced directive has been made, a relative can be given lasting power of attorney to make such decisions. Should there be no lasting power of attorney, the closest available relative should be consulted about the patient's views, and his or her opinion only be disregarded should it be felt to not represent the patient's best interests (Department of Health 2005).

A detailed examination of the physiological changes occurring in the DCD organ in the period between treatment withdrawal and circulatory death has not previously been undertaken. The study that forms the basis of this thesis will be first of its kind. Subsequently, the attitudes of donor families to research during this period are unknown, and consent rates for research of this type are unpredictable. However, it is possible to consider research studies carried out in the critically ill patient or the patient requiring emergent surgery as potential precedents, given that a. these patients are often critically ill and lack decision making capacity and b. complex decisions are being asked of next of kin at a time of substantial emotional distress and stress.

Research studies in patients who lack capacity are increasingly commonplace in the emergency setting (CRASH-2 collaborators 2011, Clark et al 2013) and in the end of life care setting (Livingston et al 2010, Mezey et al 1996, Potkins et al 2000). The European Union Clinical Trials Directive 2001/20/EC outlines the requirement for informed consent of a legal representative to be in place prior to the enrolment of an incapacitated person into a clinical trial of an investigational medicinal product (European commission 2012). The Mental Capacity Act states that following discussion with an appropriate consultee to guide decision making an incapacitated adult can be enrolled into a research study (Mental Capacity Act 2005). This legal representative is a person who, by virtue of their relationship with the patient, is in a position to act as their legal representative for the purpose of the trial and is willing to do so. Previous studies enrolling patients who lack the ability to consent have

noted the logistical difficulties inherent in the conduct of the study (Kim 2011). The UK NHS Health Research Authority (HRA) advises investigators to undertake 'Community Consultation' prior to undertaking a research study in which the participant will be unable to provide consent prior to enrollment due to 'incapacity' (HRA)

In the practice of seeking consent for organ donation, consultation with the legal representative regarding consent for donation is normal practice (Vincent et al 2012), and this is independent of whether the patient is on the organ donor register. The current organ donation consent form already asks the legal representative to consent for the patient's inclusion in a variety of research projects which perform research on patient samples and patient organs after death (Radecki et al 1997). Our proposed study is the first to seek to collect data and take blood samples during the dying process. We aimed to assess the public's opinion regarding the use of a legal representative 'Personal Consultee' to consent for participation of the potential organ donor in research by conducting a community consultation survey.

### **3.3 Survey**

#### **3.3.1 Methods**

A cross-sectional study was conducted across varying locations in Cambridge between October 2016 and May 2018. A sample of hospital outpatient attenders, patient family members and friends in the outpatient waiting area and hospital main concourse, and members of the general public on the street in central Cambridge were asked to complete a short survey.

The survey instrument included a summary of the proposed research study, an explanation of the purpose of the study and a questionnaire. The summary included a description of the process of DCD organ donation, the research question that the study was designed to answer, and a description of the necessity for 'Personal Consultee' consent for research in this patient cohort. Due to the relative complexity of the information covered in the summary, after verbal consent, the summary information and explanation of the proposed research was read to the participants, who were then given the opportunity to ask any questions about the information covered. All survey documents were worded in lay

terminology and took between 5 and 20 minutes to describe to most participants. It was emphasized that the survey participants were not making any commitment to participate in the research study. Participants were then asked some basic demographic information, and the two core survey questions in a verbal interview format:

Question 1: Would you be willing to consent for your relative to be involved in this study?

Question 2: Would you find your next of kin/ Personal Consultee giving consent for your involvement in this research study acceptable?

Survey participants responses to questions 1 and 2 were quantified using a five-point Likert scale, with available responses of 'Absolutely not, Probably not, Neutral, Probably yes and Absolutely yes. The demographic information collected included age, sex, ethnicity, religious beliefs, total household income and level of educational background. Survey participants were asked to disclose if they had any personal experience of organ donation and organ transplantation (personal experience was defined to the participant as 'close friend or family member'). The questionnaire data were collected anonymously. Survey participants were provided with contact details for the research team should they have any questions after the survey was completed.

Data were analysed using Prism (Graphpad Software, La Jolla, CA, USA). Study demographic information are given as frequencies and percentages. Non-binary characteristics were dichotomised into categories as suggested by the Office of National Statistics (ONS): Harmonised Concepts and Questions for Social Data Sources (Office for National Statistics 2017). Ethnicity categorized as: White (English / Welsh / Scottish / Northern Irish / British/ Irish/ other White background), Mixed / Multiple ethnic groups, Asian / Asian British (Indian, Pakistani, Bangladeshi, Chinese, other Asian background), Black / African / Caribbean / Black British (African, Caribbean, other Black/ African / Caribbean background), Other ethnic group (Arab, Any other ethnic group). Other categories as suggested by the ONS are as given in table 3.1 below.

The results were collated and are given below in Figure 3.1 and 3.2. Respondents were also given the opportunity to provide feedback regarding the study.

### 3.3.2 Survey Results

Two hundred and forty-eight participants completed the survey. Thirty-six people were approached and declined to take part in the survey. The characteristics of the study population are shown in Table 3.1 below. Seven percent of participants had personal experience of organ donation or transplantation. Figures 1 and 2 demonstrate the survey participants responses on willingness to consent for a relative being involved in the research study (figure 3.1) and the appropriateness of a person consultee giving consent for involvement in research (figure 3.2). Of 248 respondents 71% were positive with regard to providing consent for a relative to be involved in this research study (answering probably yes or absolutely yes). With regards to the acceptability of a personal consultee providing consent for involvement in a research study for someone who is unable to consent 75% responded positively.

The only demographic characteristic that affected the decision of whether a participant would consent for a relative being involved in the research study was religion, where respondents who stated their religion as 'Muslim' were significantly less likely to consent for involvement in research ( $p=0.023$  by Fisher's exact tests with Bonferroni correction for multiple correction)

<b>Characteristic</b>		<b>n (%)</b>
Age (years)	18-35	52 (21%)
	36-45	58 (23%)
	46-65	91 (37%)
	66-80	44 (18%)
	81+	3 (1%)
Gender	Male	109 (44%)
	Female	139 (56%)
Ethnicity	White	141 (57%)
	Mixed/ Multiple ethnic groups	61 (25%)
	Asian / Asian British	33 (13%)
	Black / African / Caribbean	6 (2%)
	Other ethnic group	5 (2%)
Religion	No religion	44 (18%)
	Christian	169 (68%)
	Buddhist	0 (0%)
	Hindu	1 (0.5%)
	Jewish	8 (3%)
	Muslim	25 (10%)
	Sikh	1 (1%)
	Other religion	0 (0%)
Total household income (£)	<20,000	45 (18%)
	20,000-40,000	109 (44%)
	40,000-60,000	72 (29%)
	>60,000	22 (9%)
Highest level of qualification	None	35 (14%)
	GCSE	47 (19%)
	A-Level	45(18%)
	Diploma/ Apprenticeship	42(17%)
	Undergraduate Degree	62 (25%)
	Post-graduate Degree	17 (7%)
Personal experience of organ donation or transplantation	Yes	17 (7%)
	No	231 (93%)

*Table 3.1: Demographic information of survey participants. Total number of respondents 248*

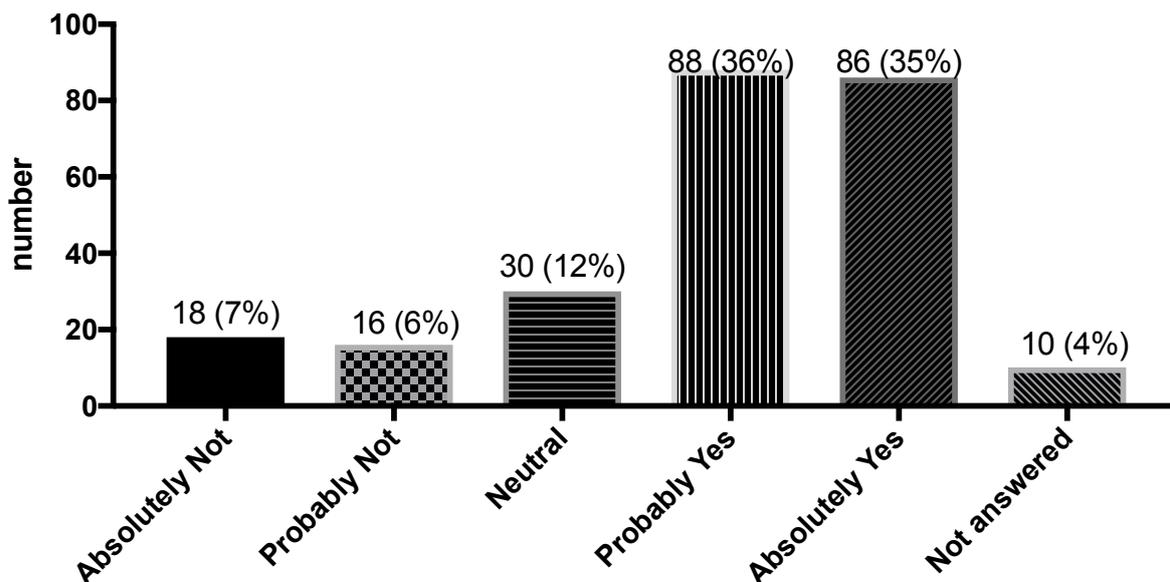


Figure 3.1: Willingness to consent for a relative being involved in this research study. Total number of respondents 248.

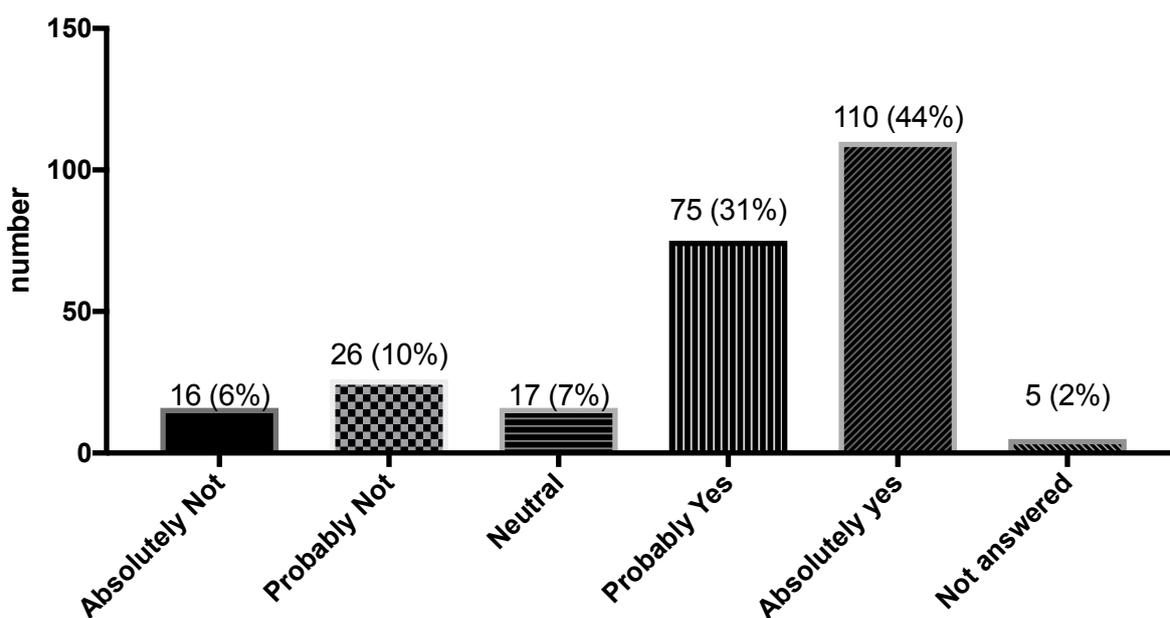


Figure 3.2: Acceptability of consent for research being provided a 'personal consultee' for a patient who cannot give consent. Total respondents 248

## 3.4 Focus group meeting

### 3.4.1 Donor Family Network meeting

A significant challenge posed in ascertaining the opinion of patient groups regarding the appropriateness of research questions in the potential DCD organ donor is that all patients included in the study are recruited in the anticipation of their death. There is no mechanism for seeking the opinion of a DCD organ donor regarding research. Traditional patient groups, consisting of patients with a specific illness, are clearly not available for a study of this nature. While living kidney donation is relatively common, and patient groups of living kidney donors exist, the decision-making process and situational stresses behind this type of organ donation are different to those underpinning DCD organ donation. However, groups of donor relatives exist (The Donor Families Network) which consist of families and friends of patients who have become organ donors. Given that it will be family members or close friends are likely to act as the patients Personal Consultee in the decision making process of consenting for research, the opinion of group members on our study question was felt to be valuable. These family members are usually the next of kin, or close family members, of previous organ donors. As such, they are likely to have previously given authorization for organ donation and are more likely than an average member of the public to be open to research that improves the quality of the organ donation experience.

The process of gaining family or carer's opinions on research questions for incapacitated patients is well established in the fields of palliative care and dementia research (Livingston et al 2010, Mezey et al 1996, Potkins et al 2000). Contact was made with the Donor Families Network, and two donor family members who had been involved in DCD donation agreed to answer some written questions and review the 'Personal consultee information sheets' produced as part of the study. These two individuals gave some anonymous feedback regarding the study documents and the lay terminology used, which were incorporated into the final document versions. They also provided the below written statements which they gave permission to be shared anonymously:

*'I find this to be an interesting study which answers questions that we had as a family in the lead up to X becoming an organ donor'*

*'The whole process of organ donation was totally overwhelming to us, and knowing that there might not even be a donation after going through going to theatre was very off putting. We really wanted X to be an organ donor, because he had been on the organ donor register. After the series of shattering events that led up to X dying, the thought that he might not be an organ donor after everything was almost unbearable.'*

*'We agreed to some research studies that the specialist nurse spoke to us about. Dad being on the donor register meant he wanted to help people if he died, and we felt that the research was just an extension of that desire'*

These statements demonstrate a willingness to consent for research that will help others in their position, and a feeling that their next of kin's altruistic attitude in signing the ODR was an expression of willingness to help other people in the event of their death.

#### **3.4.2 NIHR Blood and Transplant Research Unit Public and Patient engagement event**

A mixed group of organ transplant recipients and the general public were interviewed via Public and patient engagement meeting held for the Blood and Transplant research unit (BTRU) in Organ Donation and Transplantation held and Newcastle University. This meeting was an open invitation meeting, designed to showcase the research studies being proposed by the BTRU and was entitled:

*'NIHR Organ Donation and Transplantation Research Unit Patient and Public Involvement and Engagement Event: The Future of Transplantation Research'*.

The meeting was well attended by local patient group representatives and members, interested members of the public, and local media representatives. The meeting took the format of presenting a current problem in the field of transplantation, followed by a research study that aimed to provide answers to the problem in question. This was followed by an opportunity for the audience to ask questions about the study, make suggestions regarding its format and review the prepared patient information sheets.

The protocols for this study were presented under the title of 'Enhancing the availability of organs for transplantation' and led to focused discussion around how to predict the time to death after withdrawal of life supporting therapy in the potential DCD organ donor, and how to predict which organs will function well after transplantation and which will function poorly. This talk generated lively discussion during the following question and answer session. Patient representatives from 'LIVER NORTH' and 'Kidney Patients Association' reviewed the patient information sheets and provided input into the lay wording of several sections. It was also suggested that a website portal be produced that would allow the results of the study to be disseminated to interested members of the general public.

### **3.5 Chapter summary and discussion**

The results of the public survey presented in section 3.3.2 above demonstrate that the majority of members of the public would find pre-mortem study of potential DCD organ donors agreeable. Furthermore, it shows that our proposed method of seeking next of kin/ Personal consultee consent is considered acceptable by the majority of the group surveyed. It is worth noting that 10% of study respondents replied to the survey that they would 'absolutely not' find the proposed study acceptable. On review of the questionnaires completed by those respondents that replied 'absolutely not' or 'probably not' to the question of whether this study would be considered acceptable 19 of 34 respondents stated their religion as Muslim in the demographics section of the survey questionnaire, which represents a significant result ( $p=0.023$ ). Religious barriers and cultural barriers to organ donation have been explored in multiple previous publications (Uskun et al 2013, Da Silva et al 2015, Gillman et al 1999). Further work to explore the impact of such barriers on the consent to research rate for donor relatives would be of substantial.

An unquantifiable but surprising result of the public survey presented in 3.3.2 is the degree of misunderstanding which exists in the public domain regarding DCD organ donation. A substantial period of time was taken with each survey participant (in some cases up to 30 minutes) talking through the research proposal, outlining the particulars of DCD organ donation and answering any questions they may have about the processes of organ donation

before asking any of the survey questions. Substantial misinformation appears commonplace in the public domain, particularly around the practice of DCD organ donation.

Discussion with representatives of the Donor Family Network gave useful insights into the feelings and opinion of individuals acting in the 'Personal Consultee' role. Of particular note is the statement regarding the attitude of a donor family member towards consent for research studies, in which they state they consider research consent an extension of their relatives wish to 'help others' in the event of their death.

Given the results of this survey, and the attitude of donor families to research, it seemed likely that consent for a research study in potential DCD donors would be reasonably high. Indeed, if family members, acting as 'Personal Consultees', adopted the attitude that consent for research in this patient cohort represents an extension of the patient's altruistic wishes to help others, research consent for studies such as ours may even exceed standard research consent rates.

The opportunity to have members of the public review the personal consultee information and declaration sheets was invaluable and gave rise to changes in terminology used which were well received when the study was appraised by the Research Ethics Committee.



## **Chapter 4: Methods and Materials.**

### **4.1 Introduction and chapter overview**

This chapter will present the scientific and statistical methods used in the conduct of the research presented in this thesis. Initial consideration will be given to the practical conduct of the study, with attention given to patient identification, inclusion and exclusion criteria, the process used to consent donor families and the logistics of sample collection. The focus of this chapter will then turn to the methods utilised for sample analysis and storage before consideration is given to the statistical analyses used to present the data. Finally, the procedure undertaken for gaining ethical and institutional approval for the study is discussed.

### **4.2 Patient identification**

#### **4.2.1 Setting**

Patients were recruited from a single tertiary centre (Cambridge University Hospital NHS Foundation Trust) which contains two adult intensive care units. Unit 1, The Neurosciences Critical Care Unit (NCCU) is a 23 bedded teaching hospital Neurosciences and Trauma ICU which admits approximately 900 patients per annum, predominantly from a Neurosurgery, neuro-trauma, neurology and major trauma background. This unit has the greatest number of organ donors annually from across the east of England and has a consistently high potential donor referral rate. Unit 2 (The John Farman Intensive Care Unit) is a mixed general ICU/high dependency unit with 24 beds, admitting approximately 800 patients per annum from a mixture of medical and surgical specialties with the exception of cardiothoracic surgery. Of these, 60% require level three care, and approximately 71% stay for more than 48 hours. Level three care is defined by the Department of Health (2001) as a patient requiring two or more organ support (or needing mechanical ventilation alone). This unit has particularly specialist skills in liver transplant assessment and post-operative care of transplant patients. They have a high potential donor referral rate but a relatively low

number of proceeding donors due to a high prevalence of absolute contraindications to donation in their patient cohort.

The hospital has a team of embedded SNODs (who form part of the Eastern SNOD team) who provide daytime cover of organ donor referrals. Out of hours cover is provided by the Eastern SNOD team.

#### **4.2.2 Patient identification by SNOD team**

The routine process for identifying donors is as follows: Potential donors are identified to the SNOD team as part of the routine end of life care process. Referrals can be made 24 hours a day from both nursing and medical staff. The majority of patients referred to the SNOD team are patients admitted to the ICU, although a proportion of referrals come from Accident and Emergency Department staff. Upon receiving a patient referral, the SNOD will screen for the presence of absolute contraindications (detailed in literature review) and will ascertain the patient's status on the organ donor register. As discussed in the Chapter 1, the preference is for a collaborative approach to the patient's family/decision maker to raise the potential for organ donation. The SNOD will meet with the family/decision maker (hereafter referred to as the 'Personal Consultee' and seek consent for organ donation via their normal protocols. This consent process includes consent for national research studies such as QUOD, and for the use of organs for research which are found to be unsuitable for transplantation after retrieval. Consent for the studies described in this thesis was sought at this point in the discussions.

At this point in the consent process the SNOD introduced the 'DCD donor physiology study' and sought permission from the personal consultee for a researcher to discuss the study further with them. With their agreement, a separate approach was then made to discuss the study further. This could follow on directly from the SNOD consent or be at a later point, depending upon researcher availability and personal consultee preference. Researcher availability was 24 hours a day 7 days a week throughout the study period, both for research consent and for sample and data collection after withdrawal of life supporting treatment.

### 4.3 Inclusion, exclusion and withdrawal criteria

Patients requiring full ventilatory support following catastrophic non-recoverable injury, but who do not fulfil brainstem death criteria and for whom the supervising clinicians and next of kin agree that there is no prospect of recovery were considered for this study, with the following inclusion and exclusion criteria applied.

#### Inclusion Criteria

- Admission to the intensive care unit
- Age > 18 years
- Situation in which a consensual decision to WLST has been made and there is an anticipation of imminent death. Patients must be considered eligible for DCD according to NHS Blood and Transplant Guidance with families who have been approached regarding donation after circulatory death.
- Subjects will have a minimum of the following bedside monitors in place:
  - Pulse oximeter plethysmography
  - Continuous 3-lead electrocardiogram
  - Invasive arterial blood pressure monitoring

#### Exclusion Criteria:

The presence of any of the following precluded study inclusion:

- Declared dead by neurological criteria with a plan for DBD donation
- ICU Consultant or member of the bedside healthcare team refusal
- Personal consultee declines patient enrolment in study or unavailable to obtain consent

Criteria for withdrawal or discontinuation of the study – the subject's involvement in the study ceased if:

- death was not reached within 4 hours
- withdrawal from the study requested at any time by the next of kin, specialist nurse in organ donation or supervising anaesthetist
- the researcher decides to stop studying the subject for any reason

#### **4.4 Consent process**

This study used a model of researcher-led consent. This is the first time that this consent model had been used alongside SNOD consent in the organ donation setting. The SNOD obtained permission from the personal consultee for approach by the researcher to discuss participation in the research study. This is in keeping with Good Clinical Practice guidelines. The researcher then outlined the study objectives, the practicalities of patient enrolment in the study, the study sample and data collection requirements and answered any questions that the personal consultee may have. A 'Personal Consultee Information sheet' detailing the above information was provided to the personal consultee, with a signed dated copy being entered into the patient medical records. If the personal consultee was in agreement for the patient enrolment in the study, a 'Personal Consultee Declaration' form was filled in, and a signed dated copy of this form entered into the patient medical records. While it was anticipated that most patients being enrolled into the study would be critically ill and lacking decision making capacity, DCD donation can be offered in selected circumstances to patients who have capacity, and consequently provision was made for direct patient consent using a modification of the personal consultee form.

#### **4.5 Withdrawal of life supporting treatment.**

No modification of normal procedures or timeframes for withdrawal of life supporting treatment (WLST) were made for this study. Within the institution, protocols for the location of treatment withdrawal differ between the two ICUs, with the NCCU favouring treatment withdrawal in the theatre complex and the general ICU favouring withdrawal occurring in the ICU bedspace. This preference is based on individual clinician preference and the physical distance of the ICUs from the theatre complex. The requirements for the study were met equally in both locations.

Treatment withdrawal included tracheal extubation and cessation of inotropic or vasopressor support. Infusions of medications for treatment of symptoms of terminal agitation, breathlessness or pain were continued as part of good end of life care. Family

presence at the bed space and activities of the SNOD during the end of life were encouraged and unhindered by the study.

#### **4.6 Demographic and longitudinal information collection**

Demographic data and routine clinical information collected during the intensive care admission were recorded by the study team. This information included age, sex, past medical history, underlying pathology necessitating intensive care unit admission, length of ICU stay and treatments received while an inpatient. Immediately prior to treatment withdrawal the researcher recorded all available physiological parameters, including: heart rate, systolic and diastolic blood pressure, mean arterial pressure, ventilatory mode, inspired oxygen fraction, pulse oximetry, spontaneous respiratory rate, heart rate, inotropes administered and their doses. Neurological condition was recorded as the Glasgow coma score, with breakdown into eye opening, response to voice and motor score documented. In patients who had had a protracted intensive care admission, or who had deteriorated during their intensive care stay, summary data of trends in the above variables were collected. For parameters which were found to have varied during the intensive care admission, an average of the value during the 24 hour period prior to withdrawal was recorded. Length of stay in intensive care included stay in a general intensive care unit prior to transfer to a tertiary unit. Time to death was calculated as minutes between withdrawal of life supporting therapy and mechanical asystole and did not include the 5 minutes stand-off time between the onset of mechanical asystole and certification of death. Once treatment withdrawal had occurred, physiological data were collected at two minute intervals for the first 20 minutes post withdrawal and at five minute intervals thereafter. This included Systolic, Diastolic and Mean arterial blood pressure from the arterial line trace, oxygen saturations by pulse oximetry, heart rate from ECG trace and respiratory rate. This is the standard data collected by the SNOD team and protocols for this monitoring being established prior to treatment withdrawal are well established. These data were collected in as unobtrusive manner as possible and there was no change to the usual end of life care provided. The patient's family were able to be present throughout the study procedures as per local practice and there were no restrictions on their

activities at the bedside as a result of participation in the study. The researcher did not participate in any aspect of end of life care.

Patients were followed until certification of death or a period of four hours had elapsed. Where a patient survived beyond the four-hour timeframe allowed for donation to proceed, the patient was no longer studied but all collected data and samples were retained.

## **4.7 Blood sampling setup**

In order for the study to be feasible it was necessary to take frequent and unobtrusive blood samples from subjects after WLST. The proposed blood sampling schedule was designed not to interfere with family presence or nursing care of the potential donor at the end of life. This required the development of a novel equipment setup. In order to demonstrate to the Research Ethics Committee that frequent samples could be taken in keeping with the above restrictions, a pilot study was undertaken using dummy patients to demonstrate the effectiveness of the equipment setup.

### **4.7.1 Methods**

An equipment system was developed that utilised 2meter lengths of high pressure low volume extension tubing to distance the sampling tap from the cannulae. Such a set-up is routinely used when providing anaesthesia for patients in the MRI scanner (Serafini et al 2008) and has been demonstrated as safe, effective and undamped system (NPSA 2008). This system allowed for sampling taps to be located under a folded towel at the foot end of the bed.

In order to assess that the above system allowed for inconspicuous sampling, a pilot study was undertaken. This study replicated the environment of the anaesthetic room for withdrawal of life supporting therapy and utilised undergraduate medical student volunteers in place of family members at the bedside. Four different volunteers were recruited for each scenario. They were asked to record every instance where they were aware of blood samples being taken while normal end of life care was being undertaken. A specialist nurse in organ donation and bedside nurse then provided routine care for a volunteer who acted as a

patient and engaged the four volunteers acting as patient relatives/ next of kin in conversation. Four scenarios were run, lasting 20, 40, 90 and 180 minutes with a set number of blood samples taken during each scenario. The results of these four scenarios are summarized in table 4.1 below.

These scenarios were also used as opportunities to practice the sampling and tube labelling routines prior to enrolling the first patient. The volunteers acting as family members were asked for comments at the end of the scenario to determine whether they had felt their activities at the bedspace had been hindered by researcher presence.

### 4.7.2 Results

The results of the four trial scenarios are demonstrated in table 4.1 below:

Scenario number Scenario length (minutes)	Sampling events performed (number)	Sampling events that the 'NOK' noticed Number (%)
Scenario 1 20 mins	5	2 (40%)
Scenario 2 40 mins	7	1 (14%)
Scenario 3 90 mins	12	3 (25%)
Scenario 4 120 mins	15	3 (20%)
Total	39	10 (26%)

*Table 4.1: Results of blood sampling feasibility study using volunteers for four mock scenarios of differing length.*

This pilot study of the blood sampling process proposed for use in the study demonstrated that:

- Arterial blood pressure trace and central venous pressure traces were not damped by the addition of low volume extension tubing into the system.
- it was possible to sample from a position at the foot of the bed (or further) without difficulty
- 26% of sampling events were noticed by volunteer sitting at the bedside.
- Volunteers felt that their movements at the bedspace were unhindered by the presence of the study team.

Patients who were consented for the definitive study had arterial and potentially central venous cannula placed as part of their routine care on the intensive care units. These cannulae were used to take arterial and venous blood samples for the purposes of the study without the need of subjecting the potential donor to further vascular access procedures. However, the access points of the cannulae used to take blood samples were generally situated close to the point of vascular entry, meaning that the sampler needs direct access to the patient in order to take the sample. We felt that this would be unduly obtrusive to family members at the bedside during treatment withdrawal. To address this issue a novel equipment setup was devised as described above and subjected to a pilot study to demonstrate its effectiveness with a dummy patient cohort. This setup has shown to be safe and effective for repeated blood sampling even at very short time intervals. It also proved to be unobtrusive to volunteers acting as staff and family members at the bedside during the end of life.

## **4.8 Blood sampling**

### **4.8.1 Blood samples taken**

- Arterial blood gas – 1ml. Analysed immediately with point of care technology
- Venous blood gas (where available) -1ml. Analysed immediately with point of care technology
- EDTA tube – 2.8 mls
- Lithium-heparin tube – 2.8 mls

Total volume taken in each sampling event 17.6mls (inclusive of 5ml aspirate discarded prior to sample removal). All whole blood samples not submitted to point of care testing were kept on ice until being centrifuged at 4°C and separated within two hours of collection.

### **4.8.2 Blood sampling schedules**

Upon withdrawal of therapy an immediate blood sample was taken. Further samples were taken based upon the schedules outlined below. Samples were taken at set time points after withdrawal of therapy (Table 4.2), and also at points based upon the physiological trajectory

of deterioration shown by the patient (Table 4.3). Total blood sampling volume did not exceed 250ml prior to asystole.

<b>Time after treatment withdrawal</b>								
Time (mins)	WLST	+10	+30	+60	+90	+120	+150	+180
	X	X	X	X	X	X	X	X

*Table 4.2: Sampling schedule based upon time after withdrawal of life sustaining therapy. WLST= withdrawal of life supporting treatment*

<b>Blood pressure based schedule</b>						
SBP (mmHg)	100	80	60	40	20	Asystole
	X	X	X	X	X	x

*Table 4.3: Sampling schedule based upon systolic blood pressure.*

The two separate schedules described in the tables above were used simultaneously for each patient. One schedule dictated blood sampling events based upon the time after withdrawal of life supporting therapy. This schedule allowed for sampling at regular intervals for the patient who maintained haemodynamic stability after therapy withdrawal. The second schedule allowed for blood sampling based upon systolic blood pressure and allowed for frequent sampling in the patient who suffers a precipitous deterioration of blood pressure upon withdrawal of life supporting therapy. Integration of the two schedules allows for good temporal coverage of the period after therapy withdrawal.

## 4.9 Study flowchart

A flow chart detailing the study procedures and intervals for sample and data collection is given below (Figure 4.1).

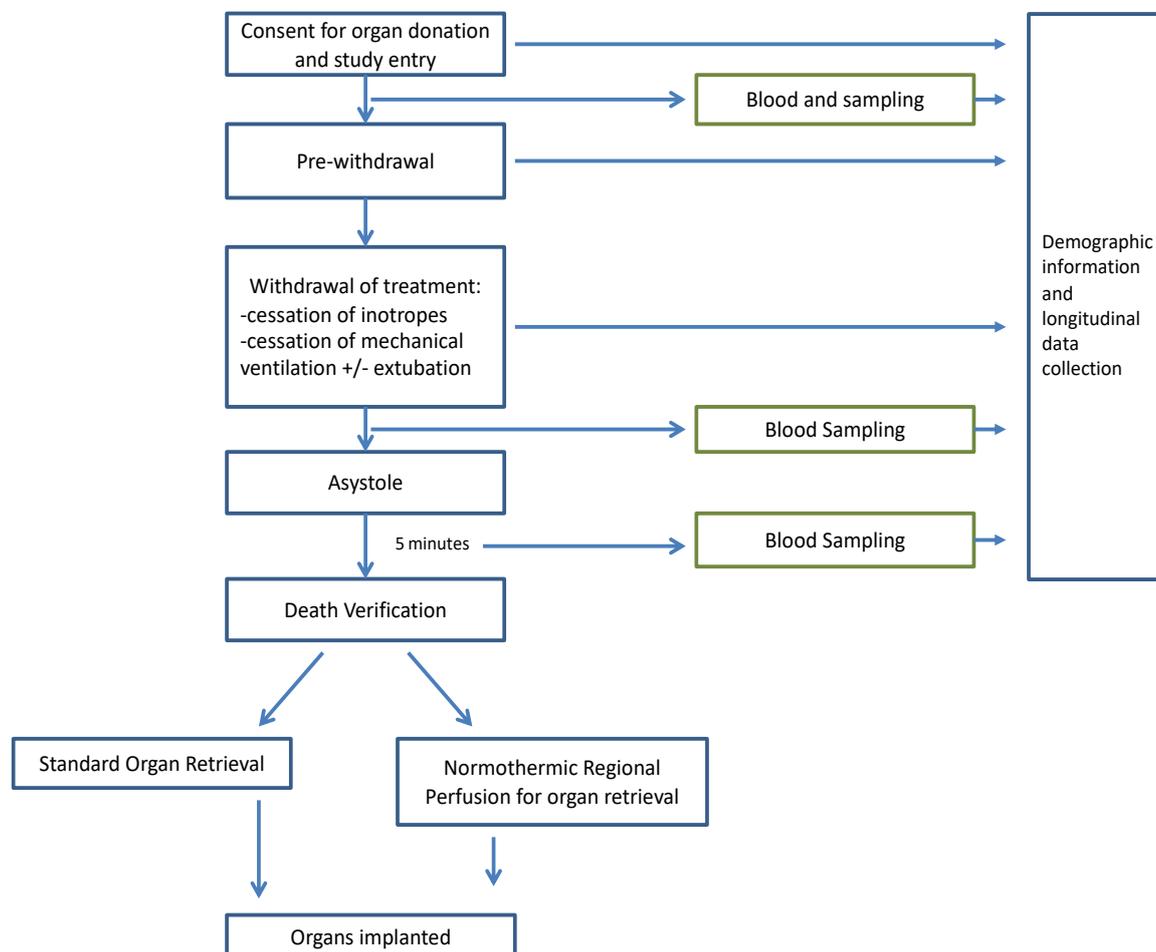


Figure 4.1 Flowchart of DCD donor physiology study

## 4.10 Analyses undertaken

### 4.10.1 Arterial and venous blood gas analysis

Samples were taken into BD Eclipse 1ml arterial blood gas syringes and analysed immediately with point of care technology. Analyser Cobas b221 (Roche Diagnostics) Compact All-In-One Blood gas analyser. This device can measure, pH, pCO<sub>2</sub>, pO<sub>2</sub>, SatO<sub>2</sub>, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, Hemoglobin (total and derivatives: O<sub>2</sub>Hb, MetHb, COHb, HHb), Hematocrit, Glucose, Lactate and Urea. The equipment was maintained by the Cambridge University

Hospital NHS Foundation Trust Point of Care Team and subject to daily calibration and maintenance. The machine performs 2 hourly self-maintenance cycles. Results are printed onto paper for assessment and automatically entered into the patient’s electronic medical record.

#### 4.10.2 Cytokines profiles

The analysis of cytokine concentrations in peripheral blood plasma was performed on the basis of commercially available kits for ELISA enzymatic immunoassay, in accordance with the instructions provided by the manufacturer (MesoScale Discovery Immunoassay). The sample of blood was taken into a lithium heparin tube, centrifuged at a speed of 3200/min for 15 minutes, and supernatant plasma removed and stored at –80°C for further testing. The cytokine panel used measures the following analytes: IFN- $\gamma$ , IL-10, IL-12p70, IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, TNF- $\alpha$ . The lower limit of assay sensitivities are given in table 4.4 below. The upper end of the calibrator curve for this panel was 10000 pg/mL for all cytokines and the lower limit of detection (LLOD) was determined as 2.5 standard deviations above the background. Concentration readings were carried out using a DIALAB ELX 808 spectrophotometer and Gen 51.10 software.

Cytokine	IFN- $\gamma$	IL-10	IL-12p70	IL-1 $\beta$	IL-2	IL-4	IL-6	IL-8	TNF- $\alpha$
LLOD (pg/ml)	0.53	0.21	0.14	0.30	0.35	0.1	0.27	0.09	0.50

Table 4.4: LLOD (Lower Limit of Detection) for cytokine panel is defined as 2.5 Standard Deviations above the background level.

#### 4.10.3 Adrenaline and noradrenaline levels

The analysis of catecholamine concentrations in peripheral blood plasma was performed using commercially available kits for ELISA enzymatic immunoassay, in accordance with instructions provided by the manufacturer (IBL Adren/NorAdren ELISA kit). Samples were initially into EDTA tubes, and centrifuged at a speed of 3200/min for 15 minutes. Supernatant plasma was stored for further tests at –80°C for batch analysis. The standard assay ranges given by the manufacturer were: Adrenaline: 0 / 0.5 - 80 ng/mL; Noradrenaline: 0 / 0.2 - 32 ng/mL. Sensitivity values for measured catecholamines are given in table 4.5 below

Catecholamine	Adrenaline	Noradrenaline
Analytical Sensitivity (pg/ml)	10	4

Table 4.5 – Analytical sensitivity of catecholamine assay

#### 4.10.4 Cortisol levels

The analysis of cortisol and Insulin concentrations in peripheral blood plasma were performed using commercially available assay kits, in accordance with instructions provided by the manufacturer (Diasorin Liaison XL assay), using the LIAISON Cortisol assay (DiaSorin S.p.A., Saluggia (VC), Italy), validated by the producer for analysis of serum cortisol levels. This is an immunoassay chemiluminescence test (CLIA) with a paramagnetic microparticle solid phase (MP). Assay methods are based on an internal Master Curve with 2-point calibration. Samples were initially collected whole blood in lithium heparin tubes, centrifuged at a speed of 3200/min for 15 minutes, and supernatant plasma for further tests was stored at  $-80^{\circ}\text{C}$  for batch analysis.

#### 4.10.5 Acknowledgements

I am grateful to the Core Biochemical Assays Laboratory (CBAL) based at Addenbrookes hospital for their assistance in acquiring the materials necessary for the above assays and their expertise in performing the above techniques.

### 4.11 Statistical analyses

Analysis was conducted using Prism (Graphpad Software, La Jolla, CA, USA). Study demographic information are provided as frequencies and percentages. Non-binary characteristics were dichotomised into categories as given in the tables. Contingency tables were analysed by Fisher's exact test (for  $2 \times 2$ ) and chi-squared (for  $>2 \times 2$ ). Difference between two non-normally distributed data sets was compared by Mann-Whitney U test (or Wilcoxon rank sum for paired data). Where more than two data sets were present Kruskal-Wallis ANOVA was used with Dunn's post-hoc test, whilst two-way ANOVA (with Bonferroni's post-hoc test) was used for analysis of values over time between two groups.  $P \leq 0.05$  was

considered statistically significant. The assistance of Dr Ari Ercole in the statistical representation of the data is gratefully acknowledged.

#### **4.12 Ethical approval and institutional review**

The study was approved by the relevant research ethics committees (RECs) and has appropriate institutional approvals in place.

The study protocol and associated documents were reviewed by the Research Ethics Committee and given a favourable review. The reference for this review is 16/WM/0179.

In order to be approved for the recruitment of NHSBT patients the study documents were reviewed and granted approval by NHSBT's Research Innovation and Novel Technologies Advisory Group (RINTAG). The study documents were reviewed by local Research and Development committees in order to approach Cambridge University Hospital NHS Foundation Trust patients and use their facilities.

The study is a National Institute of Health Research (NIHR) Clinical Research Network (CRN) registered study. It forms part of the Eastern CRN and contributes recruitment data towards the local portfolio at three monthly intervals.



## Chapter 5: Donor Demographics and physiological changes

### 5.1 Chapter overview and introduction

Chapter 3 demonstrated a high level of public and donor family approval for a research study involving the potential DCD organ donor prior to death. Such a study fits well with calls to increase donor numbers and utilisation of donated organs.

In spite of this high level of public support, there has to date been no research in humans to provide an understanding of the physiological changes that occur in the DCD donor during the withdrawal period. As is clearly illustrated using the example of brainstem death organ donation (Chapter 1 section 8.3) the understanding of donor physiology has led to post-mortem optimisation of donated organs, often through relatively simple interventions, with associated increased rates of transplant success.

As Chapter 1 demonstrates, animal models of DCD donation do exist, but they fall short in their attempts to characterise the changes that occur in human DCD donation. As previously described, much of this discrepancy is due to the often-prolonged dying process in the DCD donor. Therefore, the data presented in this chapter will provide a basis to understanding the changes seen in the DCD donor through exploration of the patterns of physiological change.

This chapter will explore the following hypotheses:

- that donor families will be willing to provide consent for their next of kin to be recruited into this study,
- that intensive study of the DCD organ donor prior to death is feasible and will generate measurable physiological data
- that the generated data may be used to make assessments regarding the physiological state of the donor

This chapter will first examine recruitment rates for the study and reasons for loss of recruited patients prior to donation. It will then go on to describe the demographic data of recruited patients. Subsequently, this chapter will examine the measured physiological data derived from intensive study of the recruited potential donors undergoing withdrawal of life supporting treatment. This will involve description of the temporal changes in the standard measured observations during treatment withdrawal (blood pressure, oxygen saturation, respiratory and heart rate) and will then go on to explore data gathered from arterial and venous blood gas analysis during the withdrawal process.

## 5.2 Patient recruitment results

Patients were recruited into the study with the inclusion and exclusion criteria given in chapter 4 section 2. A summary of patient recruitment is given below in Figure 5.1.

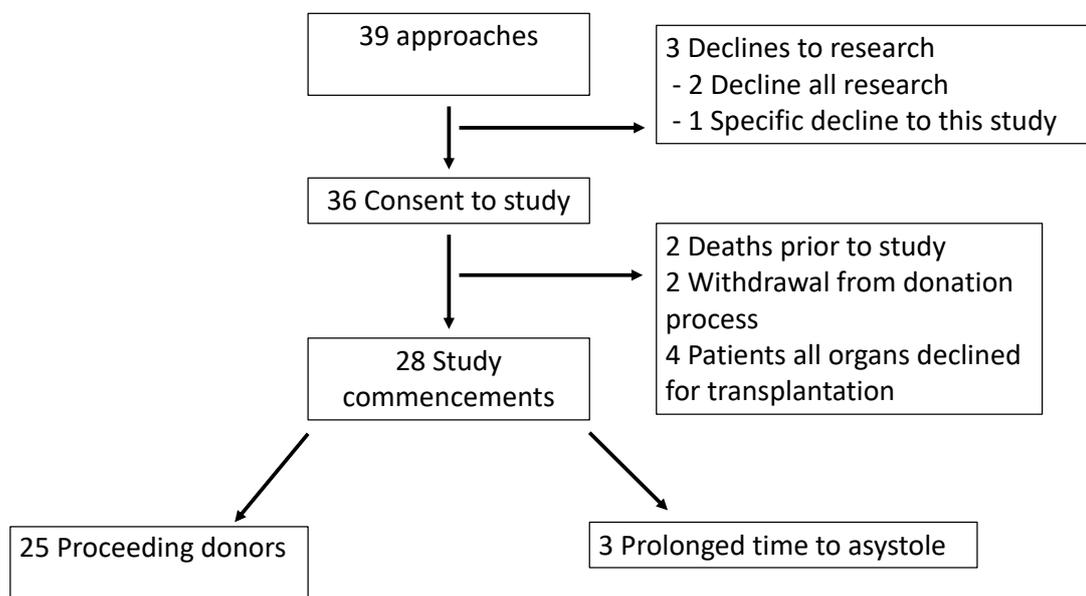


Figure 5.1: Summary of recruitment for study.

Rates of recruitment for the study echo the findings of the public and patient engagement work presented in Chapter 3, which demonstrated a high rate of support for our proposed study. The 92% consent rate for the study (36/39 approaches) is in excess of that for many reported invasive studies in critically ill patients (Sole et al 2017) and likely represents the fact that potential donor families have already made an altruistic decision to support

donation. Research to improve organ transplantation success rates could be considered an extension of this altruistic decision. Of the three families who declined enrolment in the study, 2 families declined all research opportunities available, including the use of non-transplantable organs for research and the QUOD biobank study. In only one incidence (2.6% of approaches) was consent specifically declined for this study. The rationale given for this specific decline to research was that the donor was an 'intensely private person' and the family felt an additional person (the researcher) being present at the time of death was not something they would have agreed to.

Of the 25 patients who proceeded to organ donation after treatment withdrawal, one patient subsequently had all organs declined for transplantation at laparotomy due to four quadrant peritonitis, and so has no outcome data available.

### **5.3 Patient Demographic Results**

Demographic information was collected on patients enrolled into the study as outlined in Chapter 4 section 6. The demographics of the 28 patients undergoing withdrawal of life supporting treatment is given in table 5.1 below:

Patient	Age (years)	Sex	Underlying pathology	LOS (days)	Ventilatory mode	FiO2	Vasoactive infusion NA (mcg/kg/min) Met (mcg/hr)	GCS M Score	TTD (mins)
1	55	M	ICH	2	Mandatory	0.45	Nil	1	8
2	77	M	TBI	5	Spontaneous	0.35	NA 0.05	3	45
3	25	F	TBI	2	Mandatory	0.6	NA 0.31	1	40
4*	52	F	SAH	2	Mandatory	0.8	Met 1600	1	14
5	70	F	ICH	2	Spontaneous	0.3	Nil	1	30
6*	20	M	HBI	5	Mandatory	1.0	NA 0.56	1	14
7*	52	M	TBI	8	Mandatory	0.6	NA 0.32	1	7
8	59	F	HBI	6	Mandatory	0.4	NA 0.21	2	12
9	65	M	HBI	19	Mandatory	1.0	NA 0.16	1	16
10	50	M	HBI	14	Spontaneous	0.35	NIL	1	29
11*	68	F	SAH	2	Mandatory	0.3	MET 800	1	11
12	72	M	ICH	2	Mandatory	0.3	MET 1000	2	16
13	58	M	HBI	4	Mandatory	0.4	NA 0.14	1	13
14	54	M	ICH	2	Spontaneous	0.4	NA 0.07	1	20
15	54	F	SAH	27	Spontaneous	0.28	NIL	4	PTA
16	60	F	SAH	7	Mandatory	0.3	NA 0.26	1	15
17	54	M	TBI	2	Mandatory	0.4	NA 0.23	1	105
18	45	F	HBI	5	Spontaneous	0.4	NIL	2	161
19	57	M	TBI	4	Mandatory	0.6	NA 0.18	1	11
20	68	F	SAH	4	Spontaneous	0.3	NA 0.07	1	137
21	68	F	SAH	6	Spontaneous	0.4	NA 0.15	1	83
22	72	F	TBI	7	Spontaneous	0.35	NA 0.04	4	PTA
23	41	F	ICH	7	Mandatory	0.4	NA 0.09	1	12
24	59	M	TBI	2	Mandatory	0.4	NA 0.21	1	20
25	69	M	ICH	3	Spontaneous	0.3	NIL	3	13
26	61	M	ICH	21	Spontaneous	0.4	NIL	2	63
27	58	M	HBI	8	Spontaneous	0.8	NA 0.13	1	175
28	75	M	HBI	14	Spontaneous	0.35	NIL	4	PTA

*Table 5.1: Demographic information of recruited DCD organ donors.*

*Abbreviations: LOS=Length of intensive care unit stay, FiO2= Fraction of inspired oxygen, GCS M Score – Glasgow coma scale motor score, TTD = Time to death, M=Male, F=Female. SAH= Subarachnoid haemorrhage, ICH= Intracranial haemorrhage, HBI= Hypoxic Brain Injury, TBI= Traumatic Brain Injury, NA= Noradrenaline, Met = Metaraminol, PTA= Prolonged time to asystole. \*DCD donors considered to be likely to be brain stem dead, but with contraindications to testing discussed in table 5.2 below.*

Patients marked with an asterisk in the above table represent a subset of the patient cohort who were either brainstem dead or displaying no cranial nerve reflexes on clinical examination, but with specific contraindications preventing formal brainstem criteria being undertaken. The specific conditions of each of the four patients in this cohort are given below in table 5.2.

<b>Subject number</b>	<b>Clinical details precluding Brainstem criteria testing</b>
4	FiO2 0.8 with rapid desaturation precluding apnoea element of brainstem criteria. No brainstem reflexes present on examination
6	Gross cardiovascular instability – requiring Noradrenaline 0.71mcg/kg/min and Vasopressin 20 Units/hr to maintain MAP 60. No brainstem reflexes present
7	Traumatic injury to both eyes precluding pupillary reflex and corneal reflex testing. No other brainstem reflexes present on clinical examination
11	Brainstem death testing completed, and criteria fulfilled. Family request to donate only via DCD route due to religious preferences

*Table 5.2: Clinical details in subjects 4, 6, 7 and 11 who compromise the ‘Non-typical DCD donor group’.*

Given that patients who have undergone brainstem death will reasonably be expected to behave in a different fashion to non-brainstem dead patients undergoing withdrawal of life supporting therapy, and that brainstem death involves specific physiological changes as detailed in chapter 1 section 8.3, it was decided to consider these patients as a separate cohort. This allowed for patients to be considered in two groups, those considered highly likely to be brainstem dead but not clinically able to be tested (termed ‘non-typical’ DCD donors), and those considered to be ‘typical’ DCD donors, with 4 patients falling into the non-typical DCD donor group and 18 into the typical DCD donor group.

Analysis of these two sub-groups allows for comparison of standard DCD donors undergoing the physiological changes associated with withdrawal of treatment and a brainstem dead cohort. The demographic and clinical details of patients in the two subgroups are shown below in table 5.3. For ease of reference, from here on subarachnoid haemorrhage and intracranial haemorrhage will be considered together as ‘intracranial haemorrhage’.

Parameter	Whole cohort N=28	Subgroups		
		Typical DCD N=24	Non-typical DCD N=4	p-value
Age	58.5 (20-77)	59 (25-77)	51.5 (20-68)	0.108*
Sex	M: 16 F: 12	M: 14 F: 10	M: 2 F: 2	0.8346^
Length of stay	4.5 (2-27)	5 (2-27)	2 (2-8)	0.2781*
FiO2	0.4 (0.28-1.0)	0.4 (0.8-1.0)	0.55 (0.3-1.0)	0.1788+
Vent mode	Spont 13 Mand 15	Spont 13 Mand 11	Spont 0 Mand 4	0.1074 ^
Underlying brain pathology	ICH: 13 Trauma: 7 Hypoxia: 8	ICH: 11 Trauma: 7 Hypoxia: 6	ICH: 2 Trauma: 1 Hypoxia: 1	0.862^
Motor score	1 (1-4)	1 (1-4)	1 (1-1)	0.2697+
Time to death (mins)	16 (7-175)	20 (8-175)	12.5 (7-14)	0.0325+

*Table 5.3: comparators of 'Typical DCD donor' group and 'Non-typical DCD donor' group. Data presented as median and range of values. \*p value by unpaired t test; +p value by Mann-Whitney U; ^p value by Chi Squared.*

*Vent = ventilatory, Spont = spontaneous, Mand = mandatory, ICH – Intracranial haemorrhage*

Table 5.3 demonstrates no significant difference in demographics between the 'typical' and 'non-typical' donor groups, suggesting the 'non-typical' donor group may be used as an acceptable control group for the 'typical' DCD donors undergoing treatment withdrawal as part of organ donation.

By the very definition of brainstem death, a 'non-typical' donor will be unable to breathe spontaneously and will be GCS 3 on assessment of their neurological condition. The lack of significance between the two groups on analysis of these two variables is influenced by the relatively small numbers in the 'non-typical' group and the large numbers on 'typical' DCD donors who require mandatory ventilation and have a motor score of M1. By the same logic, the significant difference in time to death between the typical and non-typical donor groups is expected, given that a brainstem dead patient is unable to breath without assistance so will die imminently after treatment withdrawal.

Examination of table 5.3 demonstrates that patients with hypoxic brain injury have a significantly longer length of ICU stay prior to treatment withdrawal (median stay of 11 days, compared to 3 and 4 days in the intracranial haemorrhage and traumatic brain injury groups respectively,  $p=0.046$ ), represented in Figure 5.2 below. This finding is explained by the requirements for initial management and subsequent neuroprognostication in the patient with hypoxic brain injury. Examination of the clinical records of the patients with cause of death listed as hypoxic brain injury revealed each had suffered an out of hospital cardiac arrest. Standard intensive care management of patients following out of hospital cardiac arrest routinely involves a period of targeted temperature management (Ford et al 2015). This is often followed by investigations to predict a prognosis, such as MRI and neurophysiology tests, which can be logistically complex to organise and time consuming to perform.

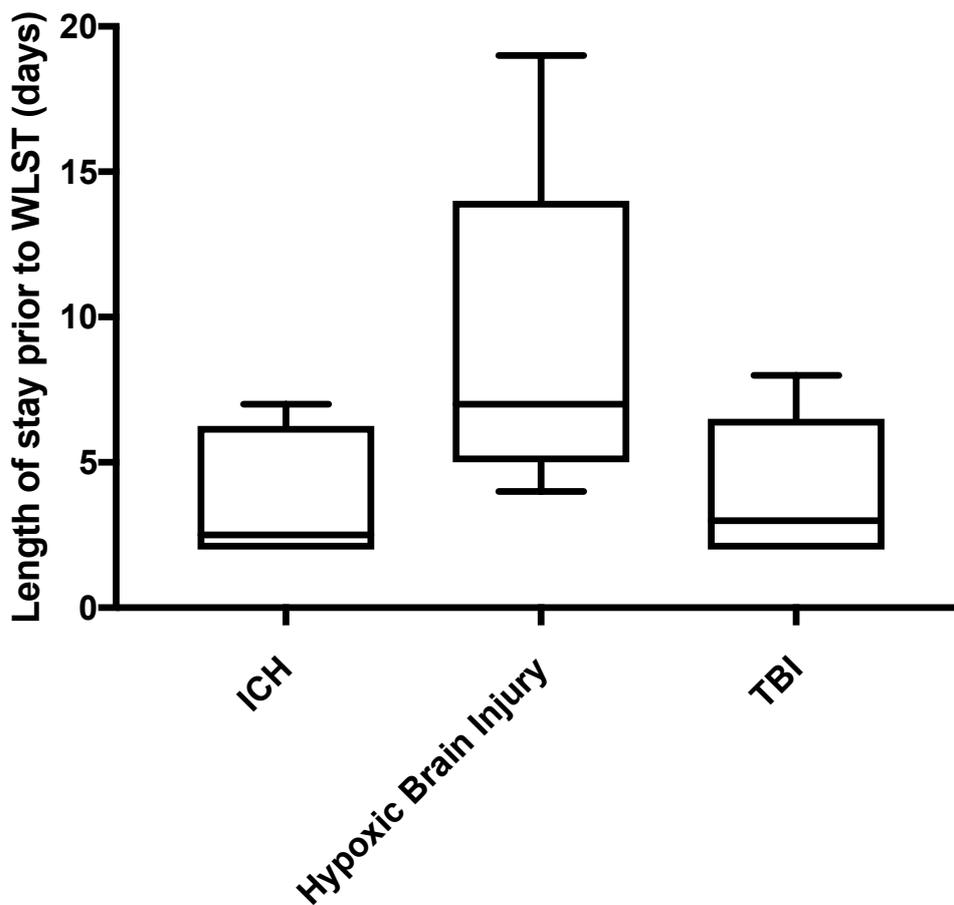


Figure 5.2: Length of stay prior to WLST in days by underlying disease pathology. SAH/ICH group median length of stay 2.5 days, range 2-7 days; hypoxic brain injury group median 8 days, range 4-19 days; traumatic brain injury group median 3 days, range 2-8 days.  $p = 0.0201$  by Kruskal Wallis with post hoc Dunns Test.  $KW = 7.812$ .

#### 5.4 Physiological changes in proceeding donors.

Physiological data from 28 patients undergoing withdrawal were collected at 2-minute intervals for the first 30 minutes and at 5 minute intervals thereafter, using routine monitoring equipment available during withdrawal of life supporting treatment (invasive blood pressure measurement via arterial line, heart rate from ECG, and oxygen saturations via pulse oximetry). Presented below are the data collected for 25 proceeding DCD organ donors who died within the timeframe that enabled organ retrieval.

### 5.4.1 Systolic Blood pressure

Graphical representation of systolic blood pressure variation with time after withdrawal of life supporting therapy in proceeding DCD donors are shown below in figures 5.3 and 5.4. It is notable that all proceeding donors had a steep terminal decline in systolic blood pressure. In the 'typical DCD donor' group (represented in the figures below by black lines) many patients experienced an elevation in blood pressure after treatment withdrawal. The same pattern is not seen in the 'non-typical DCD donor' group, who experienced a rapid decline in blood pressure with no preceding elevation. The hypothesis that this difference in pattern is due to activation of the autonomic and hypothalamic-pituitary-adrenal axis during the process of cardiovascular death in the 'typical DCD' donor which does not occur in the patient who has already undergone brainstem death (represented here by the 'non-typical DCD donor' group) will be explored in chapter 8. The use of systolic blood pressure as a marker for tissue perfusion will be considered in detail in Chapter 7.

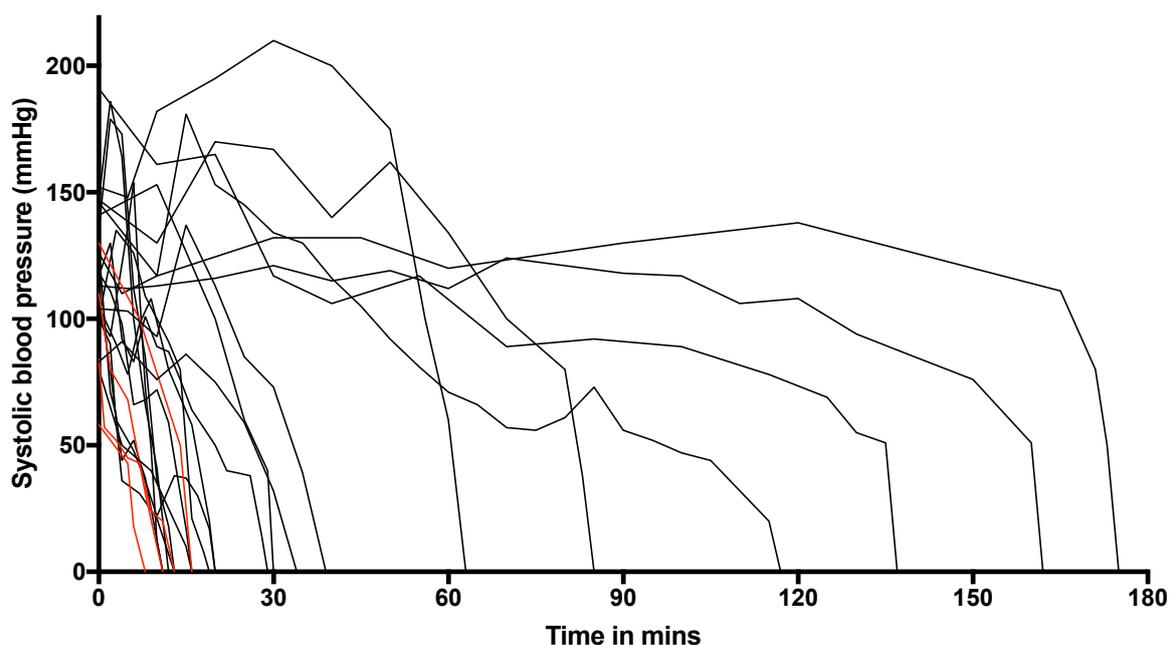


Figure 5.3: Systolic blood pressure changes over time for the 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

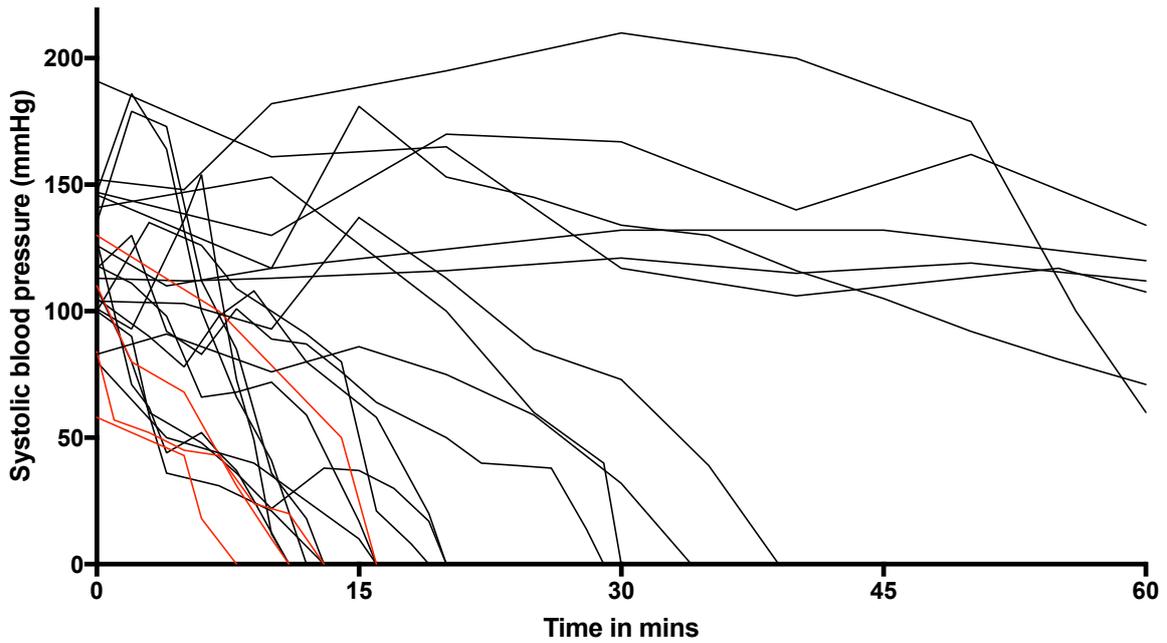


Figure 5.4: Detail of systolic blood pressure changes in the first hour for 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

#### 5.4.2 Oxygen saturation

Graphical representation of variation in percentage haemoglobin oxygen saturation (measured by finger-tip pulse oximetry) with time after withdrawal of life supporting therapy are given in figures 5.5 and 5.6 below. In all patients, haemoglobin oxygen saturation fell rapidly when using this method of assessment, notable even in patients surviving well beyond 30 minutes. The accuracy of pulse oximetry for use in oxygen saturation assessment is explored in detail in Chapter 6.

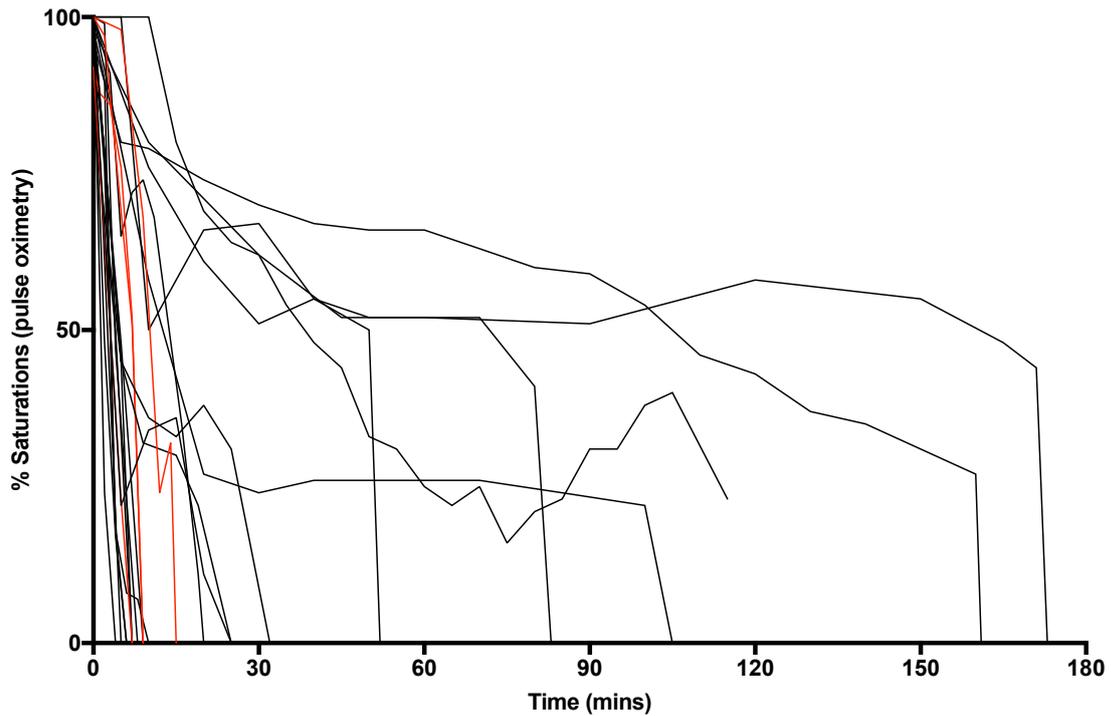


Figure 5.5: Oxygen saturation changes over time (measured by finger-tip pulse oximetry) for the 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

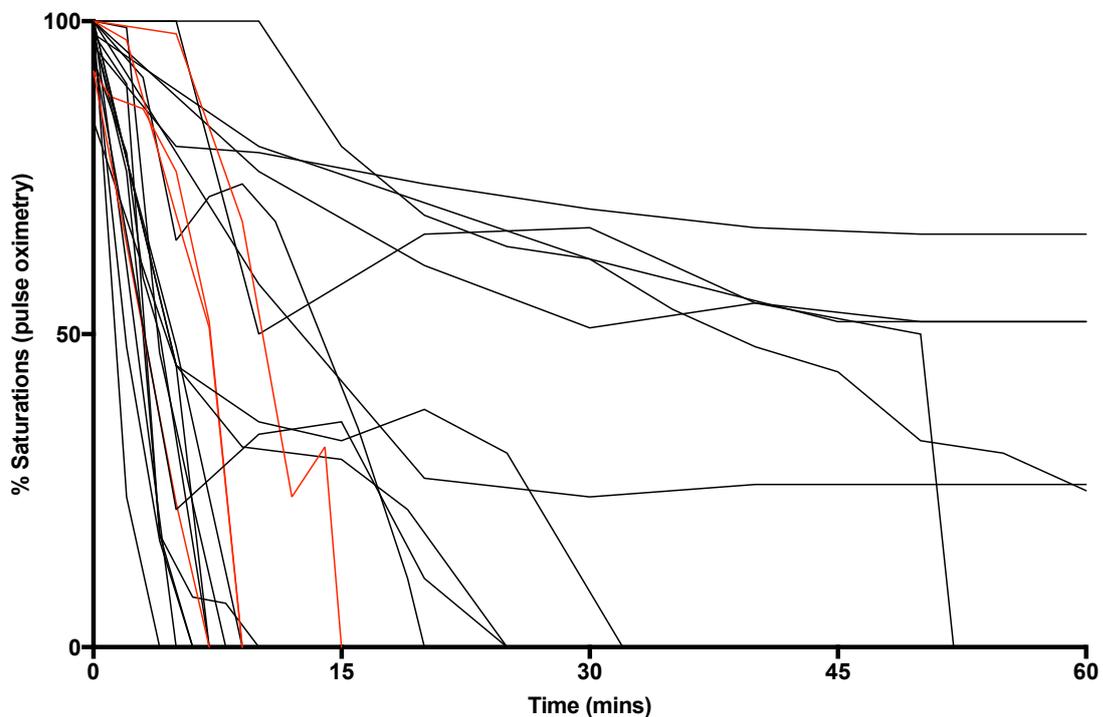


Figure 5.6: Detail of oxygen saturation changes in the first hour (measured by finger-tip pulse oximetry) for the 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

### **5.4.3 Arterial and venous blood gas data**

As part of this study, extra information was collected from donors undergoing withdrawal of therapy through assessment of arterial and venous blood gas samples at regular intervals. This is additional information which is not collected routinely by the SNOD team during treatment withdrawal. All samples were taken from pre-existing lines, with no new lines placed for the purposes of this study. All 28 recruited patients had arterial lines in place at the time of treatment withdrawal. 24 patients had central venous cannulae in place prior to treatment withdrawal, the remaining four patients had peripheral cannulae only. Of the 24 patients with central venous cannulae in place, it was not possible to aspirate samples for analysis from 2 cannulae, consequently 22 patients provided information regarding venous oxygen saturations

Samples were taken according to the sampling schedule outlined in 4.8.2 and analysed using point of care methodology as outlined in 4.10.1.

### **5.4.4 PaO<sub>2</sub>**

Graphical representation of arterial oxygen partial pressure (PaO<sub>2</sub>) variations with time after withdrawal of life supporting treatment are given below in figures 5.7 and 5.8. Review of the graphical data represented below demonstrates that after treatment withdrawal, the PaO<sub>2</sub> in all patients dropped precipitously. Normal levels of PaO<sub>2</sub> are suggested as being 10-14kPa, and hypoxic cerebral vasodilatation occurs at a oxygen saturation threshold of ~90% in healthy volunteers (which equates to a PaO<sub>2</sub> of ~ 8.5 kPa (Gupta)). All patients had PaO<sub>2</sub> levels drop to below 8kPa within 10 minutes of treatment withdrawal. Those patients who survived a substantial period of time after withdrawal did so with critically low levels of PaO<sub>2</sub>. The consequences of this rapid decline in PaO<sub>2</sub> after treatment withdrawal, and its implications for tissue oxygenation are considered in chapter 6.

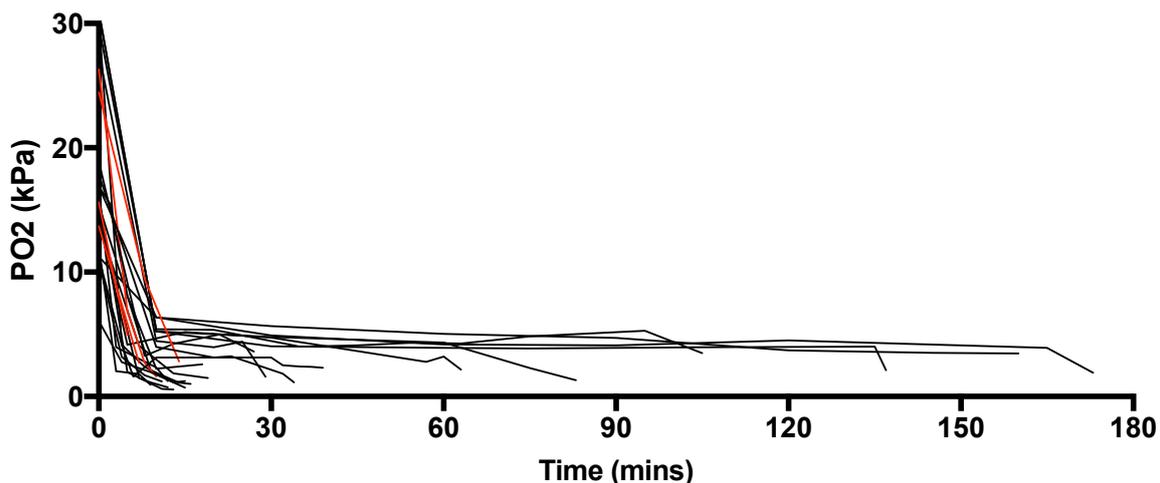


Figure 5.7: Arterial partial pressure of oxygen changes over time from 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

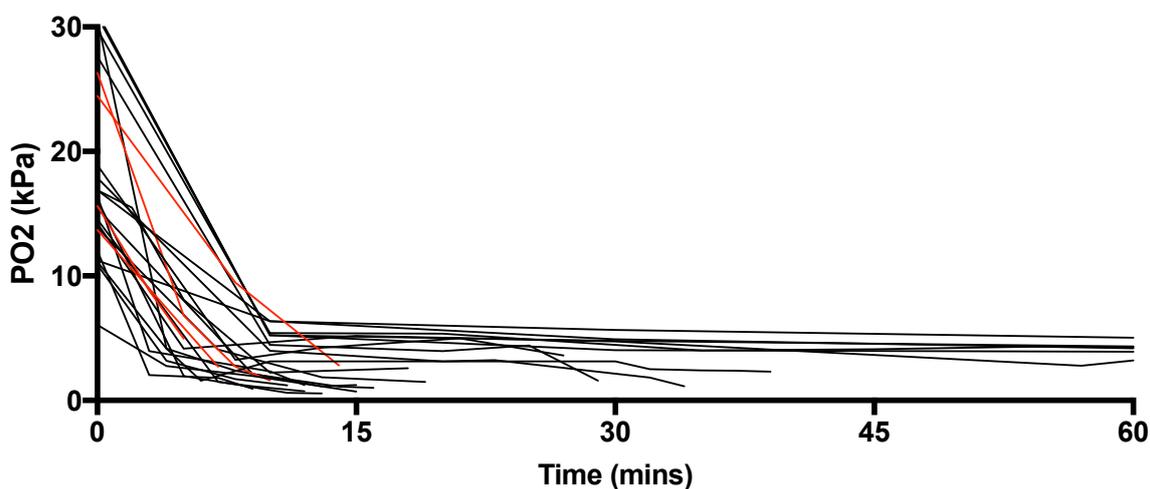


Figure 5.8: Detail of arterial partial pressure of oxygen changes in the first hour from 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

#### 5.4.5 PaCO<sub>2</sub>

Graphical representation of arterial partial pressure of carbon dioxide (PaCO<sub>2</sub>) with time after withdrawal of life supporting therapy are given in figures 5.9 and 5.10 below. A normal range of PaCO<sub>2</sub> is taken as being 4.5-6.0kPa. From inspection of the data represented below, it is apparent that donors who died rapidly after treatment withdrawal had rapid elevation in PaCO<sub>2</sub> levels. As expected, donor death was associated with a rise in PaCO<sub>2</sub>.

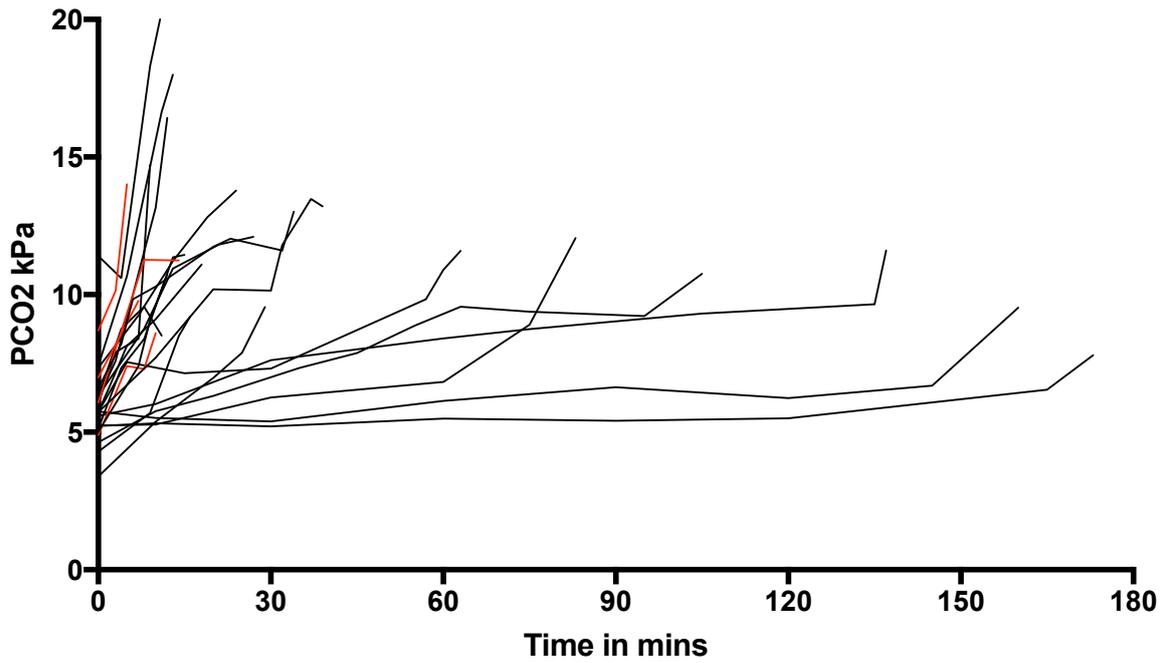


Figure 5.9: Change in arterial partial pressure of carbon dioxide ( $\text{PaCO}_2$ ) over time for the 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group ( $n=21$ ), red lines representing 'Non-typical DCD donor' group ( $n=4$ )

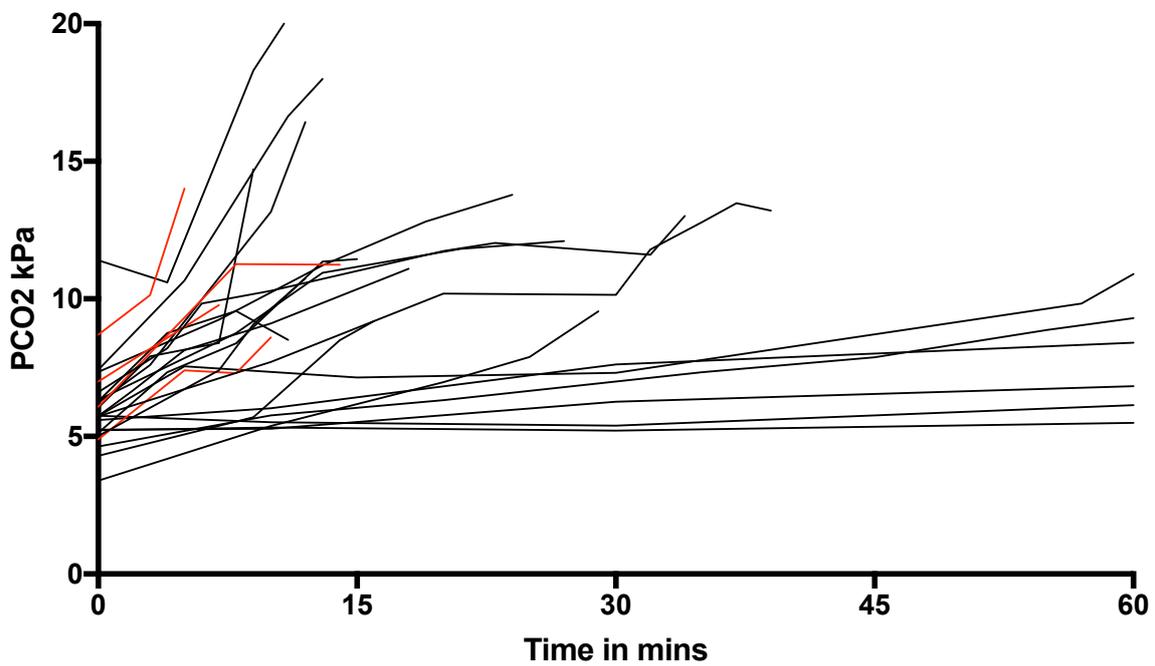


Figure 5.10: Change in arterial partial pressure of carbon dioxide ( $\text{PaCO}_2$ ) over the first hour for the 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group ( $n=21$ ), red lines representing 'Non-typical DCD donor' group ( $n=4$ )

### 5.4.6 Lactate

Graphical representation of variation of the lactate concentration with time after withdrawal of life supporting treatment are given in figures 5.11 and 5.12 below. Of note, all patients with the exception of patient 6 had initial lactate levels within the normal range (<2.2 mmol/L). Those donors who died rapidly after treatment withdrawal had rapid elevation in blood lactate levels, while those who survived longer after treatment withdrawal had a more gradual rise in blood lactate. No donor died with a lactate level within the normal range. The clinical condition of donor 6 is outlined in table 5.2 above, the initial elevated lactate level in this donor can be attributed to gross cardiovascular instability and substantial inotrope and vasopressor use suggesting ongoing ischaemia at the time of treatment withdrawal. The implications of elevation in lactate level during the process of cardiovascular death and its potential for use as a marker for tissue perfusion in the proceeding DCD organ donor are explored in detail in chapter 7.

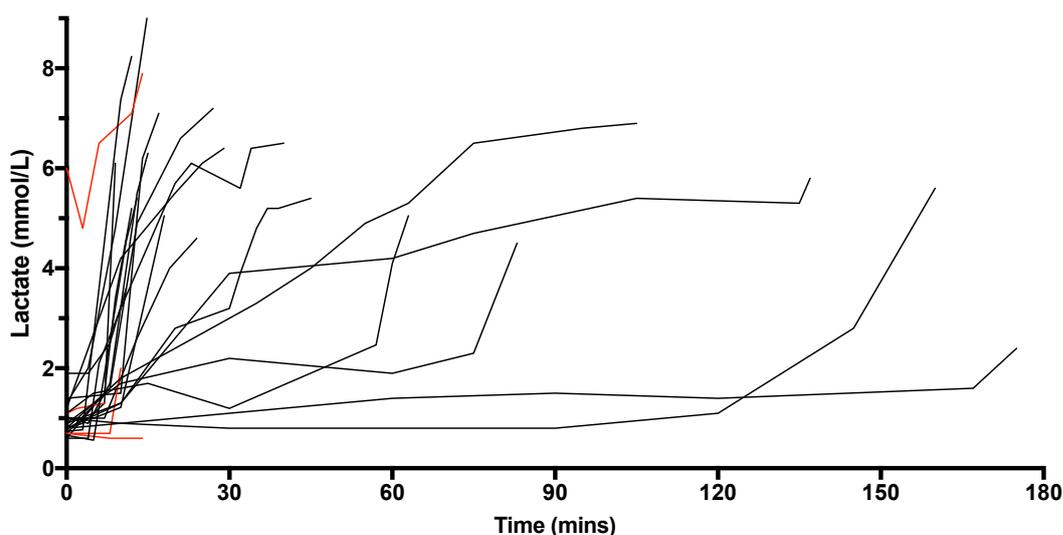


Figure 5.11: Blood lactate changes over time for 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

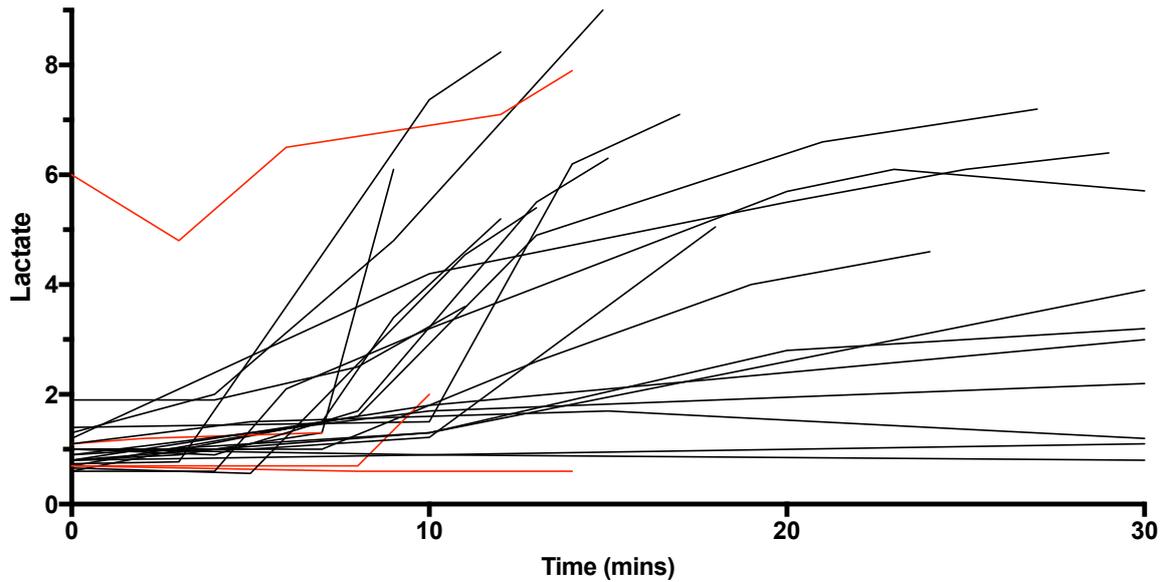


Figure 5.12: Blood lactate changes in first 30 minutes for 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

#### 5.4.7 pH

The change in pH with time after withdrawal of life supporting treatment is illustrated in figures 5.13 and 5.14 below. Normal pH is 7.35-7.45. Review of the data presented below are notable for the fact that donors dying rapidly had a rapid decrease in pH, while those who survived longer after treatment withdrawal survived with a normal pH. Death occurred rapidly in all patients once a pH of 7.3 was achieved.

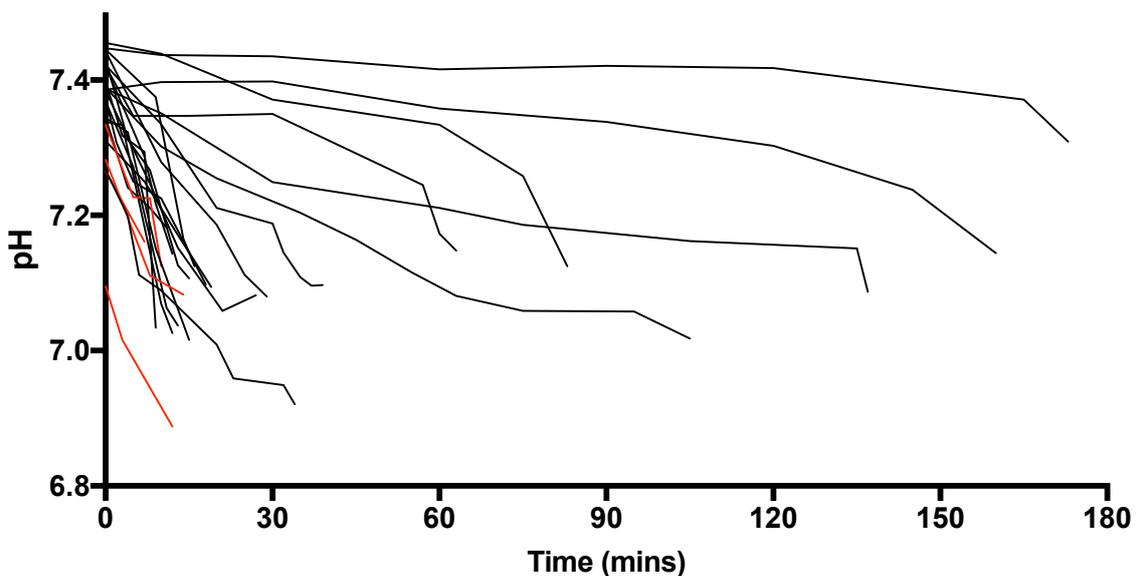


Figure 5.13: Graphical representation of pH changes over time from 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

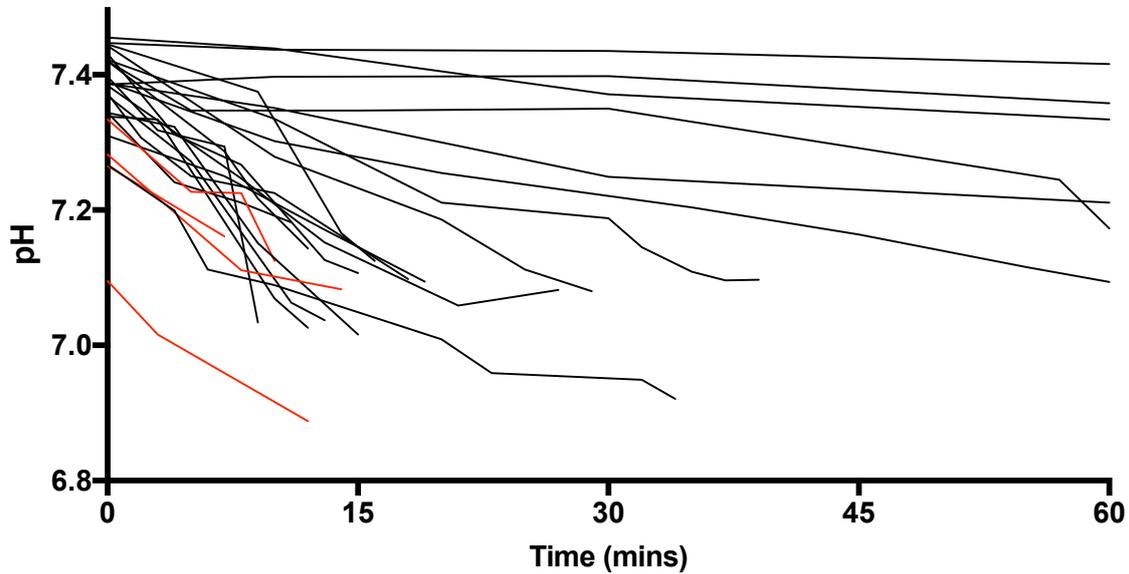


Figure 5.14: Change in pH in the first 60 minutes for 25 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=21), red lines representing 'Non-typical DCD donor' group (n=4)

#### 5.4.8 Venous oxygen saturation

The change in venous oxygen saturation with time after withdrawal of life supporting treatment is illustrated in figures 5.15 and 5.16. Normal venous oxygen saturation is 60-80% but varies with the site of venous sampling. In this study sampling was presumed to be from the superior vena cava rather than being a true mixed venous oxygen sample which would have required pulmonary artery catheterisation. No patient recruited into the study had a pulmonary artery catheter in place as part of their routine intensive care. All donors had rapid decreases in venous oxygen saturation after treatment withdrawal. Those donors who survived for a longer period after treatment withdrawal did so with levels of venous oxygen saturation below normal levels. The implications of low venous oxygen saturations in the proceeding DCD organ donor are considered in detail in results chapter 7 – identification of cardiovascular physiology in the DCD donor.

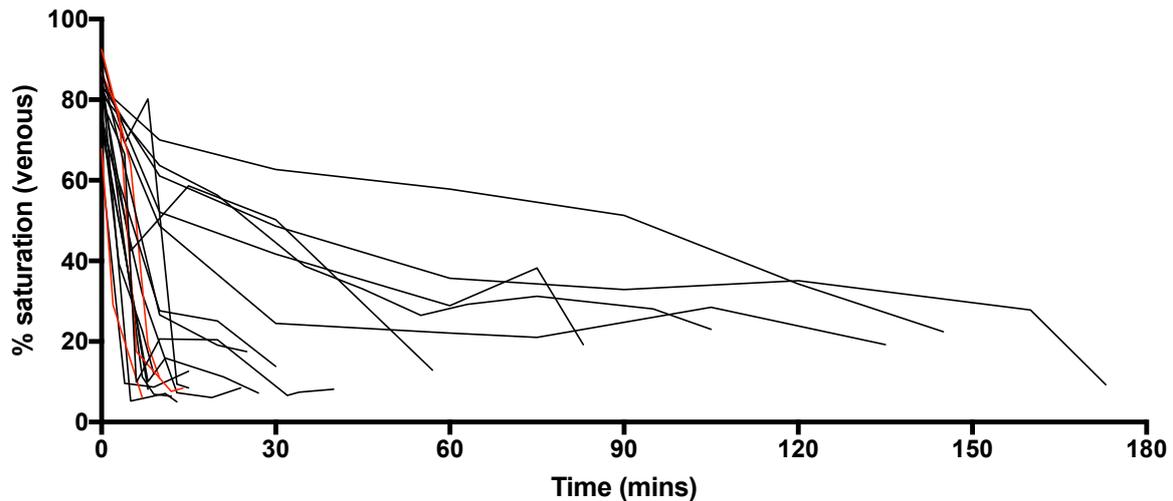


Figure 5.15: Change in venous oxygen saturation over time for 22 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=18), red lines representing 'Non-typical DCD donor' group (n=4)

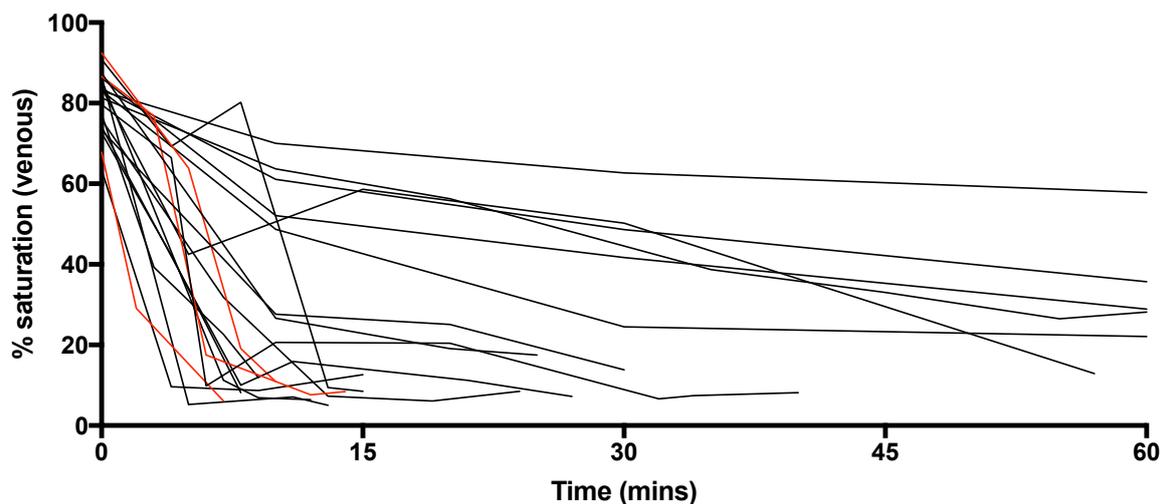


Figure 5.16: Change in venous oxygen saturation changes in the first 60 minutes for 22 patients undergoing withdrawal of life supporting treatment. Black lines representing 'typical DCD donor' group (n=18), red lines representing 'Non-typical DCD donor' group (n=4)

## 5.5 Chapter summary and discussion

This study has been the first study run with a DCD donor cohort that has used specific researcher consent for study enrolment rather than using the traditional model of consent for research by the SNOD. The high rate of consent for this study, in excess of 92%, suggests that this model can be used successfully within the organ donation framework. Extension of consent models to include researcher consent gives the opportunity for complex studies to

be undertaken in organ donors whilst ensuring that families are given optimal study information. In the case of studies involving the organ donor prior to death, such as this study, early researcher involvement with the family has been advantageous. It has allowed substantial time to discuss the study in detail without detracting from the already busy job of the SNOD and has allowed the researcher who will be present at the time of death to meet the family and form a relationship with them in advance of treatment withdrawal.

The rate of potential organ donors not proceeding to donation due to prolonged time to asystole is lower in this study than in the 2017/18 NHSBT Potential donor audit. This publication gives the rate of prolonged time to asystole at 19.8% (221 of 1115 consented donors) compared to the rate in this study of 8.3% (3 of 36 consented donors). Our study has been performed entirely in a tertiary hospital, with 24 of the 28 donors undergoing withdrawal of life supporting treatment coming from a specialist neuro-intensive care unit. Such units have substantial expertise in neuroprognostication, and it is possible that they have 'filtered' out patients who are unlikely to die within the four-hour time frame prior to consent for donation.

The physiological changes during the withdrawal period demonstrated in section 5.4 above represent information seen in human DCD donors for the first time. Arterial and venous gas analysis has not previously been undertaken in patients undergoing withdrawal of treatment and circulatory death. These data demonstrate that donors who lived longer after treatment withdrawal did so with protracted periods of hypoxia, hypotension and low arterial and venous oxygen partial pressures. Those donors who lived longer did so with less rapid deterioration in physiological parameters than donors who died rapidly. Examination of this data in greater detail will form the subject of the following chapters.



## **Chapter 6: Measurements of arterial oxygen saturation in the potential donor**

### **6.1 Introduction and chapter overview**

As described in Chapter 1, current decisions to stop proceeding with the DCD process for reasons of organ hypoxic burden are based on thresholds for oxygen saturation and systolic blood pressure. However, this approach has several shortcomings. First, these thresholds are based on consensus between transplant surgeons rather than rational physiological principles and have not been validated against the function or survival of transplanted organs. Second, because of this approach, there is substantial variation in thresholds between national guidelines – it is possible that none of these is optimal. Finally, though the physiological principles underpinning these thresholds may be rational, the way in which they are measured may not be optimal.

This chapter looks at the assessment of arterial oxygen saturation during the withdrawal period for potential DCD organ donor. As noted in the introduction, arterial oxygen saturation is commonly used by the National Organ Retrieval Service (NORS) to make decisions regarding the period of time that organ retrieval teams will wait for asystole to occur. This requires ‘realtime’ information regarding saturations which can be relayed to the NORS teams for decision making. At present, these decisions are frequently made based upon pulse oximetry, with a threshold arterial oxygen saturation of 50% (Messer 2017) being set as the onset of warm ischaemia for cardiothoracic organ retrieval.

The aims of this study were to examine the accuracy of pulse oximetry when used in this context. The hypothesis being explored was pulse oximetry information from potential DCD organ donors undergoing withdrawal of life supporting therapy is inaccurate and leads to the period of time the potential donor spends with saturations of under 50% being overestimated. I will then show that this limitation can be overcome with the use of arterial blood gas (ABG) analysis of arterial haemoglobin saturation.

The results and discussion in this chapter focus on the following issues:

1. Both ABG analysis and pulse oximetry can be used to measure oxygen saturations in the potential DCD organ donor.
2. There are broad correlations between arterial oxygen saturations when measured by pulse oximetry and ABG analysis.
3. However, despite broad correlations, the two methods show important differences in critical thresholds and calculated metrics of oxygen debt.
4. This lack of agreement between measures of arterial oxygen saturation may have important implications for organ donation.

## **6.2 Patients**

Patients were recruited as outlined in Chapter 3 section 2. Data from the 28 consented potential donors are presented below. The demographic and clinical details of donors are shown in table 5.1.

## **6.3 Measurement of oxygen saturations:**

### **6.3.1 Pulse oximetry measurement of oxygen saturations (SpO<sub>2</sub>)**

Pulse oximetry relies upon the fact that oxygenated haemoglobin and deoxygenated haemoglobin have a conformational difference in structure, meaning that they absorb light at different spectra (Jubran et al 1990). Current clinical pulse oximeters contain two different light emitting diodes, one of which emits light in the visible red spectrum with a wavelength of 660nm and one which emits light in the infrared spectrum with a wavelength of 940nm. These specific wavelengths were chosen due to the different light absorbance spectra of oxygenated and deoxygenated haemoglobin at these wavelengths, allowing for differentiation of their proportions in the circulation. Oxygenated haemoglobin has preferential absorption in the red light spectrum, while deoxygenated haemoglobin absorbs preferentially in the infrared spectrum. There is a photo detector on the opposite side of the

probe to the light emitting diodes. The diodes pulse around 30 times per second, with each switching on in sequence, and a pause with both diodes off to allow for signal compensation for ambient light.

The signal from arterial blood is isolated by subtracting the non-pulsatile part of the signal, which is made up of soft tissue and venous blood absorption, leaving only the pulsatile component of the signal which represents the arterial blood. The ratio of red and infrared absorption of the arterial blood is then compared against a calibration curve stored within the digital microprocessor of the pulse oximeter, allowing for an estimation of the arterial saturation (Wukitisch et al 1988). These calibration curves were calculated by rendering healthy volunteers hypoxic and making direct measurements of their arterial blood oxygenation, and creating an algorithm that related the pulse oximeter signal with the arterial blood oxygenation.

### **6.3.2 Arterial blood gas analysis measurement of oxygen saturations (SaO<sub>2</sub>)**

The arterial blood gas analyser is a point of care technology which allows for immediate analysis of a blood sample obtained from an arterial or venous cannula. The Cobas b221 benchtop analyser (Roche) used in this study for blood gas analysis gives the following information:

- pH
- Partial pressure of carbon dioxide (pCO<sub>2</sub>). Available as temperature corrected and standardised temperature values.
- Partial pressure of oxygen (pO<sub>2</sub>). Available as temperature corrected and standardised temperature values.
- Bicarbonate concentration (HCO<sub>3</sub>)
- Base excess concentration (BE)
- Sodium concentration (Na<sup>+</sup>)
- Potassium concentration (K<sup>+</sup>)
- Chloride concentration (Cl<sup>-</sup>)
- Calcium concentration (Ca<sup>2+</sup>)

- Glucose concentration (Glu)
- Lactate concentration
- Haematocrit (Hct)
- Haemoglobin concentration (tHb)
- Oxyhaemoglobin concentration (O<sub>2</sub>Hb)
- Carboxyhaemoglobin concentration (COHb)
- Methaemoglobin concentration (MetHb)
- Saturated haemoglobin concentration (SaO<sub>2</sub>)

In this study, samples for arterial blood gas analysis were taken into dedicated sampling syringes (BD Eclipse) which contain electrolyte balanced heparin as an anticoagulant. Samples were analysed within 10 minutes of collection and underwent frequent agitation to prevent sample separation leading to inaccurate reporting of parameters.

The gas analyser contains a co-oximeter which allows for the direct measurement of SaO<sub>2</sub> using spectrophotometric analysis of the haemoglobin species released from a haemolysed sample of blood. Each haemoglobin species has a characteristic spectrum of light absorption, meaning its concentration can be directly measured by measurement of the quantity of light absorbed at prespecified wavelengths. Knowledge of the concentration of the haemoglobin species then allows SaO<sub>2</sub> to be calculated.

## **6.4 Comparisons of Pulse oximetry saturations and arterial oxygen saturation measurements**

The first step in comparing the two methods of measuring oxygen saturations was to compare the correlation between pulse oximeter measurements and arterial blood gas measurements.

A correlation of saturations obtained by pulse oximetry and arterial blood gas analysis (figure 6.1 below) showed good correlations ( $r^2 = 0.85$ ;  $p < 0.0001$ ), but several readings differed by more than the 2% margin of error stated by manufacturers (Milner et al 2012, Hinkelbein et

al 2005), with 49% showing higher values by ABG analysis/SaO<sub>2</sub> and 16% showing higher values by Pulse oximetry/SpO<sub>2</sub>.

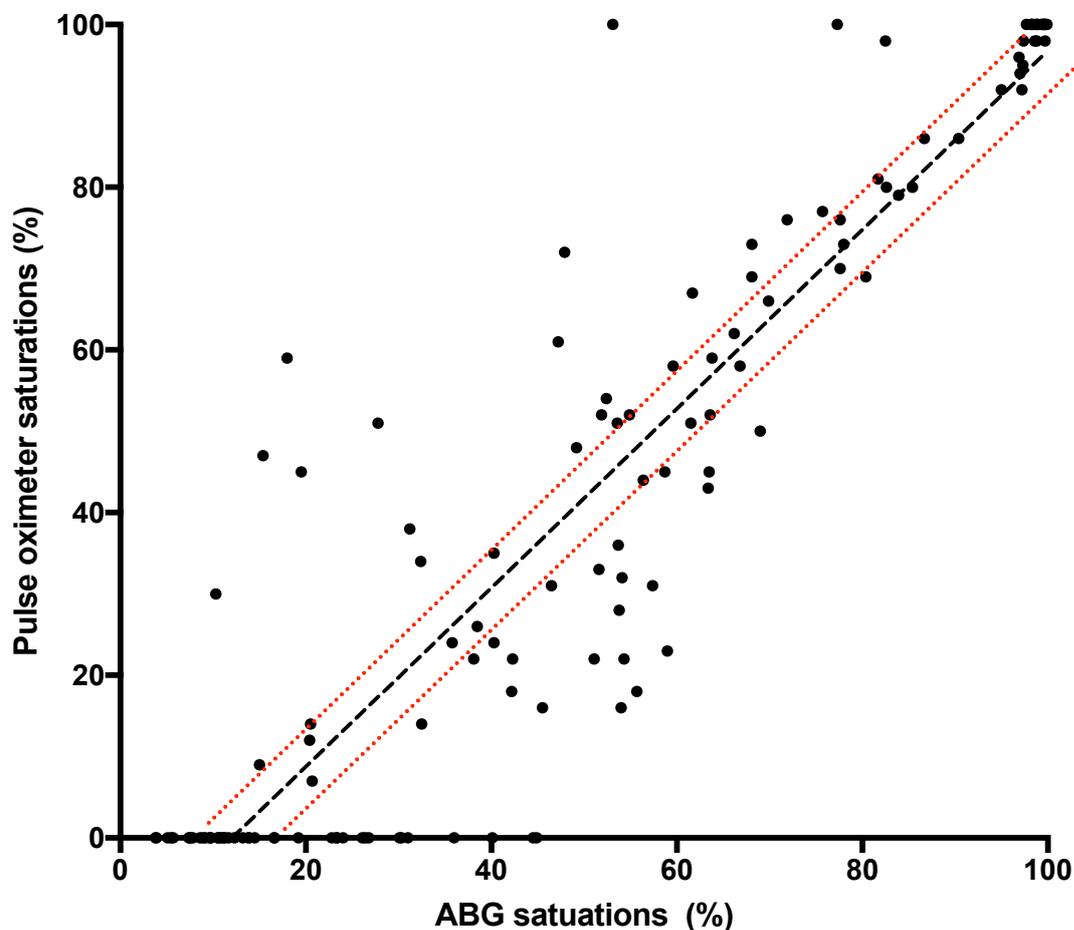


Figure 6.1: Correlation for oxygen saturations measured by pulse oximeter and as part of the blood gas analysis.  $r^2= 0.8467$ ,  $p<0.0001$  by Pearson  $r$  correlation. Red dotted line +/- 2% (Manufacturers reported bias of sats probe when SBP>80mmHg as per Hinkelbein et al 2005) Data collected from 138 paired ABG saturation and pulse oximeter saturation readings from 25 proceeding potential donors.

Having confirmed the relationship between pulse oximetry and ABG measurement of saturations, the next step was to evaluate the agreement between the two methods of measuring oxygen saturations. This was done by evaluation of all data collected from consented patients in a Bland-Altman plot. This plot and analysis is a technique used to compare two measurements techniques of the same variable, and is a statistical method for differentiating between correlation and agreement between two measurements. It is created by plotting the means of each pair of measurements (x-value) versus the difference between measurements (y-value) as is shown below in figure 6.2.

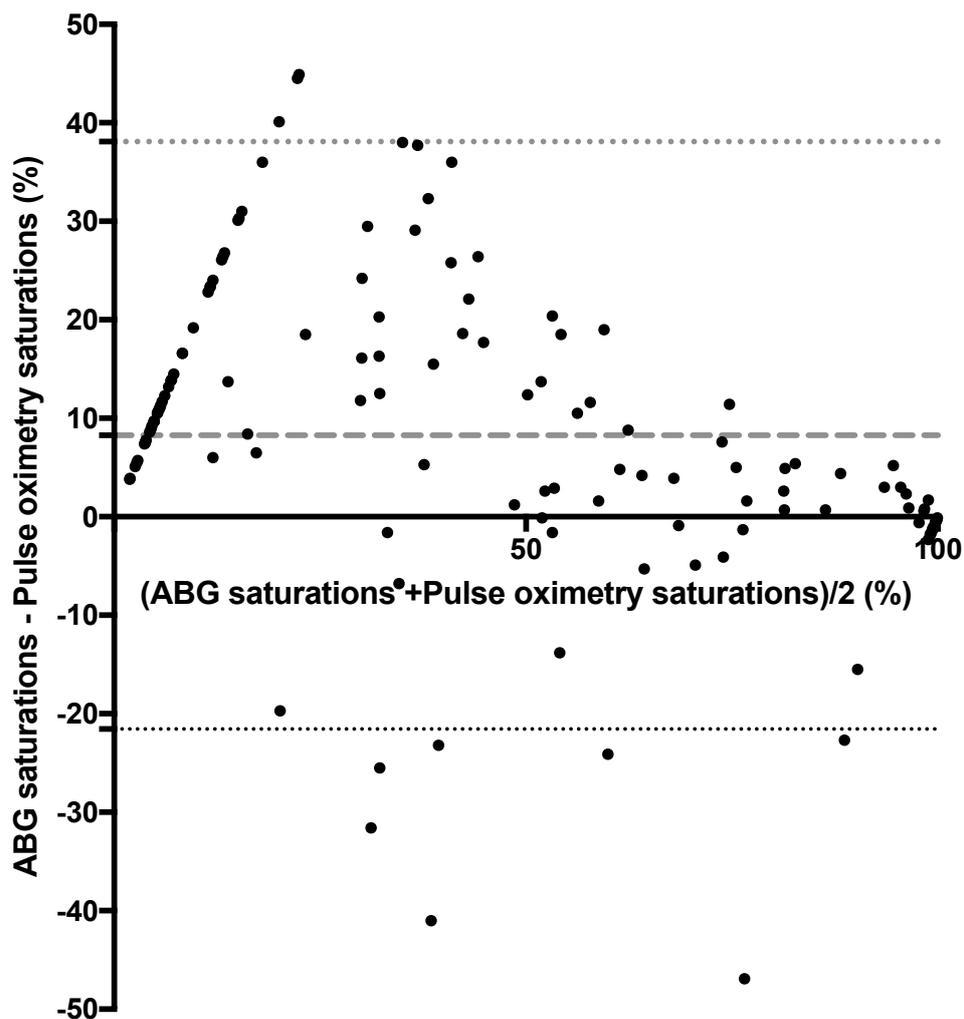


Figure 6.2: Bland-Altman plot showing difference between ABG saturation measurement and Pulse oximetry measurement (Y axis) and the mean value of the paired values of SpO<sub>2</sub> and SaO<sub>2</sub>. Dotted lines represent 95% confidence intervals, dashed line represents bias of data = 8.262. Data collected from 138 paired ABG saturation and pulse oximeter saturation readings from 26 consented potential donors

Having demonstrated the correlation between pulse oximeter saturation measurements and ABG saturation measurements in figure 6.1 and seen the bias of the measurements towards higher ABG saturation readings in figure 6.2, the next step was to compare the implications of the difference observed in figure 6.2 for individual patients. Arterial blood gases were taken in the schedule outlined Chapter 5, and the pulse oximeter reading was recorded at the corresponding time. Graphical representations of this data are given in figures 6.3 and 6.4 below. These figures demonstrate that oxygen saturations can be readily measured by both the techniques being examined.

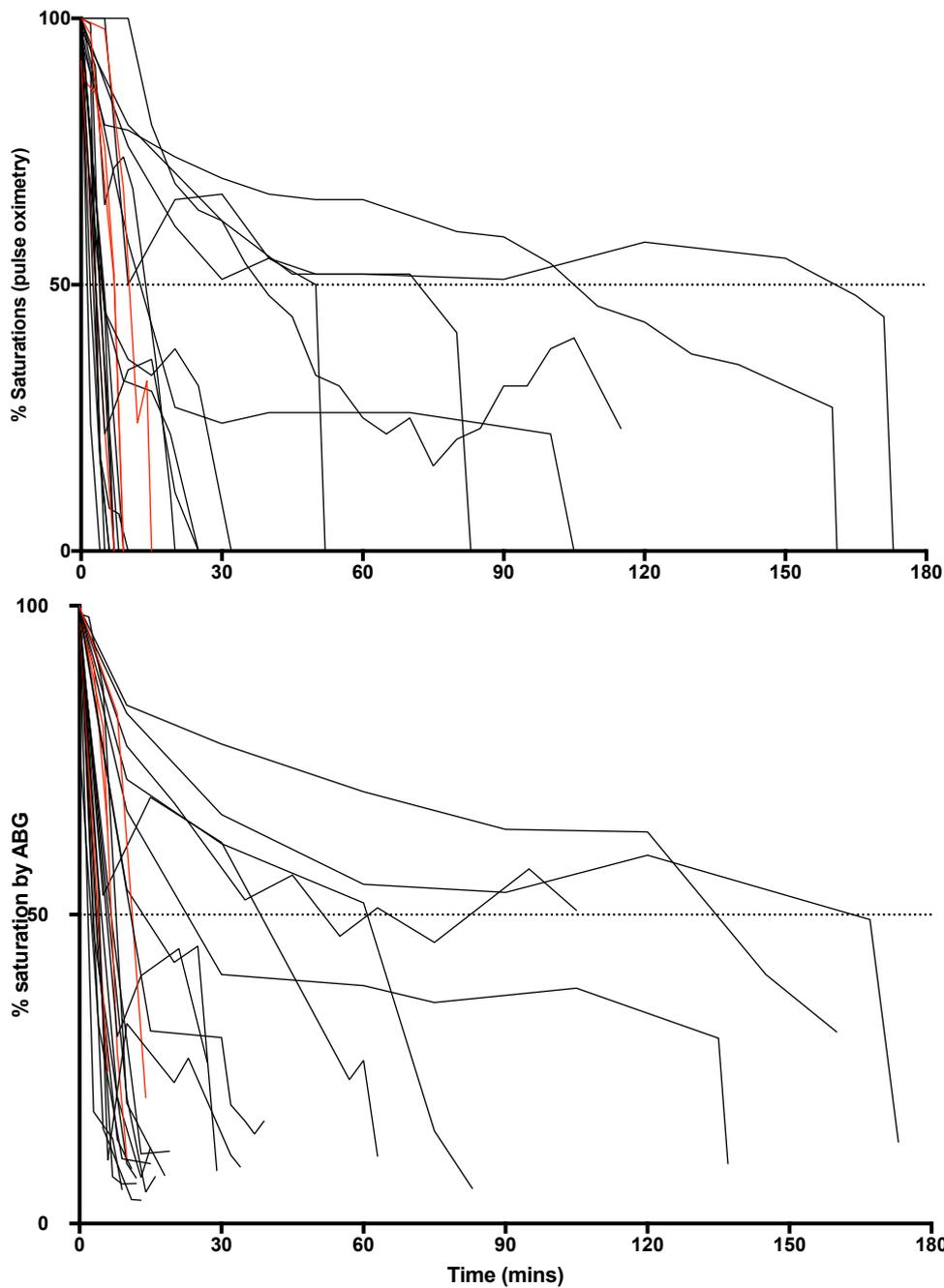


Figure 6.3 (top) and 6.4 (bottom) demonstrating graphical representation of oxygen saturations measured by pulse oximetry and arterial blood gas analysis respectively. Data derived from 25 preceding DCD organ donors. Dotted line represents saturation level of 50%. Solid red lines represent non-typical DCD donors.

A plot of the variation between  $SaO_2$  and  $SpO_2$  for an individual donor is given below in figure 6.5. This clearly demonstrates the potential difference between the two measurement

techniques for an individual. The implications of this difference, and the clinical implications for organ procurement are examined in the following section.

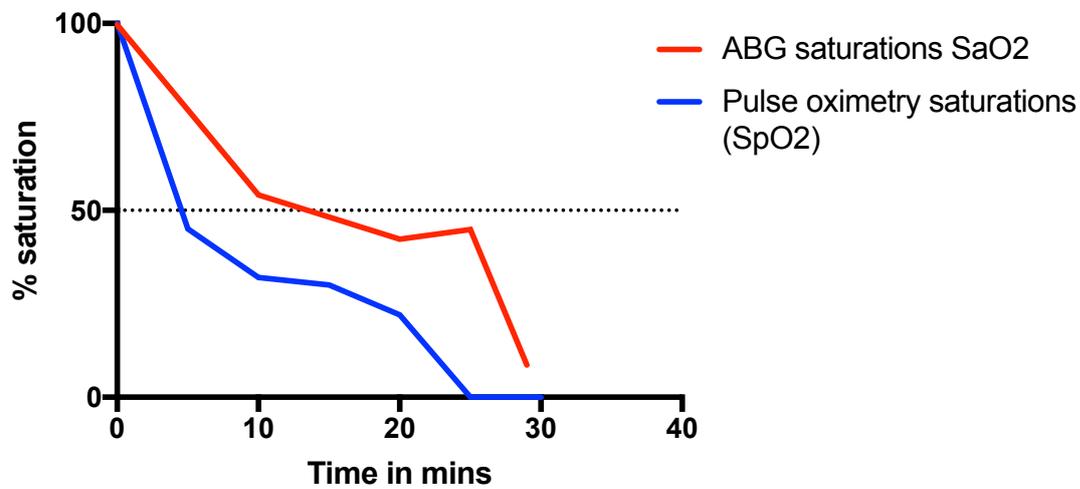


Figure 6.5- A comparison of arterial oxygen saturations from a single patient, subject 5, demonstrating the typical observed variation between saturations recorded by pulse oximetry (blue line) and arterial blood gas (ABG) analysis (red line)

## 6.5 Clinical implications of inaccuracy in saturation measurements

Decisions made by National Organ Retrieval Service (NORS) surgeons regarding how long they will wait for asystole during the agonal period are often made based upon oxygen saturation information provided by pulse oximeter readings. A specified period of time spent with saturations below a predetermined (although arbitrary) level is permitted before the retrieval team stand down due to a perceived ‘prolonged period of hypoxia’ in the donor. In this study threshold saturations of 50% have been used to examine the implications of the variations between SpO<sub>2</sub> and SaO<sub>2</sub> readings.

Of the 25 patients included for analysis in this chapter, cardiothoracic attendance for retrieval took place in 6 cases. The 19 remaining donors were ineligible to donate thoracic organs due to specific contraindications as outlined in section 1.5.1. Cardiothoracic organs were retrieved in three cases with retrieval teams being stood down in the other three cases. Reasons for stand down as recorded by NHSBT are as given below:

- Subject 13: 'Lungs declined due to poor function and copious secretions on bronchoscopy'
- Subject 14: 'Heart stood down due to long hypoxic time'
- Subject 18 'Heart declined due to prolonged hypoxic period'

Examination of the pulse oximetry and arterial blood gas analysis of two of these three patients is revealed in figure 6.6 below:

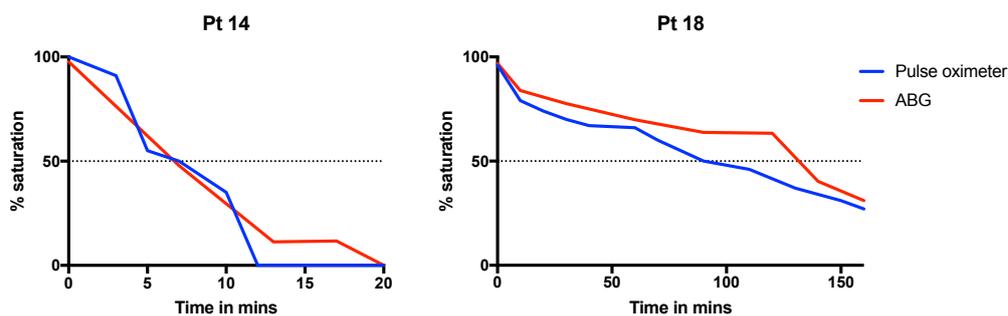


Figure 6.6: Comparison between saturations during the agonal period when measured by pulse oximetry and ABG analysis in two patients in which the Cardiothoracic retrieval teams stood down during the agonal period due to 'prolonged hypoxia'.

Pt 14: Saturations <50% at 7 mins by pulse oximetry and ABG analysis

Pt 18: Saturations <50% at 90mins by pulse oximetry, <50% at 134mins by ABG analysis

The data for Patient 18 in figure 6.6 above demonstrates that with use of ABG analysis of saturations, the patient would be considered eligible to donate their cardiothoracic organs for 44 minutes longer. In the case of patient 18, asystole occurred at 160 minutes, and depending upon the time after saturations drop below 50% allowed by the retrieving team, which is variable depending upon the accepting centre for the cardiothoracic organs, these organs could have been retrieved and transplanted had oxygen saturations levels from ABG analysis been utilised.

Overall, the data presented above demonstrates that SpO<sub>2</sub> readings tended to more frequently be lower than the ABG values for SaO<sub>2</sub>, with the consequence that the arterial oxygen saturation threshold for unacceptable hypoxic burden (with a threshold of 50% being examined in this study) was reached more quickly with SpO<sub>2</sub> monitoring than ABG monitoring (Fig 6.7 below). Consequently, if these data are confirmed acceptance of ABG derived arterial oxygen saturation thresholds would have resulted in 3 more opportunities for cardiac retrieval (maximum acceptable hypoxic time 30 mins), 2 more opportunities for

lung and liver retrieval (maximum acceptable hypoxic time 60 min) should all 25 proceeding patients be capable of donating the above. This assumes that the NORS service utilise the BTS guidelines for permissible hypoxic periods prior to standing down organ retrieval and is likely to be influenced by the use of normothermic regional perfusion techniques which allow in situ organ function assessment and result in greater flexibility of permitted hypoxic times by retrieving surgeons.

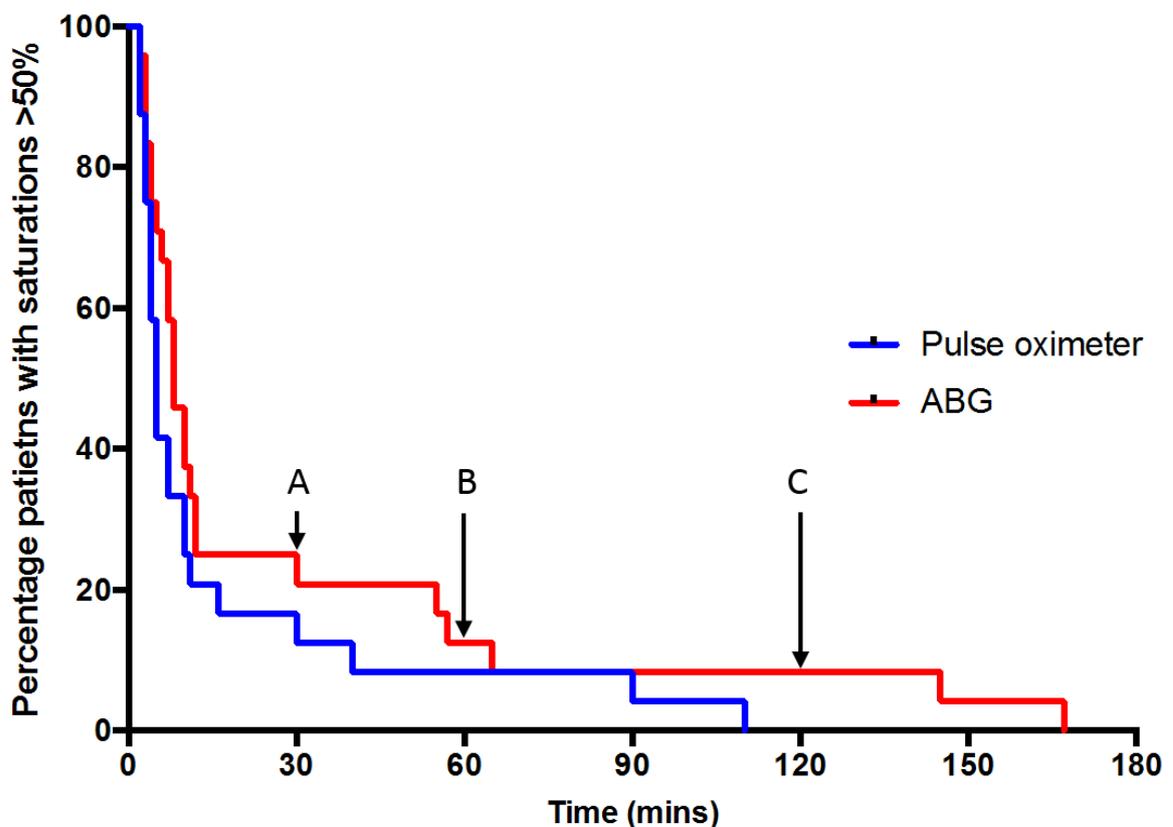


Figure 6.7: Patient survival with saturations greater than 50%. Data derived from 25 proceeding DCD donors. Additional organs available for transplantation at following time points: A: Time = 30 minutes, 9% more organs available; B: Time = 60 minutes, 5% more organs available; C: Time = 120 minutes, 8% more organs available.  $p=0.0387$  by Log-rank (Mantel-Cox) test

Given that SpO<sub>2</sub> accuracy is reduced by hypotension (Hinkelbein et al 2005), presumably by reducing peripheral perfusion, and since ABG derived SaO<sub>2</sub> measurements would be a change in practice, I then investigated whether the product of SpO<sub>2</sub> and SBP might correlate better with SpO<sub>2</sub>, thus providing a means of relating the two measures of arterial oxygenation. However, the correlation of SpO<sub>2</sub> vs. [SaO<sub>2</sub> x SBP] (data not shown) was worse

than that for SpO<sub>2</sub> vs SaO<sub>2</sub> ( $r^2$ : 0.72 vs. 0.85, respectively) which suggests that systolic driving pressure was not the main reason for the discrepancy.

## **6.6 Chapter summary and discussion**

### **6.6.1 Chapter summary**

Monitoring of oxygen saturation is currently used by retrieval surgeons to assess the degree of hypoxia being experienced by the potential donor (Peters-Sengers et al 2018). Traditional monitoring of oxygen saturation during the withdrawal period is with pulse oximetry (NHS Blood and Transplant National Standards for Organ Retrieval from Deceased Donors (2012)). An alternative to pulse oximetry to measure saturation is with frequent blood sampling for ABG analysis. The data presented in Figures 6.3 and 6.4 demonstrate that both techniques can be used to gain frequent assessment of oxygen saturation. While Figure 6.1 demonstrates that there is a correlation between the two techniques, Figure 6.2 demonstrates that there is not agreement between pulse oximetry and ABG saturation measurements when used in this context, and that there is bias towards higher values being recorded from ABG measurements. The non-equivalence of these two techniques is important when considering the use of pulse oximetry in this setting.

Figures 6.5, 6.6 and 6.7 demonstrate that there are significant differences in the data gathered from the two techniques. Inaccurate saturation data may lead to inappropriate decisions being made by organ retrieval teams, who rely upon non-invasive monitoring techniques to make clinical decisions. The two key sources of inaccuracy in pulse oximeter measurements of oxygen saturations are considered below:

### **6.6.2 Discussion of sources of pulse oximeter inaccuracy**

#### **a. Hypoxaemia**

The technology underpinning pulse oximetry was developed in 1937 by Matthes (Severinghaus et al 1986) but took until the 1980s to reach widespread clinical use since when it has become an essential form of monitoring for patients across all disciplines worldwide. It has been widely proved to be reliable and cost effective in the critical care settings (Jubran 2012) and is a recommended standard of care in anaesthesia and critical

care (Checketts et al 2017, ARDSNET 2000). However, inaccuracy of pulse oximetry in certain clinical settings is well established (Benson et al 1995).

Previous studies which have compared time matched SpO<sub>2</sub> with SaO<sub>2</sub> have found large differences can occur, particularly in critically ill patients (Louw et al 2001). Severinghaus induced transient hypoxaemia down to SpO<sub>2</sub> of 80% in a group of healthy volunteers and observed mean errors in excess of 6% with a standard deviation of greater than 10% with pulse oximeter saturation measurements (Severinghaus et al 1986). A meta-analysis conducted by Jensen et al included 74 studies concluded that pulse oximeters were accurate to within 2% ( $\pm 1$  SD) or 5% ( $\pm 2$  SD) of *in vitro* oximetry in the range of 70% to 100% SaO<sub>2</sub>. It did acknowledge that the majority of studies were based upon healthy volunteers, and also suggested that pulse oximeters may be inaccurate during severe or rapid desaturation, hypotension, hypothermia, dyshemoglobinaemia, and low perfusion states (Jensen et al 1998).

The inaccuracy of pulse oximetry saturations below 70% is attributed to the difficulty faced by companies responsible for creating algorithms for gathering calibration data for humans in conditions of extreme hypoxia. The accuracy of the algorithms used in pulse oximetry are clearly limited by the range of saturations that are ethically and safely obtained in volunteers. There are multiple algorithms used by companies who produce pulse oximeters, the majority of which use calibration algorithms tested on volunteers only down to oxygen saturations of 70%. Consequently, reported saturations of below 85% are based on extrapolation of the known data with incorporation of data from animal models (Jubran et al 1998). As is clear from figures 6.3 and 6.4, hypoxaemia is an inevitable feature of the proceeding DCD donor.

#### b. Hypotension

Pulse oximetry-based saturation measurements have been noted to be inaccurate in patients with haemodynamic instability (Ibanez et al 2001, Vicenzi et al 2000) and as DCD donation by definition requires circulatory arrest, haemodynamic instability clearly occurs in all proceeding donors. Hypotension results in peripheral vasoconstriction and central redistribution of blood flow, leading to a decrease in the pulsatile component of the signal

detected by pulse oximeter probe. This effect has been noted to be more significant for finger pulse oximeter probes than for ear probes (Das et al 2010). A study by Hinkelbein et al suggests the threshold at which hypotension renders pulse oximetry less accurate is 80mmHg (Hinkelbein et al 2005). Other probe locations not commonly used in routine UK clinical practice have been noted to be significantly less affected by vasoconstriction (Clayton et al 1991). Forehead probes have been suggested as being the least affected during hypotension (Nessler et al 2012, Sugino et al 2004). Furthermore, the presence of vasoactive drugs has been demonstrated to decrease the accuracy of pulse oximetry (Ibanez et al 1991) through the same mechanisms described above. As described in table 5.1, 71% of potential donors in this study receive vasoactive drugs in the lead up to withdrawal of life supporting treatment, not to mention the catecholamine release that occurs on withdrawal and which is discussed later.

Inaccuracies due to anaemia have also be reported in the literature (Severinghaus et al 1990) but are unlikely in this study as no patient had a haemoglobin level below 72g/L and accuracy has been suggested down to a haemoglobin level of 52g/L (Jay et al 1994). Further well established sources of error in pulse oximetry include carboxyhaemoglobinemia (Barker et al 1987), methaemoglobinemia (Glass et al 1986), hypothermia (Tremper et al 1985) and the use of surgical dyes (Barker et al 1987). However, the extensive screening undertaken by the SNOD team prior to the withdrawal of life support means it is unlikely that these factors play a role in this study.

In the setting of assessment of donor physiology, much animal work has focussed on saturation assessment (White et al 2016) by pulse oximetry, which is subject to the same sources of inaccuracy set out above. Recent work by Peters-Senger et al aims to compare DCD donor haemodynamics to kidney graft outcomes and uses a cut off of saturations below 60% by finger tip pulse oximetry to represent the onset of warm ischaemia in the donor (Peters-Senger et al 2018). They noted that the period of  $SpO_2 < 60\%$  was not associated with increased risk of delayed graft function (DGF), however, the period of hypotension, defined as  $SBP < 80\text{mmHg}$ , correlated with the rate of DGF. The data represented by figure 6.7 suggests it likely that the period of saturation below 50% would be different when calculated by arterial blood gas analysis and new correlates may need to be drawn.

### 6.6.3 Chapter Summary and Conclusions

It is not clear which of these two measures of oxygen saturation provide the better measure of true hypoxic burden. The ABG measure is a more direct (and arguably “gold standard”) measurement of true arterial oxygen saturation. Further, though manufacturers claim a 2-3% error rate for SpO<sub>2</sub> measurement between SpO<sub>2</sub> values >70%, in practice, many devices show substantial errors even in this range (Milner & Mathews 2012), and are likely to perform worse at lower saturations, making measurements at 50% more likely to be affected by error. These errors are also more likely to be dependent on blood pressure, with greater inaccuracy below a systolic blood pressure of 80 mmHg (Hinkelbein et al 2005), since peripheral perfusion may affect the accuracy of pulse oximetry.

However, it is important to sound one cautionary note. Even if reductions in blood pressure affect the accuracy of SpO<sub>2</sub> readings, these inaccuracies may not necessarily translate into poorer prediction of the overall burden of hypoxia and post-transplant function in donated organs. This is because the very factors that make SpO<sub>2</sub> measurements of SaO<sub>2</sub> inaccurate might integrate information about peripheral circulatory efficiency that is unavailable from the accurate gas exchange information delivered by ABG-derived SpO<sub>2</sub>.

The data provided in this chapter (and the discussion here) relate primarily to a SpO<sub>2</sub> threshold of 50% (which is in common use in UK cardiothoracic transplant centres). An SpO<sub>2</sub> threshold of 70% is often adopted in abdominal retrieval centres as a basis for practice. However, all of these thresholds have been derived by expert consensus and are not evidence based. The rational approach to determine what threshold should be used, and (in the light of this chapter) how this should be measured, would be to look at organ outcomes in a large series of patients to determine critical oxygen saturation thresholds that influence organ function. However, we have no such data, and the many covariates and confounders that affect transplant organ function would necessitate an extremely large prospective data collection to allow robust estimation of such hypoxic thresholds.

I have demonstrated in figure 6.7 the gains in organ retrieval numbers that can be made by maximising the retrieval potential of each donor using a potential change of protocols to ABG assessment of donor oxygen saturations ( $\text{SaO}_2$ ) rather than pulse oximetry ( $\text{SpO}_2$ ). Such a change would be cheap and uses point of care technology readily available in the donor hospital. This contrasts with current interests in reconditioning donated organs either in-situ using normothermic perfusions techniques or ex-situ cold perfusion techniques which are successful in increasing the numbers of transplantable organs but are expensive and highly specialised.

The results of this chapter raise the question of the appropriateness of the use of pulse oximetry to monitor oxygen saturations during the withdrawal period and suggests that more accurate data may be seen with regular ABG saturation assessment. Clearly this would have implications for current practice and protocols used during the withdrawal period to gather data from the potential donor. Of note, these protocols utilise thresholds that are based solely on consensus opinions of retrieving surgical teams – there is no evidence to support that a particular threshold of oxygen saturation is associated with worse donated organ condition. However, the fact that clinical decisions regarding organ retrieval are made on what this chapter demonstrates to be less accurate information leaves a clear potential for increased organ retrieval numbers if more accurate methods of monitoring oxygen saturations in low oxygen conditions are used routinely.

An alternative approach would be to use a well-accepted metric of tissue hypoxia in the form of plasma lactate levels as an endpoint for assessing the applicability of different oxygen saturations thresholds, and means of measuring these. Lactate levels are responsive to the physiological burdens presented by both arterial hypoxaemia and tissue hypoperfusion, and though only available at a global level in arterial samples, still provide a whole body readout of the impact of these two insults, and hence a means of exploring physiology in this context. The next chapter will therefore describe the changes in lactate levels in proceeding DCD donors, and explore the physiological drivers of lactate elevation in this context.



## **Chapter 7: Assessing the onset of tissue hypoxia in the DCD donor**

### **7.1 Chapter Introduction and overview**

In chapter 6 I demonstrated that the dying process in the DCD organ donor is associated with the development of progressive hypoxaemia. The limitations in the current practice of using pulse oximetry data to determine oxygen saturations were explored, and alternative methods for assessing hypoxaemia were suggested.

Simply demonstrating the development of hypoxaemia does not sufficiently characterise the burden of abnormal physiology that organs are exposed to, since tissue oxygen delivery is dependent on both perfusion and arterial oxygen content. Therefore, this chapter sets out to explore the relationship between development of hypoxaemia and inadequate oxygen delivery to the tissues.

In this chapter, we have used the basic physiological principles outlined in chapter 5 to calculate arterial and venous oxygen content, and oxygen extraction using information obtained from arterial and venous blood gas analysis. We have then correlated these markers against systolic blood pressure and lactate to assess their relationship with tissue hypoxaemia. The experimental literature has, in addition estimated cardiac output using a model of a fixed stroke volume, and used these estimates to calculate oxygen delivery (DO<sub>2</sub>). I do not believe that these estimates are accurate, and so have not used these calculations in my results. However, in order to provide context for comparison with experimental studies, I have included some of the results of such estimations in the discussion section of this chapter

Blood lactate was chosen as a marker of tissue hypoxaemia, as its concentration represents the balance between lactate production and lactate clearance in the body. As discussed in Chapter 1, lactate is formed as an end product of the glycolytic pathway under anaerobic conditions, with the aim of providing ATP for essential metabolic processes in the presence of low oxygen conditions. This is inefficient by comparison to aerobic metabolism which

yields 38 ATP for each molecule of glucose metabolised. The measurement of lactate provides a measure of this impact of hypoxaemia on cellular metabolism, which is detectable in peripheral blood samples (Cain et al 1994).

In order to assess the factors implicated in elevation of blood lactate, I will explore how various measurable and calculable oxygenation parameters are related to its rise. The clinical relationship between blood lactate level and tissue hypoxia was first described by Meakins in 1927 (Meakins 1927) and has formed the basis for understanding the degree of organ dysfunction caused by hypoxia and monitoring its resolution once optimal oxygenation has been restored. The clinical use of blood lactate levels to monitor the degree of tissue hypoxia, forms the basis of multiple guidelines to diagnose and manage patients with impaired oxygen delivery to the tissues (Singer et al 2016, Rivers et al 2001). The association between elevated serum lactate and poor patient outcomes due to organ hypoxia is well established across multiple clinical areas (Stacpoole et al 1994) and has received some attention in animal model of DCD donation. Measurement of lactate levels is used by groups using Normothermic Regional perfusion (NRP) to recover organs to indicate successful restoration of perfusion to intra-abdominal organs, and in particular the liver (Baroncelli et al 2017). However, the rise of lactate during the dying process in the DCD organ donor has not previously been the subject of intensive study.

The importance of warm ischaemia in causing organ damage is well established, and while it is clearly occurring following asystole, there is a period before asystole when harmful tissue hypoxia is occurring. The difficulty in defining the onset of warm ischaemia before asystole for a particular patient represents a significant clinical challenge. Given the onset of elevation of serum lactate occurs at the point of impaired oxygen delivery to the tissues, I aim in this chapter to explore the potential of using lactate as surrogate marker for the onset of warm ischaemia.

The results and discussion in this chapter focus on the following:

1. Demonstration that blood oxygenation and serum lactate can be measured frequently and accurately in the potential DCD organ donor after treatment withdrawal.
2. Demonstration of the correlation between serum lactate and arterial oxygen content.
3. Demonstration of the relationship between serum lactate and venous oxygen content.
4. Demonstration of the relationship between serum lactate and oxygen extraction ratio

The hypotheses being tested in this chapter are:

- that a decreasing oxygen delivery to the tissues will correlate well with elevation of blood lactate levels, and
- that lactate levels will provide an objective biochemical marker of the onset of anaerobic metabolism in in the DCD donor.

## **7.2 Methodology of lactate assessment**

Samples were acquired at times and systolic blood pressure intervals as described in Chapter 4. Samples were taken from arterial cannulae using specific heparinised syringes and analysed immediately using point of care blood gas analysers.

The blood gas analyser measures lactate using an enzyme receptor probe. Within the probe, the blood sample reacts with lactate oxidase to form pyruvate and  $H_2O_2$  which is subsequently oxidized. This process creates a potential difference, the size of which is proportional to the lactate concentration in the sample. The blood gas analyser undertakes automatic calibration cycles every 120 minutes, and has a daily quality control and reagent assessment performed by specially trained staff. The machine is able to detect serum lactate in the range of 0 to 20 mmol/l with an accuracy of +/- 5% for readings below 10.0mmol/l and 10% for readings above 10.0mmol/l. The normal range for lactate is 0.5-2.0 mmol/l when this assay is used.

### 7.3 The role of hypotension in the elevation of lactate

The correlation between blood lactate level and hypotension is well established clinically but has previously not been assessed in DCD donors. In order to test our hypothesis that hypotension during the dying process in the DCD donor is also associated with elevation of blood lactate, arterial blood samples were acquired from 27 patients undergoing withdrawal of life supporting treatments. Samples were taken using the methodology outlined above and taken in a time and systolic blood pressure-based schedule as outline in chapter 3.

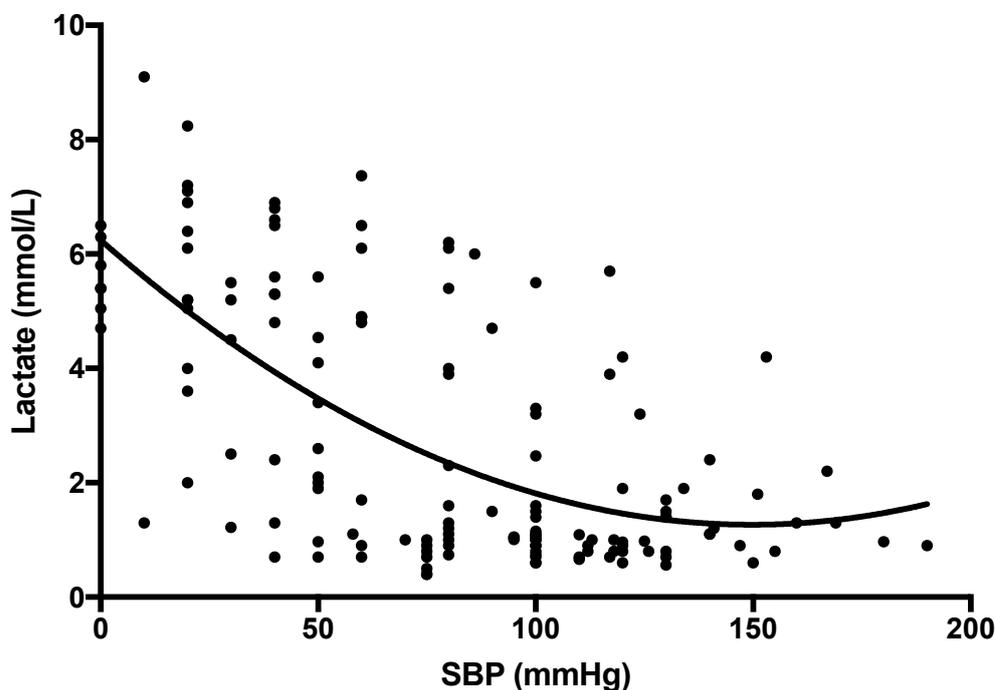


Figure 7.1: Correlation between Lactate level and Systolic blood pressure. Solid line represents non-linear (second order polynomial) fit of data  $R^2= 0.4072$ .  $Y= 6.243+(-0.0665X)+0.00222X^2$ . Data from  $n=28$  consented DCD donors undergoing withdrawal of treatment.  $p= <0.0001$  by spearman rank correlation.

Figure 7.1 shows a negative correlation between systolic blood pressure and lactate level in the proceeding DCD donors. Systolic blood pressure is the analysed marker displayed here as this is the marker used by the transplant community and NORS surgical teams. However, given that mean arterial blood pressure (MAP) is the physiological target most frequently used in the intensive care setting, I then investigated whether MAP might correlate better with lactate. However, the correlation of MAP vs lactate (data not shown) was marginally worse than that for SBP ( $r^2$ : 0.38 vs. 0.41 respectively)

Figure 7.1 demonstrates that there are some donors in whom there is a relatively high lactate despite a high systolic blood pressure. Clearly the elevation of lactate in the dying process is a complex and multifactorial relationship. Further consideration of factors that influence lactate levels such as hypoxia and catecholamine are considered during the remainder of this chapter and chapter 8.

## 7.4 The role of arterial and venous oxygenation

Given that lactate levels correlated poorly with SBP, I examined how measures of arterial oxygenation and oxygen extraction/utilisation correlated with lactate levels. In order to determine which measure of arterial oxygenation best correlated with arterial lactate (as a metric of global tissue oxygen debt), I examined the relationship of arterial lactate levels to SpO<sub>2</sub>, ABG-derived SaO<sub>2</sub>, and [SaO<sub>2</sub> x SBP]. All three analyses showed a nonlinear relationship, with lactate levels within normal limits (<2.0 mmol/l) at normal SaO<sub>2</sub> and SpO<sub>2</sub> levels, but with an overall progressive rise in lactate as arterial oxygen saturation fell. Overall, the correlation of lactate concentrations was better with SpO<sub>2</sub> compared to SaO<sub>2</sub> ( $r^2$ : 0.56 vs. 0.44;  $p < 0.0001$  for both; Fig 7.2 and 7.3). However, it was noticeable that several instances in which the SpO<sub>2</sub> was unrecordable (represented as 0% in Fig 7.2). Using the [SaO<sub>2</sub> x SBP] product resulted in even worse correlations ( $r^2$ : 0.42; data not shown), suggesting that including SBP as a measure of driving pressure did not add explanatory power.

In all cases, the relationship of SpO<sub>2</sub> (or SaO<sub>2</sub>) to lactate was far more variable at SpO<sub>2</sub> values <50%, with some patients showing marked elevations in lactate, and others showing normal lactate levels, even at SpO<sub>2</sub> or SaO<sub>2</sub> values  $\leq$  25%. It is tempting to attribute a portion of this variability to the rate of decline in physiological parameters, however examination of figure 5.12 demonstrates that even in donors dying in the shortest time periods there remains scope for significant serum lactate elevation.

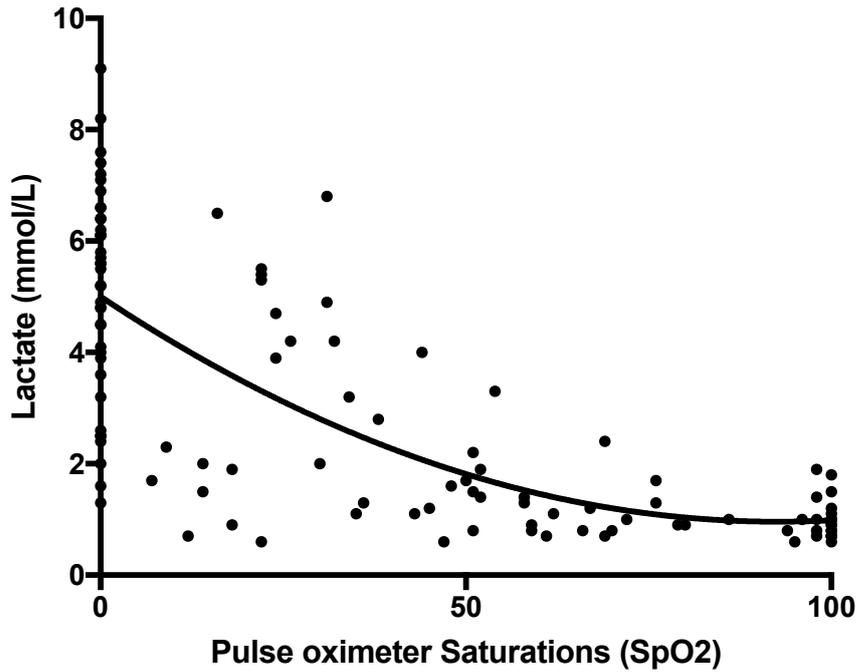


Figure 7.2: Relationship between pulse oximeter saturations ( $SpO_2$ ) and lactate levels. Data derived from 121 paired values from 25 preceding DCD donors.  $R^2 = 0.5599$ ;  $UY = 5.001 + (-0.08726X) + 0.000471X^2$

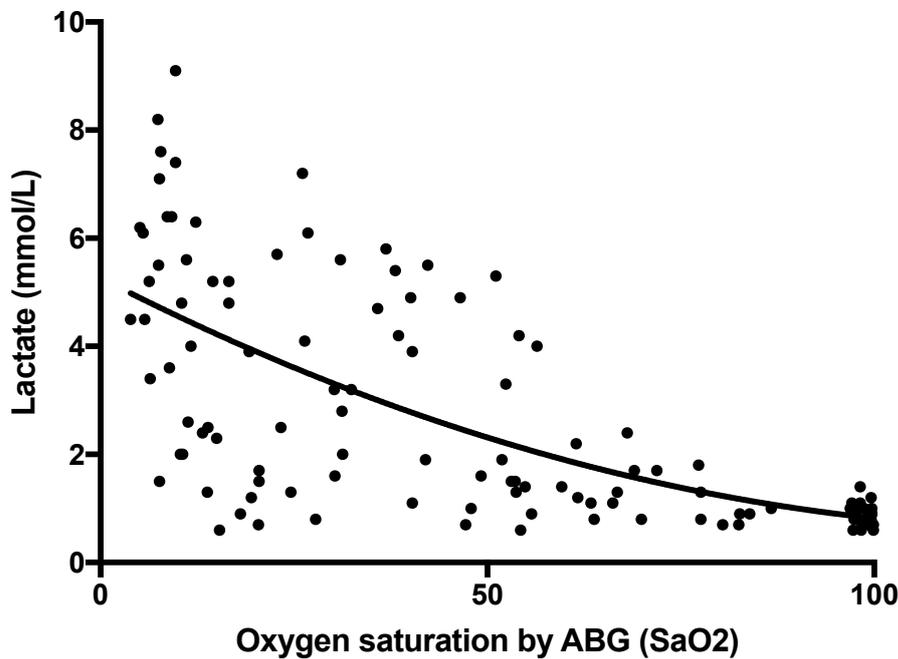


Figure 7.3: Relationship between oxygen saturation by ABG ( $SaO_2$ ) and Lactate. Solid line represents non-linear regression of data.  $Y = 5.264 + (0.07355X) + 0.00291X^2$   $R^2 = 0.4381$ . Data derived from 125 paired samples from 25 preceding DCD donors.

Given this heterogeneity in  $SaO_2$ -lactate relationship, I explored other drivers of lactate elevation in this context. While measurements of oxygen delivery and consumption would

have provided useful measurements in this context, the absence of cardiac output measurements made calculation of these metrics impossible. I therefore examined the relationship of arterial lactate to arterial oxygen content ( $CaO_2$ ; which would compensate for differences in Hb levels), venous oxygen saturation ( $SvO_2$ ), venous oxygen content ( $CvO_2$ ), oxygen extraction ratio (OER) and arteriovenous oxygen difference ( $AVDO_2$ ).  $SvO_2$  levels were taken directly from venous blood gases, when available, at the schedules outlined in chapter 4. Formulae used to calculate the remaining metrics are listed below:

$$CaO_2: CaO_2 = (1.39 \times Hb \times SaO_2) + (0.003 \times PaO_2)$$

$$CvO_2: CvO_2 = (1.39 \times Hb \times SvO_2) + (0.003 \times PvO_2)$$

$$OER: OER = (SaO_2 - SvO_2) / SaO_2$$

$$AVDO_2: AVDO_2 = CaO_2 - CvO_2$$

*(where Hb = Haemoglobin,  $SaO_2$  = arterial oxygen saturation of blood,  $PaO_2$  = Oxygen partial pressure of arterial blood,  $SvO_2$  = venous blood oxygen saturation,  $PvO_2$  = oxygen partial pressure of venous blood)*

The use of venous oxygen saturations and venous oxygen content in the presence of circulatory shock is well established (Nguyen 2013). A true mixed venous sample is only available by sampling from the pulmonary artery via a pulmonary artery flotation catheter (PAFC), the use of which has decreased over recent years. The difficulty in interpreting venous oxygen saturation measurements from central venous catheters (CVC) placed in the superior vena cava or right atrium is well established (Edwards, et al 1998). Equal difficulty comes in interpretation of measurements from central venous catheters placed in the femoral artery (Van Beest et al 2012). Many of the cohort recruited for this study had a CVC placed as part of their routine care in the intensive care unit. In order to assess if venous oxygen saturation information from these lines provided clinically useful information during the withdrawal of life supporting treatment, line position was surveyed in 28 potential DCD donors consented for enrolment in this study (table 7.1 below)

Line position	Number of Patients
CVC – Neck	16
CVC - Femoral	4
CVC - PICC	4
Peripheral cannulae only	4

Table 7.1: Location of venous lines for consented donors.

As shown in Table 7.1, there is a variation in CVP line type and location in the studied DCD organ donors. No donor had a PAFC in order to measure true mixed venous oxygenation or cardiac output. To examine the relationship between line location and venous oxygen saturations, which has been demonstrated in previous studies of patients undergoing cardiac surgery or resuscitation from circulatory shock (Van Beest et al 2010), the differences between initial venous oxygen saturations and line location were performed (figure 7.4 below). The low numbers of femoral CVC lines in proceeding donors, combined with the fact that several of these lines were unable to be aspirated to provide samples during the withdrawal period meant that I was unable to perform stratification of CvO<sub>2</sub> and OER by line location.

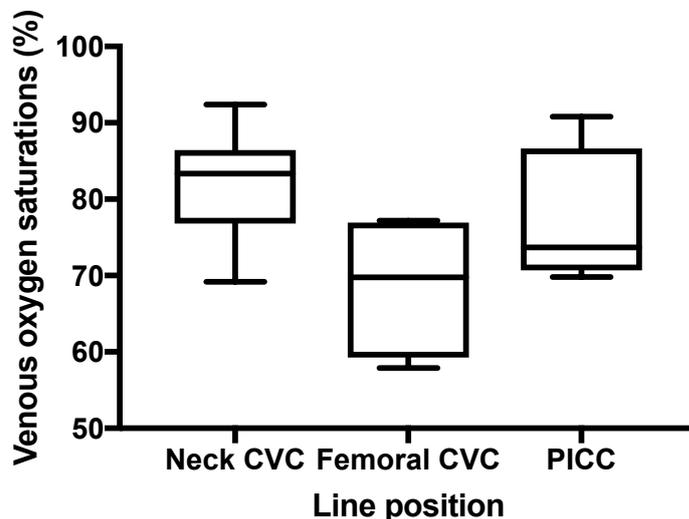


Figure 7.4: Venous oxygen saturation by line location. Data presented as medians, inter-quartile ranges (box) and range (whiskers),  $p=0.0763$  by Kruskal-Wallis test. Data derived from 24 patients (16 Neck CVC, 4 Femoral CVC, 4 PICC)

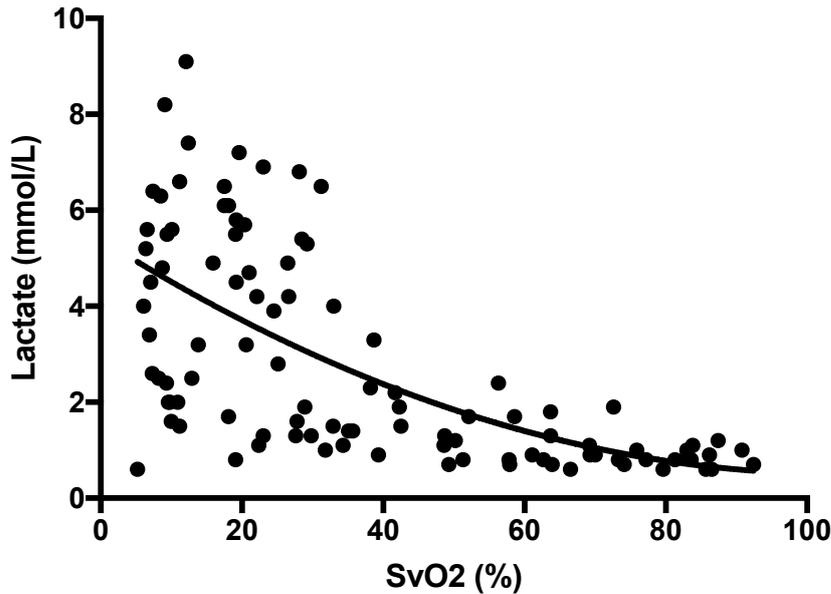


Figure 7.5: Relationship between venous oxygen saturations ( $SvO_2$ ) and lactate. Solid black line represents non-linear fit of data.  $R^2 = 0.4161$ .  $Y = 5.4 + (0.09332X) + 0.00445X^2$ . Data derived from 20 proceeding DCD donors.  $p < 0.0001$  by Spearman rank correlation

The  $CaO_2$ -lactate relationship, demonstrated below in figure 7.6, was very similar to the  $SaO_2$ -lactate plot, with a worse  $r^2$  (0.41 vs. 0.44) suggesting no improvement by allowing for variations in Hb levels.

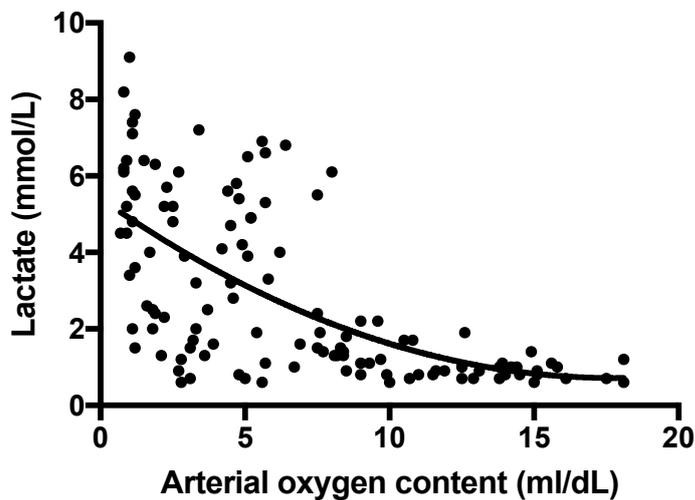


Figure 7.6: Relationship between arterial oxygen content of blood (ml/dl) and blood lactate level (mmol/l) Solid line represents linear regression of data with  $R^2 = 0.4065$ .  $Y = 5.412 + (-0.5283 * X^2)$  Data from 121 samples from 24 proceeding DCD donors after withdrawal of life supporting therapy.  $p < 0.0001$  by Spearman rank correlation

Serum lactate levels showed a better correlation with CvO<sub>2</sub> than CaO<sub>2</sub> (r<sup>2</sup>: 0.44 vs. 0.41), but this remains similar to the r<sup>2</sup> values for the association with SaO<sub>2</sub>.

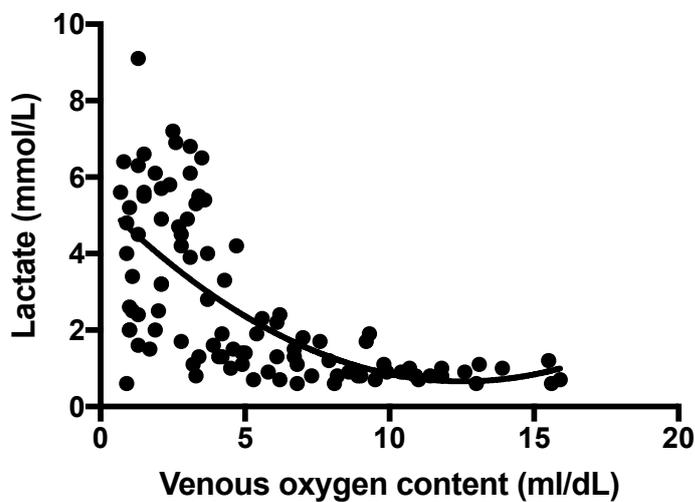


Figure 7.7 Relationship between venous oxygen content and blood lactate level. Solid line represents non-linear regression analysis of data, with  $R^2=0.4433$ .  $Y=5.38+0.7525*X^2$ . Data derived from 99 paired samples from 20 proceeding DCD donors.

## 7.5 Calculation of Oxygen Extraction ratio

Oxygen extraction ratio is typically taken at being 0.25 in resting conditions, rising to 0.7 in periods of exercise or circulatory stress (Nebout et al 2012). As discussed earlier, as oxygen consumption increases, or delivery of oxygen falls, the oxygen extraction ratio rises in order to maintain adequate oxygen for aerobic metabolism. At a critical threshold of oxygen delivery, the maximum oxygen extraction ratio is reached, and further decline in oxygen delivery leads to tissue hypoxia, and anaerobic metabolism. This anaerobic metabolism manifests as an increase in lactate level. To demonstrate that this phenomenon described in critically unwell patients applies to the potential DCD donor (who by definition could be considered critically ill), correlations between lactate level and oxygen extraction ratio were undertaken in 20 proceeding DCD organ donors (Figure 7.8 below)

Arterial lactate showed only a weak relationship to OER (Fig 7.8) and no significant relationship to AVDO<sub>2</sub> (Fig 7.9)

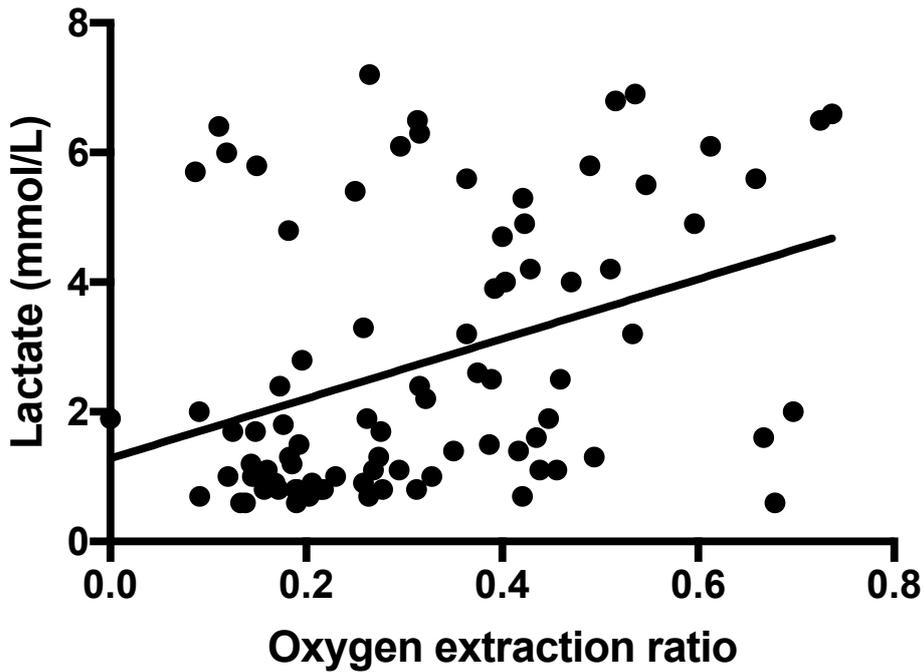


Figure 7.8: Relationship between oxygen extraction ratio and lactate. Solid line represents linear regression of data with  $R^2=0.1381$  and  $p=0.0004$ .  $Y=4.607*X+1.282$ .  $p=0.0004$  by spearman rank correlation

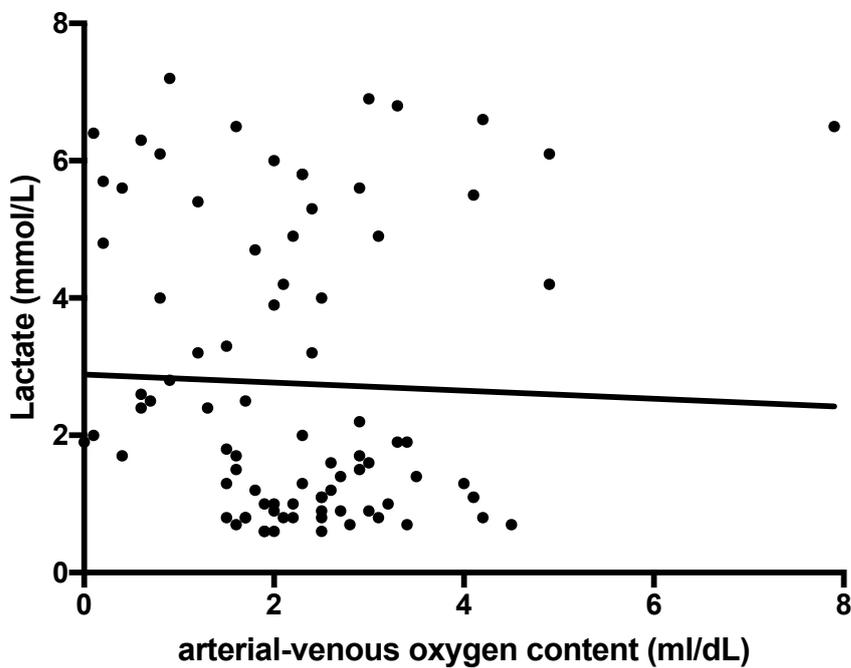


Figure 7.9. Correlation between arterial- venous oxygen content (ml/dL).  $R^2= 0.001299$   $Y = -0.05888*X + 2.885$ . Data collected from 99 paired samples from 20 proceeding DCD donors.  $p=0.1257$  by spearman rank correlation

## 7.6 Chapter Summary and Discussion

The work described in this chapter gives new insight into the cardiovascular physiological changes that occur in the DCD organ donor during the process of circulatory death.

Figures 7.1 to 7.9 above demonstrate that frequent paired samples taken from arterial and venous lines during the dying process are feasible and translate to information that can be used clinically to describe the progression of physiology within the donor from aerobic to anaerobic metabolism.

Figure 7.1 demonstrates the poor correlation between systolic blood pressure and lactate. The use of systolic blood pressure assessment during withdrawal of life supporting treatment by clinical teams responsible for organ retrieval is standard clinical practice. Hypotension below an arbitrary threshold is used as marker for the onset of 'warm ischaemia' which can be considered as anaerobic metabolism occurring in the normothermic donor. No international consensus exists on a threshold figure with considerable variation in practice, but UK NORS teams use a figure of 50mmHg to represent the onset of warm ischaemia (BTS guidelines 2012). Figure 7.1 demonstrates a correlation, albeit poor, between decreasing systolic blood pressure and arterial lactate concentrations, but there remain some donors who have high levels of lactate despite a normal or supranormal systolic blood pressure. In this context, blood pressure can be considered a function of flow and resistance, and while blood pressure increases may represent increased flow, it may equally represent increased resistance caused by vasoconstriction and flow that is similar or reduced. These potential factors affecting blood pressure will be considered further in Chapter 9.

The remainder of this chapter has considered the relationship between lactate and tissue oxygenation. The data represented in this chapter demonstrate that frequent lactate levels can be taken during the dying process after withdrawal of life supporting therapy and can yield clinically useful information regarding the onset of anaerobic metabolism. This gives rise to the concept that they could be used to provide 'real time' information regarding the onset of anaerobic metabolism in the potential donor.

The calculation of arterial and venous oxygen content yields useful information in the DCD donor. Figures 7.6 and 7.7 demonstrate that arterial and venous oxygen content can change rapidly in the DCD donor. The finding that falling arterial and venous oxygen content is related to elevation in lactate level is well established in human physiology, but has not previously demonstrated in DCD donors.

It is important to note the wide variation in haemoglobin levels in the proceeding donors enrolled in this study (7.3-12.9 g/dL) which will have a clear influence in arterial oxygen content and oxygen delivery to the tissues. While the rationale in avoiding transfusion in intensive care patients with haemoglobin above 7 is clear (UK Blood Transfusion Services Transfusion Handbook 2014) it is possible that low haemoglobin levels leading to low oxygen delivery could be deleterious to organs donated by DCD donors. However, examination of the relationship between lactate level and  $\text{CaO}_2$  revealed a marginally worse correlation than the relationship between  $\text{SaO}_2$  and lactate ( $r^2 = 0.41$  vs  $0.44$  respectively) suggesting that incorporation of haemoglobin level into the models has no effect on lactate levels. However, it is conceivable that haemoglobin level in the donor may have an effect on graft survival and further studies incorporating graft outcomes data would be valuable.

In this study, there was not the significant difference between venous oxygen saturation between central venous lines placed in differing locations that has been reported in previous studies (Fig 7.4). However, no patient in the group had PAFC for comparisons, and the numbers of patients with femoral and PICC lines were low and compounded by frequent malfunction of these lines during low pressure states. The relationship between venous oxygen content and lactate is consistent with findings from studies in critically ill patients with sepsis and cardiogenic shock (Rivers et al 2001, Stacpoole et al 1994). The difference between the venous oxygen saturation when measured by PAFC and CVC has been demonstrated in multiple studies (Edwards) and the poor approximation of CVC venous oxygen saturation to the mixed venous oxygen saturation provided by PAFC has been well documented (Van Beest et al 2010). No patient consented for this study had a PAFC placed as part of their routine care, and the ethical approval gained for this study precluded the placement of new invasive lines. It is conceivable that the lack of mixed venous oxygen saturations causes difficulty in the interpretation of parameters relying on  $\text{CvO}_2$ .

The lack of cardiac output data for the cohort enrolled in this study means that calculations of delivery of oxygen ( $DO_2$ ) and consumption of oxygen ( $VO_2$ ) have not been possible. Falling levels of  $DO_2$  have been previously demonstrated to be associated with worse outcomes in other patient cohorts, with a figure of 600ml/min being suggested as a threshold below which survival worsened in post-operative surgical patients (Shoemaker et al 1988). A normal  $DO_2$  is considered to be 900ml/min for a healthy 75kg subject at rest.

Animal studies (White et al 2016) demonstrate that cardiac output deteriorates throughout the withdrawal period following a similar trend to heart rate (HR). Consequently, some example calculations were undertaken using a fixed stroke volume (SV) of 70ml to assess the potential relationship of  $DO_2$  with lactate and systolic blood pressure. Variation in stroke volume during treatment withdrawal is inevitable, hence this model was used as an example of potential relationships and justification for consideration of further work that allows accurate measurement of cardiac output during treatment withdrawal. The formulae used to calculate these metrics are given below:

$$CO = HR \times SV$$

$$DO_2 = CaO_2 \times CO.$$

In line with the falling  $CaO_2$  after treatment withdrawal (data not shown here)  $DO_2$  also falls rapidly after treatment withdrawal in these model calculations.

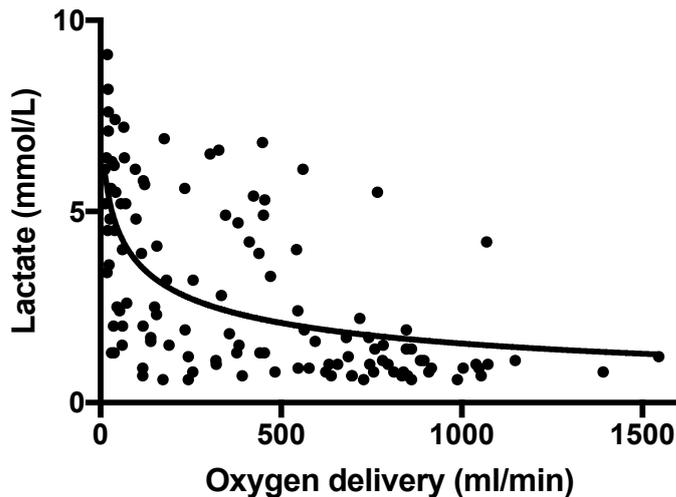


Figure 7.10: Correlations between the blood lactate level and oxygen delivery using model data for cardiac output calculations. Solid line represents non-linear regression of the data with  $R^2=0.3596$ .  $Y=(15099/X)*\exp(0.5*(\ln(X/4152805)/\ln(49.59))^2)$ . Data from 121 paired samples collected from 24 proceeding DCD donors after withdrawal of life supporting treatments.  $p<0.0001$  by spearman rank correlation

At present, clinical teams responsible for organ retrieval place much emphasis upon systolic blood pressure during withdrawal of life supporting therapy in the potential DCD organ donor, with an impression that a 'normal' systolic blood pressure means that oxygen delivery to the tissues will be maintained. Work from animal models of DCD donation (White et al 2016) would suggest this not to be the case, and that oxygen delivery falls to significantly subnormal levels before systolic blood pressure falls. In order to assess the hypothesis that a similar relationship between systolic blood pressure and oxygen delivery exists in the DCD organ donor undergoing withdrawal of therapy, a correlation of the two parameters was undertaken (see Figure 7.11 below)

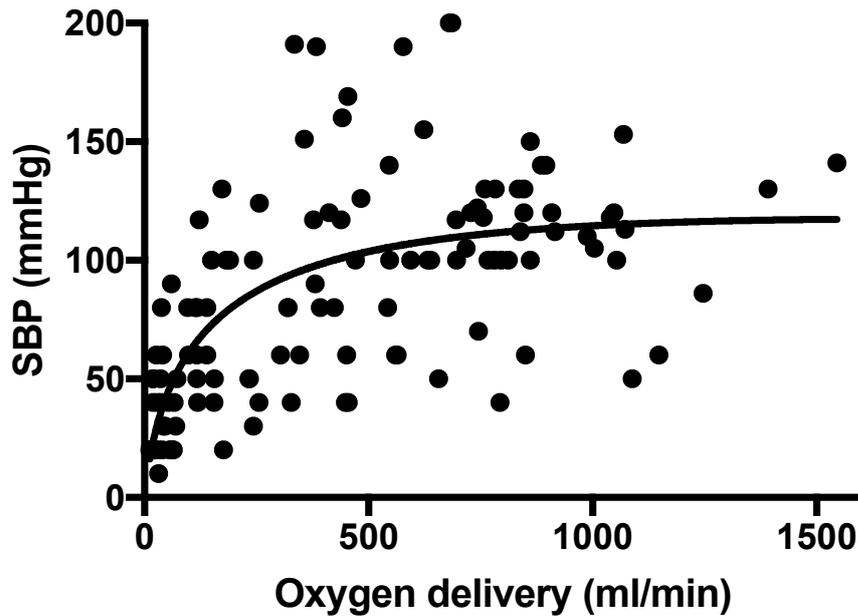


Figure 7.11: Relationship between oxygen delivery and systolic blood pressure using model data for cardiac output calculations. Solid line represents non-linear fit of data with  $R^2=0.4521$ .  $Y=(4897114/X)*exp(-0.5*(ln(X/988928)/ln(12.39))^2)$ . Data derived from 124 paired data points from 24 preceding DCD organ donors.  $p<0.0001$  by spearman correlation

The relationships demonstrated above between  $DO_2$  and blood lactate levels and SBP replicate the relationships found in animal models of DCD donation, and were they able to be replicated in donors using accurate CO calculations would provide further evidence for the fact that a 'normal' systolic blood pressure does not necessarily equate to adequate oxygen delivery

Oxygen extraction ratio can be accurately calculated in this study using the formula outlined above and is independent of cardiac output. An inspection of the lactate-OER plot revealed substantial physiological heterogeneity between patients. Some pairs of data showed normal physiology (OER  $\sim$  0.3 and lactate  $<$  2.0 mmol/l, identified by the red overlay in Fig 7.12) and others showing elevations in OER which correlated with expected increases in arterial lactate (identified by the blue overlay in Fig 7.12, signifying that OER increases were no longer adequate to maintain aerobic metabolism in the face of reduced oxygen delivery). However, the relationship between OER and lactate was poor, even within these data points that broadly conformed to expected classical physiology. Intriguingly, some data points showed complete dissociation between lactate levels and OER – with maintenance of normal lactate despite OER values in the 0.5-0.75 range (identified by the purple overlay in Fig 7.8),

or elevated lactate levels despite OER values below 0.3 (identified by the brown overlay in Fig 7.12). The former presumably represents patients in whom oxygen extraction (and by inference microcirculatory dynamics and mitochondrial oxygen utilisation) was highly efficient in the face of reductions in  $DO_2$ . However, the explanation for the latter is less certain, though microcirculatory ischaemia (with diffusion hypoxia) and/or mitochondrial dysfunction remain possibilities.

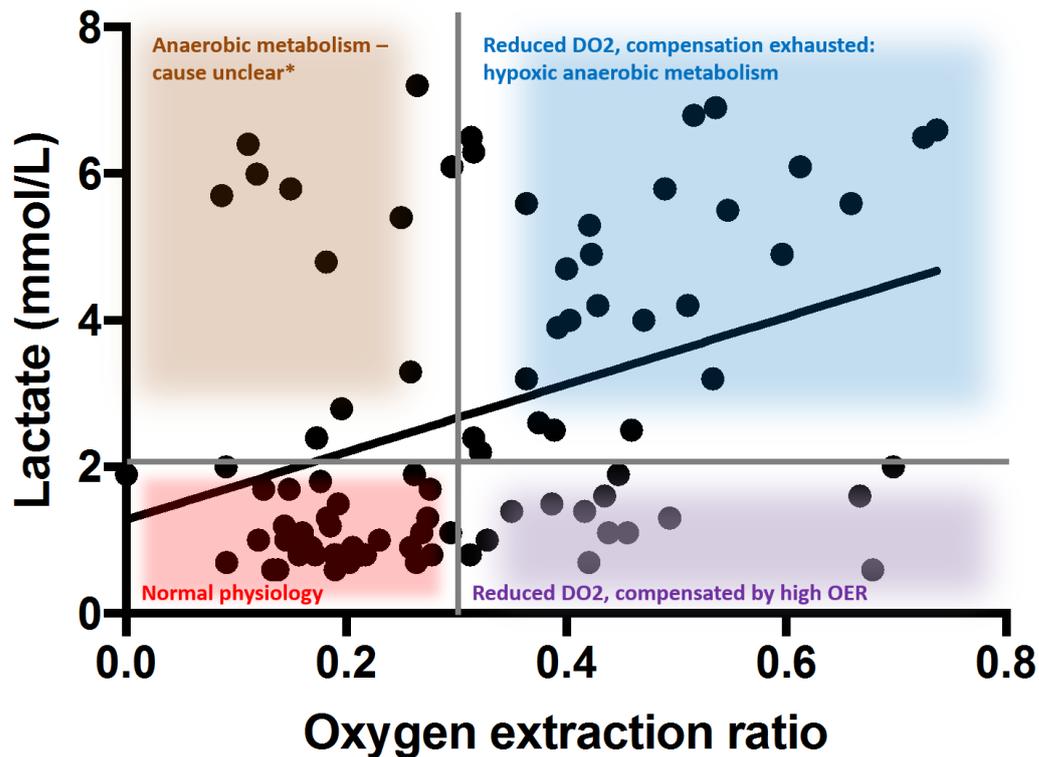


Figure 7.12: Replication of figure 7.8 Relationship between oxygen extraction ratio and lactate with additional highlights added to illustrate discussion.

Other causes of elevation in lactate level during the dying process require consideration. Impaired hepatic metabolism due to poor liver function is associated with an elevated lactate level (Almenoff et al 1989), as the predominant pathway for lactate metabolism is via the liver, which removes up to 70% of lactate via a monocarboxylate transporter. Within the hepatocytes, lactate is metabolised via gluconeogenesis and oxidative reactions to  $CO_2$  and water. This process is impaired in liver failure. However, as described in Chapter 1, the potential DCD organ donor has been extensively investigated and screened for contraindications to donation, which include major organ dysfunction. The development of acute liver failure during the dying process cannot be ruled out. However, those patients

who donated livers for transplantation donated organs which worked well upon transplantation, hence acute organ failure during withdrawal of treatment can be discounted as a cause of the elevated serum lactate. Furthermore, in donors where circulation to the intraabdominal organs was restored using normothermic regional perfusion, there was minimal enzyme release from the liver, suggesting there was no significant non-recoverable hepatic insult during the withdrawal period. In those donors who did not die within the required timeframe for donation, or in whom the liver was found to be unsuitable for transplantation, it is feasible that liver failure leading to impaired hepatic metabolism of lactate accounts for a proportion of the lactate elevation seen. It is conceivable that as the circulation fails leading up to death, decreased renal blood flow could influence the rate of lactate clearance. However, given renal clearance accounts for only 5% of lactate clearance it is unlikely to play a substantial role in the elevation of lactate seen in the above chapter. There is a well established link between catecholamine release and elevation in lactate levels in experimental studies (James et al 1999), with patients undergoing surgical resection of pheochromocytoma being the best studied clinical group (Suzuki et al 2014, Wu et al 2017). The catecholamine response to circulatory death has never been studied in a human DCD donor, however animal work by White et al. suggests that adrenaline and noradrenaline release during the dying process is likely, and indeed this is demonstrated in the following chapter.

The data presented in this chapter conclude that further assessment of elevation in lactate during the withdrawal period may prove it to be an appropriate marker for the onset of anaerobic metabolism, and hence the onset of warm ischaemia in the DCD organ donor.

It is to this topic of other body system responses to hypoxia that the next chapter turns, to further consider the response of the DCD donor to critical hypoxia and to pursue the hypothesis that circulatory death leads to catecholamine and stress hormone release.

## **Chapter 8: The stress and HPA axis response to circulatory death**

### **8.1 Chapter introduction and overview**

In Chapter 7 the association of hypotension and impaired delivery of oxygen to tissues with elevated blood lactate levels in DCD organ donors was demonstrated. This was further expanded in section 7.4 to consider how alternative measures of impaired oxygenation were related to elevations in lactate level. The chapter reached the conclusion that an elevated lactate level in the DCD donor could be used as a surrogate marker for the onset of anaerobic metabolism and hence the onset on warm ischaemia in donated organs.

Chapter 7 also notes that there are additional factors implicated in the elevation of blood lactate levels that require consideration, drawing particular attention to the well-established relationship between elevation in catecholamine levels and elevation in lactate level (James et al 1999, Gjedsted et al 2011). Work from animal models of DCD donation, which are mainly porcine models of the DCD heart (White et al 2016, Ali et al 2011) suggest that the hypoxic conditions experienced by the donor are associated with a large increase in plasma catecholamine levels. The fact that hypoxia stimulates catecholamine release has been demonstrated in a variety of animal models (Favier) going back many years (Becker et al 1986). This association has also been demonstrated in humans undergoing hypoxia at altitude (Rostrup 1998, Chen et al 2008).

The work outlined in chapters 5, 6 and 7 demonstrate that circulatory death can be considered to be a 'stressful' experience for the body, with hypoxaemia and hypotension being well established causes of physiological stress (Goldstein 2010). As demonstrated in chapter 1, physiological stress is associated with activation of the sympathetic nervous system, leading to catecholamine release and activation of the hypothalamic-pituitary-adrenal axis leading to, amongst others, cortisol release. This physiological response to acute stress is seen to occur in both experimental (Julien et al 2016) and clinical settings (Arafah 2006, Peng et al 2010). Therefore, this chapter explores the patterns of sympathetic nervous system activation and hypothalamic-pituitary-adrenal system activation in the

proceeding DCD organ donor by examining the end products of those systems which are measurable in the plasma.

The hypotheses examined in this chapter are that:

- Cardiovascular death is associated with activation of the sympathetic nervous system.
- The activation of the sympathetic nervous system seen in the DCD donor during death is greater than that seen in brainstem dead subjects undergoing circulatory arrest.
- Cardiovascular death is associated with activation of the hypothalamic – pituitary – adrenal axis, leading to an increase in stress hormone levels.
- The activation of the HPA axis in the DCD donor is greater than that seen in brainstem dead controls undergoing circulatory arrest.

This chapter will examine plasma concentrations of adrenaline, noradrenaline and cortisol in turn. I will initially demonstrate how those levels change in a temporal fashion after withdrawal of life supporting treatment. I will then compare how peak levels differ between standard DCD donors and a 'control' group of non-typical DCD donors undergoing withdrawal of life supporting treatment. The final consideration will be examination of the relationship between activation of these pathways and elevation in serum lactate levels, which Chapter 7 has established as a potentially valuable marker of anaerobic metabolism in the proceeding DCD organ donor.

## **8.2 Patient selection and sample collection**

Twenty two eligible potential DCD organ donors were recruited to the study and consented via the procedures set out in chapter 4. All patients recruited to the study met the eligibility criteria outlined in Chapter 4 section 4.3. Of the 22 recruited patients, 4 had a high clinical suspicion for being brainstem dead but were unable to be tested to fulfil brainstem criteria (Academy of Medical Royal Colleges Guidelines) due to a variety of reasons as outlined in

table 5.2. These patients were considered as 'Non-typical DCD donors'. Two consented patients did not die within the four hour timeframe required for donation to proceed.

In order to assess the activation of the sympathetic nervous system during the withdrawal of life supporting treatment samples were collected at time points from recruited DCD donors following the sampling schedule outlined in in Chapter 4.8.2 Samples were taken, stored and analysed as outlined in chapter 4 sections 4.10.3 and 4.10.4

### **8.3 Adrenaline release during the withdrawal period in DCD organ donors**

In order to quantify elevations in adrenaline levels during the withdrawal period in the potential DCD organ donor samples taken from the 20 patients described in 8.2 were analysed using the methodology described above. A rise in adrenaline level over time was noted in the 'typical DCD patient group', depicted in black in Figure 8.1 below. No donor was receiving MAP augmentation with adrenaline immediately prior to treatment withdrawal. No rise in adrenaline level between withdrawal of treatment and death was noted in the non-typical DCD donor group, represented by the solid red lines in Figure 8.1 below.

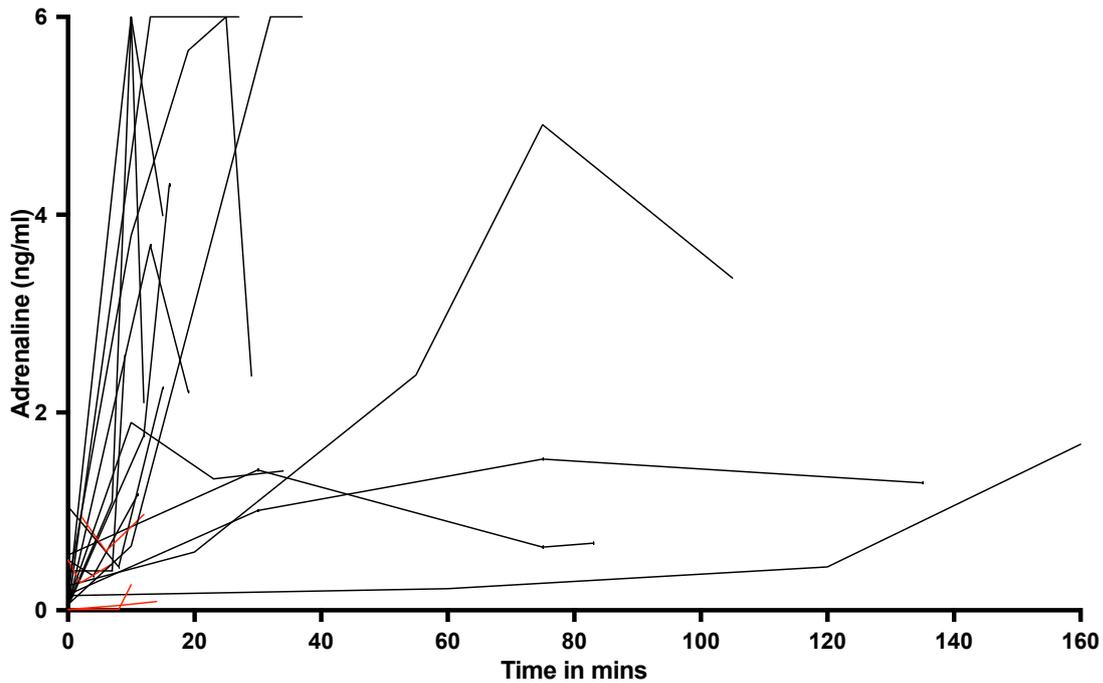


Figure 8.1: Graphical representation of change in adrenaline level against time for 20 proceeding DCD organ donors. Solid black lines represent Typical DCD subgroup (n=16). Upper limit of assay 6ng/ml. Solid red lines represent Non-typical DCD group (n=4)

#### 8.4 Peak adrenaline levels during the withdrawal period

To understand the significance of the relationship identified in Figure 8.1, a comparison in peak adrenaline levels was undertaken between the typical DCD donor group and the non-typical DCD donor group (figure 8.2). This demonstrated a significant difference between the two groups, with the typical DCD donor group patients experiencing a substantial elevation in circulating adrenaline levels during the withdrawal period, while those from the non-typical group had no significant rise in adrenaline during the withdrawal period. Patients in the non-typical group had a mean adrenaline level within the quoted normal range ( $<0.4 \pm 0.04$  ng/ml ) by Griffiths et al (Griffiths et al 1970). This suggests that there is significant release of adrenaline during the dying process in the DCD organ donor which may not occur in the already brainstem dead patient.

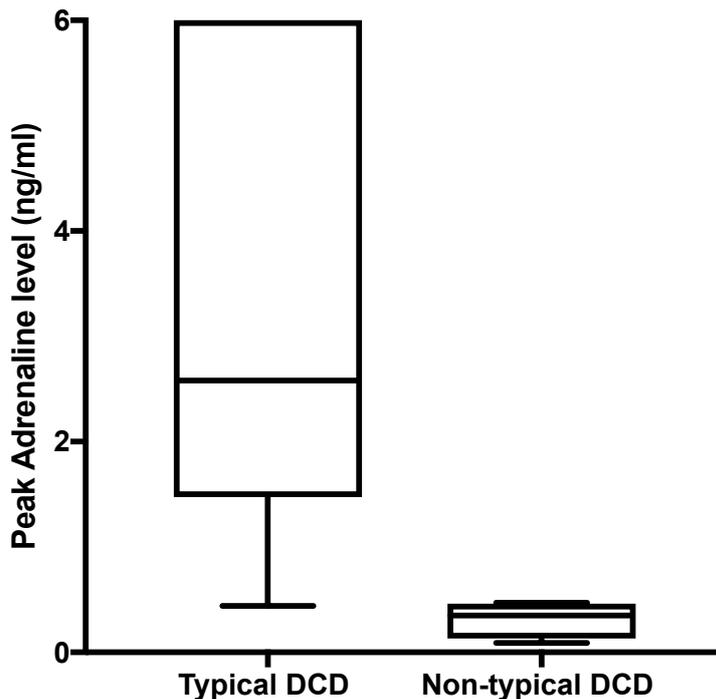


Figure 8.2: Comparison of Peak Adrenaline levels from Typical DCD and Non-typical DCD donors. Data are presented as median, inter-quartile ranges (box) and range (whiskers). Upper limit of Adrenaline assay is 6.0 ng/ml. Median for Typical DCD group = 2.58/ml. Median for Non-Typical DCD group = 0.35ng/ml.  $p = 0.0005$  by Mann-Whitney test. Data derived from 16 proceeding typical-DCD donors and 4 non-typical-DCD patients

## 8.5 Changes in adrenaline level during the withdrawal period in DCD donors

Figure 8.2 above shows non-typical DCD donors maintain a plasma adrenaline level within normal levels during the withdrawal period. In order to assess whether the non-typical donors in the study were able to mount an adrenaline response to withdrawal of life-supporting treatment and subsequent cardiovascular decline a comparison between the maximum change in adrenaline level in the typical DCD donor group and the non-typical DCD donor group (Figure 8.3 below). Maximum change in adrenaline level was calculated by subtraction of the initial level from the peak level. A statistically significant difference exists between the two groups, with the typical DCD donor group mounting a substantial but variable release in adrenaline, while the non-typical DCD donor group did not release adrenaline in response to treatment withdrawal and circulatory death. This is in accord with historical studies showing that adrenaline release in response to physiological stress is impaired following brainstem death.

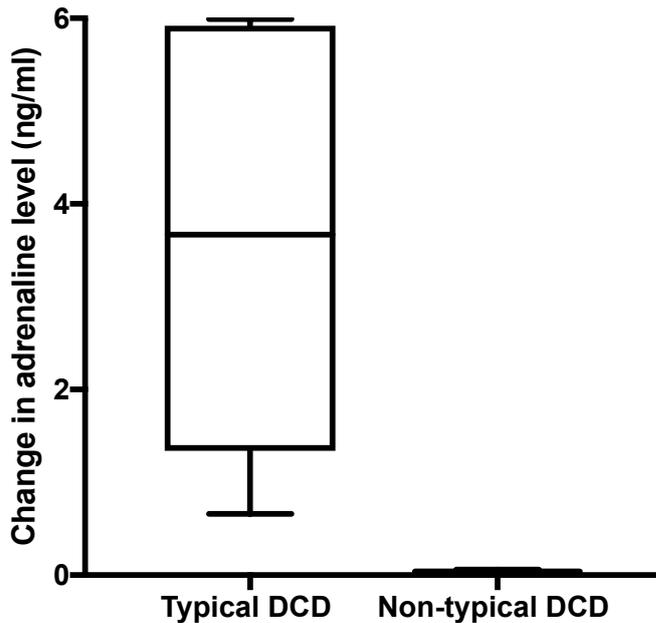


Figure 8.3: Comparison of change in Adrenaline levels between withdrawal of treatment and death from Typical DCD and Non-typical DCD donors. Data are presented as median, inter-quartile ranges (box) and range (whiskers). Upper limit of Adrenaline assay is 6.0 ng/ml. Median for Typical DCD group = 3.67ng/ml. Median for Non-typical DCD group = 0.04ng/ml.  $p = 0.0005$  by Mann-Whitney test. Data derived from 16 proceeding typical-DCD donors and 4 non-typical-DCD patients

## 8.6 Relationship between plasma adrenaline level and lactate level

Further evidence of the complex physiological interactions leading to lactate release in the DCD organ donor come from the analysis of the relationship between plasma adrenaline level and lactate during the withdrawal period leading up to death. As a potent vasoconstrictor via its  $\alpha_1$ -adrenoceptor effects and direct stimulator of lactate production due to accelerated aerobic glycolysis mediated by  $\beta_2$ -adrenoceptors in skeletal muscle (Levy), elevation in lactate during a period of enhanced adrenaline secretion is expected. Furthermore, this expected effect can be clearly demonstrated in the proceeding DCD organ donor (Figure 8.4 below) and provides evidence of the significant release of catecholamines during the dying process which has not previously been documented in human subjects.

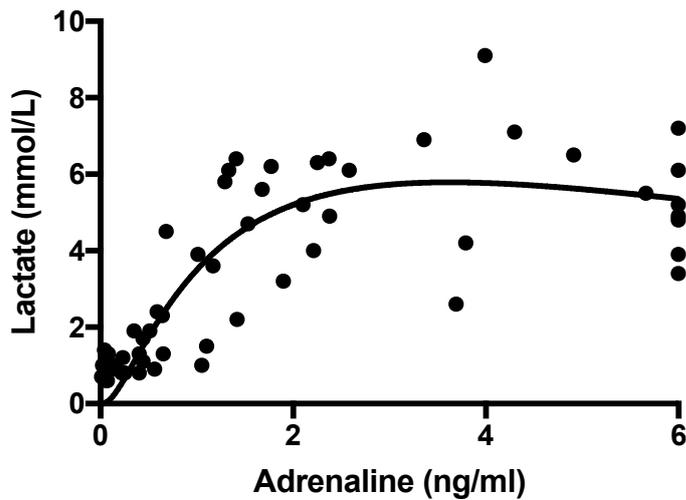


Figure 8.4: Relationship between adrenaline level and lactate. Solid line represents second order polynomial non-linear relationship of data with  $R^2=0.7087$ .  
 $Y=0.5692+2.96X+(0.3692X^2)$   $p<0.0001$  by spearman correlation  
 Data derived from 66 paired samples from 20 proceeding DCD donors.

## 8.7 Noradrenaline release during withdrawal of life supporting treatment

The pathways explored above that result in adrenaline secretion are also responsible for noradrenaline secretion. Consequently, the physiological stresses that occur during the withdrawal period and which are implicated in the stimulation of sympathetic nervous system and adrenaline release from the adrenal medulla are also expected to result in noradrenaline release. In order to quantify the magnitude of elevation in circulating noradrenaline levels during the withdrawal period in the potential DCD organ, donor samples taken from the 20 patients described above in section 8.2 were analysed using the methodology described above. A rise in noradrenaline level over time was noted in the 'typical DCD patient group', depicted in black in Figure 8.5 below. No rise in noradrenaline level between withdrawal of treatment and death was noted in the non-typical DCD donor group, represented by the solid red lines in Figure 8.5 below. One non-typical DCD donor had initially high noradrenaline levels due to the therapeutic administration of the drug to maintain blood pressure, but these rapidly dropped after cessation of the infusion.

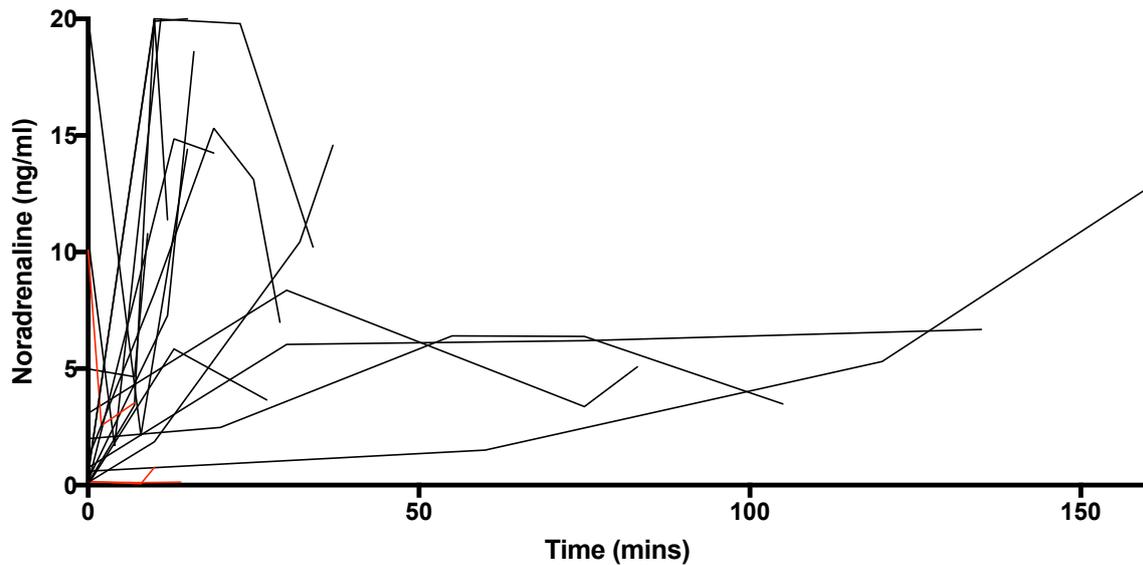


Figure 8.5: Graphical representation of change in noradrenaline level against time for 20 proceeding DCD organ donors. Solid black lines represent Typical DCD subgroup (n=16). Solid red lines represent Non-typical DCD group (n=4). Upper limit of assay 20ng/ml

## 8.8 Peak noradrenaline levels during the withdrawal period

To understand the significance of the relationship identified in Figure 8.5, a comparison in peak noradrenaline levels was undertaken between the typical DCD donor group and the non-typical DCD donor group. Time zero noradrenaline level was discounted for donors receiving noradrenaline MAP augmentation prior to treatment withdrawal (n=14) with time t+5 taken as the starting level for those donors. Noradrenaline half-life in the circulation was taken as two minutes (Beloeil et al 2005), and calculations were undertaken (not shown here) to ensure the t+5 value would not represent a significant infused noradrenaline remainder. This demonstrated a significant difference between the two groups, with the typical DCD donor group patients experiencing a substantial elevation in noradrenaline levels during the withdrawal period, while those from the non-typical group had no significant rise during the withdrawal period. Patients in the typical group had a mean noradrenaline level that remained above the quoted normal range ( $0.24 \pm 0.09$  ng/ml) by Griffiths et al (Griffiths et al 1970). This suggests that there is significant release of noradrenaline during the dying process in the DCD organ donor which may not occur in the already brainstem dead patient undergoing circulatory arrest.

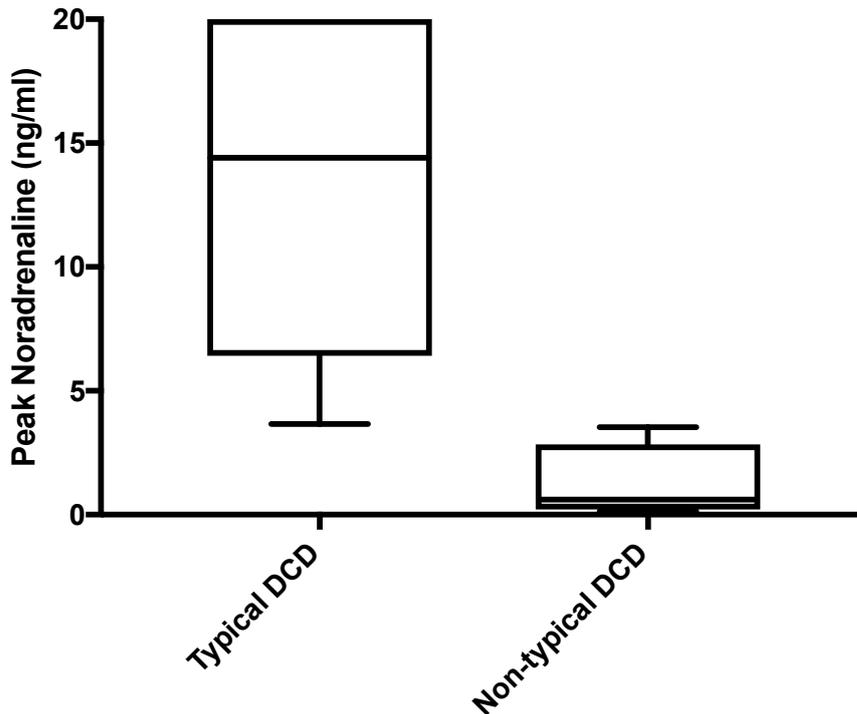


Figure 8.6: Comparison of Peak Noradrenaline levels from Typical DCD and Non-typical DCD donors. Data are presented as median, inter-quartile ranges (box) and range (whiskers). Upper limit of Noradrenaline assay is 20.0 ng/ml. Median for Typical DCD group = 14.41ng/ml. Median for Non-Typical DCD group = 0.61ng/ml.  $p = 0.0005$  by Mann-Whitney test. Data derived from 16 proceeding typical-DCD donors and 4 non-typical-DCD patients

## 8.9 Changes in noradrenaline level during the withdrawal period in DCD donors

Figure 8.6 above shows Non-typical DCD donors maintain a plasma noradrenaline levels marginally above normal levels during the withdrawal period. These levels are significantly lower than typical DCD donors. In order to assess whether the non-typical donors in the study were able to mount a noradrenaline response to withdrawal of life supporting treatment and subsequent cardiovascular decline a comparison was performed between the maximum change in noradrenaline level in the typical DCD donor group and the non-typical DCD donor group (Figure 8.7 below). Maximum level change was calculated as the difference between initial noradrenaline (determined as outlined in 8.8 above) and the peak noradrenaline level. A statistically significant difference exists between the two groups, with the typical DCD donor group mounting a substantial but variable release in noradrenaline,

while the Non-typical DCD donor group demonstrated a decline in noradrenaline in response to treatment withdrawal and circulatory death. This may represent the fact that the two patients of the Non-typical DCD donor group were receiving high dose noradrenaline MAP augmentation immediately prior to treatment withdrawal. The lack of rise in noradrenaline in response to circulatory death in this cohort suggests the mechanisms by which noradrenaline is released in response to physiological stress may be impaired in the brainstem dead organ donor and will be explored further in Chapter 10.

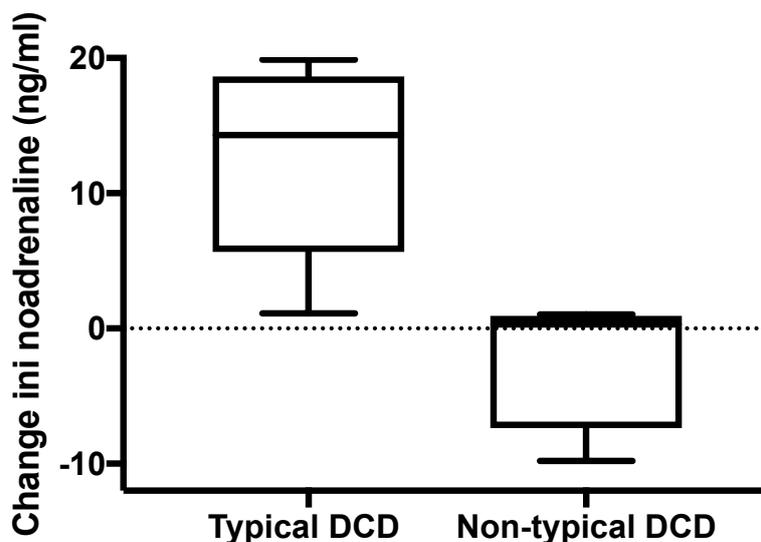


Figure 8.7: Comparison of change in Noradrenaline levels between withdrawal of treatment and death from Typical DCD and Non-typical DCD donors. Data are presented as median, inter-quartile ranges (box) and range (whiskers). Upper limit of Noradrenaline assay is 20.0 ng/ml. Median for Typical DCD group = 14.31ng/ml. Median for Non-typical DCD group = 0.29ng/ml.  $p = 0.0007$  by Mann-Whitney test. Data derived from 16 proceeding typical-DCD donors and 4 non-typical-DCD patients. Two Non typical DCD donors receiving noradrenaline at time of treatment withdrawal, 12 typical DCD donors receiving noradrenaline at time of treatment withdrawal.

## 8.10 Relationship between plasma noradrenaline level and lactate level

Further evidence of the complex physiological interactions leading to catecholamine release in the DCD organ donor come from the analysis of the relationship between plasma noradrenaline level and lactate during the withdrawal period leading up to death. As a potent vasoconstrictor via its  $\alpha$ -adrenoceptor effects elevated plasma noradrenaline is associated with elevation in lactate levels due to decreased blood flow to body tissues

(Qvisth et al 2008). Consequently, an elevation in plasma lactate during a period of elevated noradrenaline secretion is expected. This expected effect can be clearly demonstrated in the proceeding DCD organ donor (Figure 8.8 below) and provides evidence of the significant release of catecholamines during the dying process in the DCD organ donor which has not previously been documented in human subjects. The data I present below demonstrating correlation between noradrenaline elevation and serum lactate elevation is in keeping with previously published works, but the actual processes underlying the lactate elevation may be more complex.

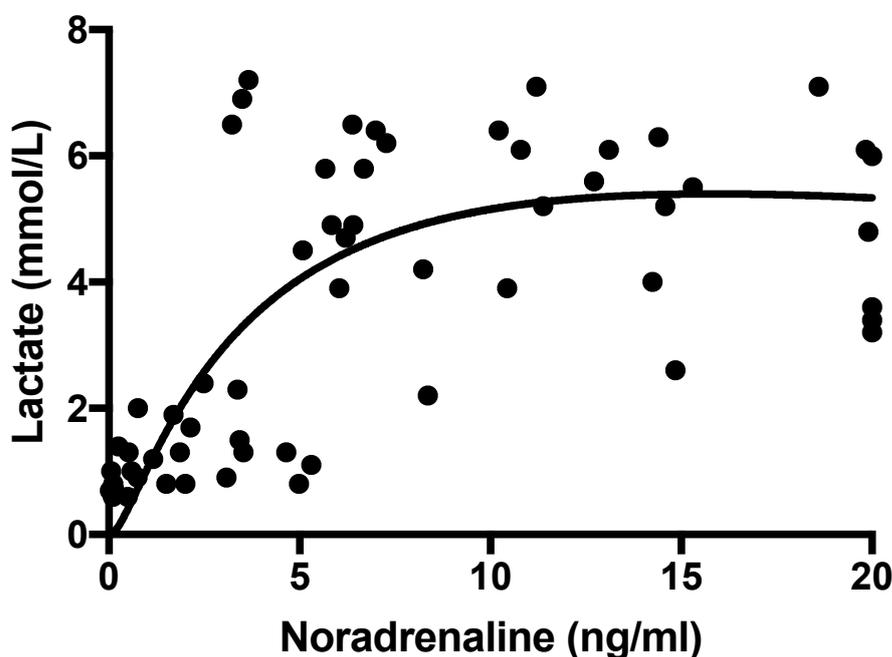


Figure 8.8: Relationship between Noradrenaline level and lactate. Solid line represents second order polynomial non-linear relationship of data with  $R^2=0.5509$ .  $Y=0.762+0.714X+(-0.02505X^2)$ .  $p<0.0001$  by spearman correlation. Data derived from 58 paired samples from 16 proceeding Typical DCD donors. Time zero samples from 6 donors receiving noradrenaline supplementation prior to withdrawal discounted from analysis.

Given the observed relationship between elevation in both adrenaline and noradrenaline with lactate elevation, a correlation was performed between the combined value of both catecholamines (as a multiplier of their upper limit of the normal level) with lactate level. This produced a similar relationship to that seen for each individual catecholamine (data not shown here) but with a worse  $R^2$  (0.4639 vs 0.5509 for noradrenaline and 0.7087 for adrenaline). This lack of improved fit by composite measure may be related to differing

vasoconstrictor potency between adrenaline and noradrenaline depending upon serum level.

### **8.11 Cortisol release during withdrawal of life supporting treatment in the proceeding DCD donor**

Following the demonstration in sections 8.3- 8.10 above that cardiovascular death in the proceeding DCD organ donor is a physiologically stressful event, with activation of the sympathetic nervous system and catecholamine release, I turned my attention to other end results of activation of the hypothalamic-pituitary-adrenal axis. Cortisol is a steroid hormone secreted from the zona fasciculata of the adrenal gland in response to stress. This represents one of the endpoints of activation of the hypothalamic-pituitary-adrenal axis, which can be considered part of the body's adaptive response to attempt physiological stability in the face of stress. Serum cortisol levels are known to rise during periods of acute physiological stress (Kaushik et al). Therefore, the effect of the previously demonstrated 'stress' of circulatory death in the proceeding DCD organ on serum cortisol levels was examined (figures 8.9, 8.10 and 8.11 below). As described in section 8.2 above, patients were split into 'typical DCD' and 'Non-typical DCD groups' to isolate patients who had a high likelihood of being brainstem dead and hence exhibiting different physiology during the period following withdrawal of life supporting treatment. Two donors were excluded from analysis due to being on high dose steroid replacement therapy, one donor was excluded from the typical DCD group and one from the non-typical DCD group. This assessment of cortisol levels demonstrated that cortisol release occurs during the dying period in the proceeding DCD organ donor (figure 8.9).

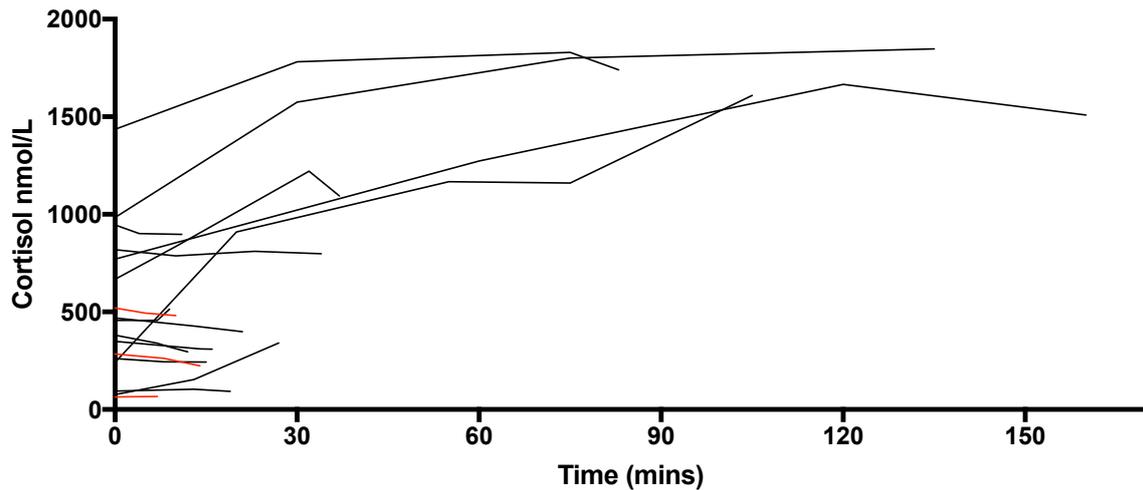


Figure 8.9: Graphical representation of change in serum cortisol level against time for 18 proceeding DCD organ donors. Solid black line represents Typical DCD subgroup (n=15). Solid red lines represent Non-typical DCD group (n=3).

The graphical representations of serum cortisol change with time demonstrate that cortisol does appear to rise during the dying process in some of the typical DCD organ donors, but not those with the shortest time to death. Statistical examination of the peak cortisol levels and change in cortisol levels between the typical and non-typical DCD donor groups does not show a significant difference between the two groups (Figures 8.10 and 8.11 below, represented by \*). It is well established that the time for cortisol levels to peak in response to an acutely stressful event is 15-30 minutes (Levine). All non-typical DCD donors suffered a circulatory arrest within 20 minutes of withdrawal of life supporting therapy, hence are unlikely to have been able to mount a serum cortisol response to stress in that time frame even if they were not already brain stem dead. In order to consider the time taken for cortisol to peak in response to acute stress, the typical DCD donor group was subdivided into two groups: typical DCD donors surviving greater than 30 mins after withdrawal of life supporting therapy and typical DCD donors surviving less than 30 minutes after withdrawal of life supporting therapy. Subgroup analysis was performed which demonstrated a significant difference in both peak cortisol levels and change in cortisol levels between those donors surviving greater than and less than 30 minutes (Figures 8.10 and 8.11 below, represented by \*\*).

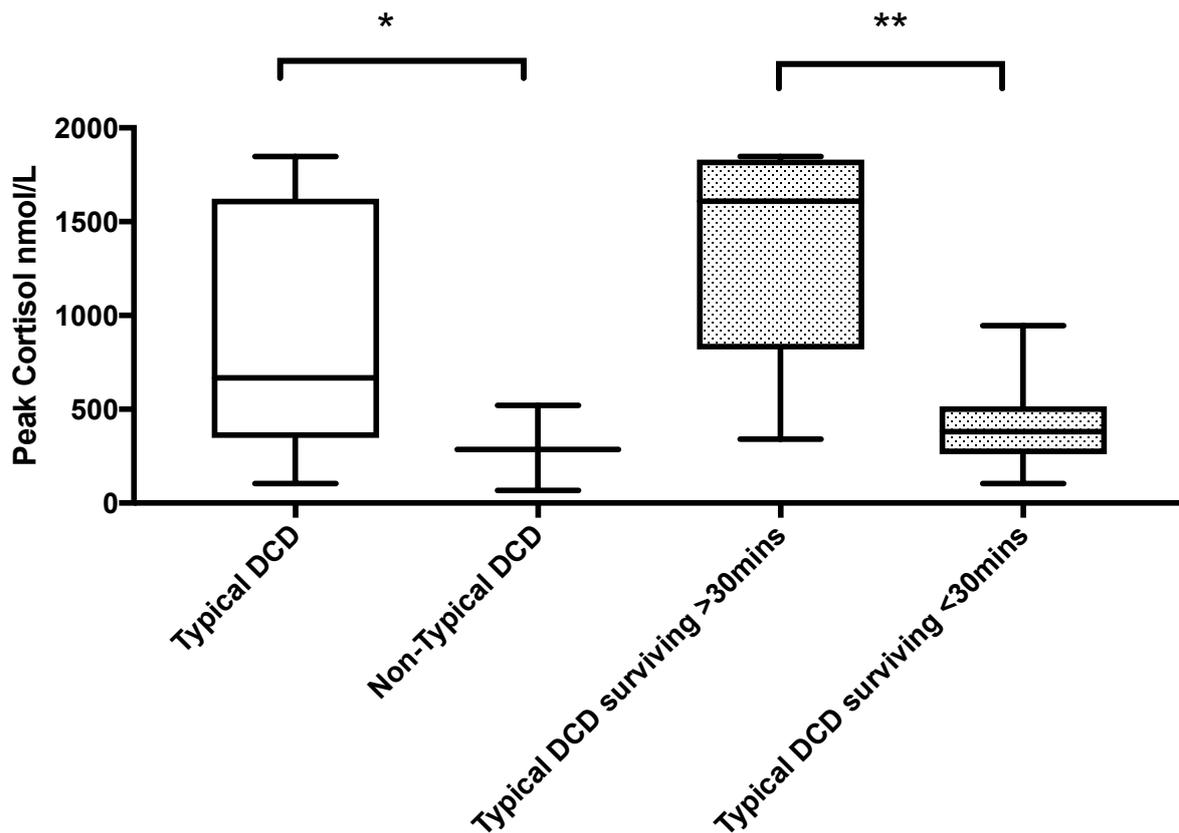


Figure 8.10: Comparison of Peak Cortisol levels between withdrawal of treatment and death from Typical DCD and Non-typical DCD donors. Shaded boxes represent subgroup analyses of Typical DCD donors. Data are presented as median, inter-quartile ranges (box) and range (whiskers). Upper limit of Cortisol assay is 2208 nmol/L. \*Median for Typical DCD group = 667nmol/L. Median for Non-typical DCD group = 285nmol/L.  $p = 0.1559$  by Mann-Whitney test. Data derived from 15 proceeding typical-DCD donors and 3 non-typical-DCD patients. \*\*Subgroup analysis of Typical DCD donors comparing those surviving >30mins and <30mins. Median for Typical DCD donor surviving >30mins =1609nmol/L. Median for Typical DCD donor surviving <30mins = 381nmol/L.  $p = 0.0175$  by Mann-Whitney test. Data derived from 8 Typical DCD donors surviving >30mins and 7 Typical DCD donors surviving < 30mins. 2 donors removed from analysis as receiving high dose enteral hydrocortisone treatment, one from Typical DCD group, one from Non-typical DCD group.

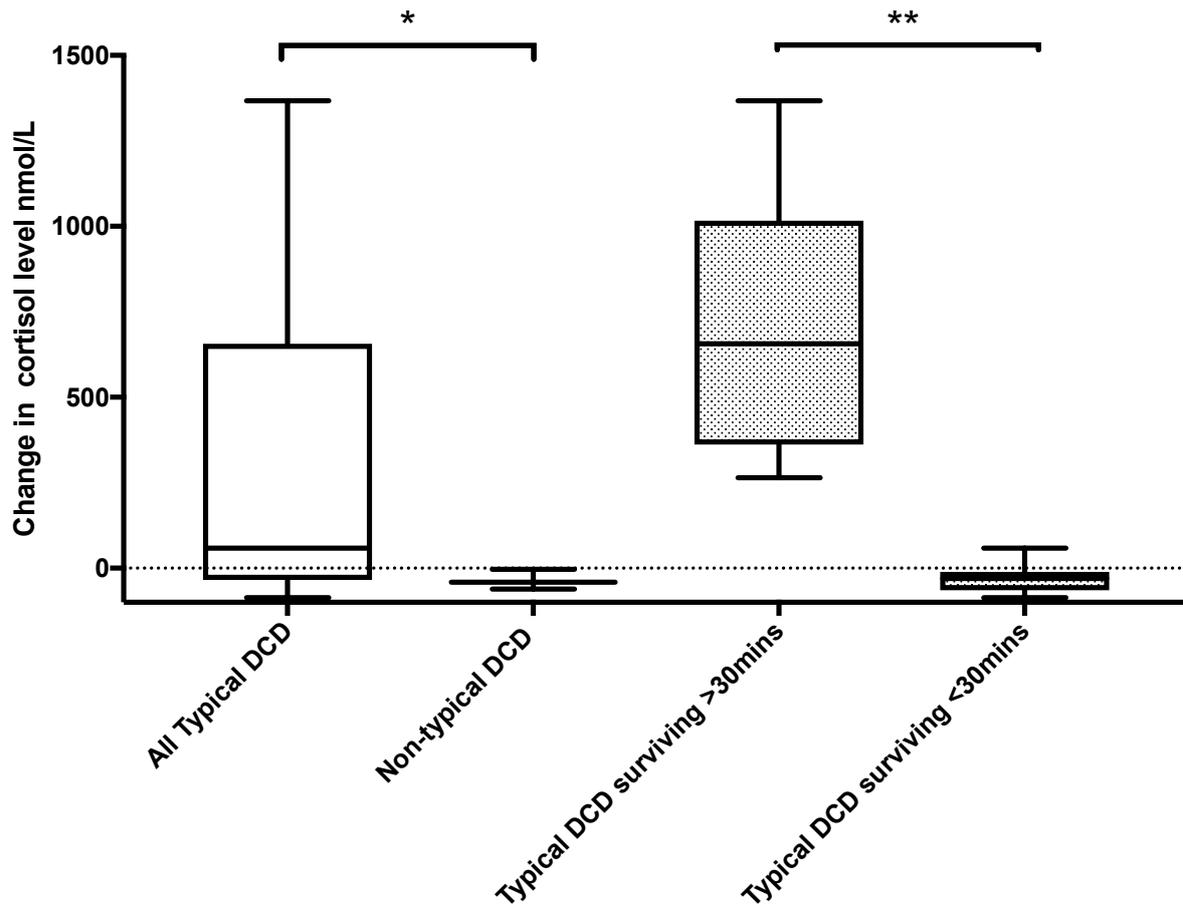


Figure 8.11: Comparison of Change in Cortisol levels between withdrawal of treatment and death from Typical DCD and Non-typical DCD donors. Shaded boxes represent subgroup analyses of Typical DCD donors. Data are presented as median, inter-quartile ranges (box) and range (whiskers). Upper limit of Cortisol assay is 2208 nmol/L. \*Median for Typical DCD group = 59nmol/L. Median for Non-typical DCD group = -41nmol/L.  $p = 0.2964$  by Mann-Whitney test. Data derived from 15 proceeding typical-DCD donors and 3 non-typical-DCD patients. \*\*Subgroup analysis of Typical DCD donors surviving >30mins and <30mins. Median for Typical DCD donor surviving >30mins = 657nmol/L. Median for Typical DCD donor surviving <30mins = -30nmol/L,  $p = 0.0007$ . Data derived from 8 Typical DCD donors surviving >30mins and 7 Typical DCD donors surviving < 30mins. 2 donors removed from analysis as receiving high dose enteral hydrocortisone treatment, one from Typical DCD group, one from Non-typical DCD group.

## 8.12 Chapter summary and Discussion

In chapters 6 and 7 the development of progressive hypoxaemia and hypotension in the proceeding DCD organ donor was demonstrated. In chapter 7 the relationship between these variables and the onset of anaerobic metabolism in the proceeding DCD organ donor as demonstrated by plasma lactate elevation was established. These chapters draw the

conclusions that circulatory death in the proceeding DCD organ donor is a physiologically 'stressful' experience for the donor and raises the question of the influence of a stress hormone response on lactate levels.

This chapter addresses these two questions. Firstly, it demonstrates that circulatory death in the proceeding DCD organ donor is associated with secretion of adrenaline and noradrenaline. Through the comparisons of DCD organ donors with a comparator group of probable brainstem dead donors undergoing treatment withdrawal it is possible to determine that the peak and total change in catecholamine level during the period leading up to circulatory arrest is far higher in the DCD organ donor compared to the minimal change seen in the comparators. It is, however, important to note that while both noradrenaline and adrenaline levels are seen to rise during the withdrawal period, and to correlate with lactate elevation, the current data do not provide evidence of a causal link. The mechanism underpinning lactate elevation is complex and multifactorial, and though a link to the catecholamine surge that I demonstrate during circulatory death is entirely physiologically plausible, the processes may be independent, and simply share the same temporal narrative as the patient proceeds to circulatory death.

These findings, which are supported by previous animal DCD model work (White, Ali), are reported here in human organ donors for the first time. As noted in the introduction, the deleterious effects of catecholamine excess on body tissues are well established (Hariskov et al 2013, Nef et al 2007, Movahed et al 1994) and are known to cause acute structural damage to organs, with the most notable research being related to cardiac muscle function (Ranieri et al 2018). This finding has clear implications for organ retrieval teams and transplant surgeons interested in the assessment of donated organs prior to their transplantation. These findings also give rise to the question of whether sympathetic blockade during the withdrawal period in the DCD organ donor might influence the degree of organ dysfunction seen in donated organs. As described in chapter 2 such an intervention is not currently permitted in human DCD donors in the UK, but it has long been practised in parts of the USA following animal work by Belzer's group in the 1970s.(Pryor et al 1971)

Of note in this chapter, the division of proceeding DCD donors into 'typical' and 'non-typical' groups has potential weaknesses as a concept. The non-typical donors were not able to be confirmed as brainstem dead for the reasons outlined in table 5.2, so in 3 out of 4 cases cannot be definitively confirmed as different to the typical DCD group. Other options for a comparator group undergoing monitored circulatory death were not available, as potential patients identified as part of a potential control group were either dying of multiple organ failure, extreme age, or in an unmonitored location meaning that they were not eligible organ donors and lacked the invasive lines necessary for frequent blood sampling. Consideration was given to approaching these groups for enrolment into the study as a control group, but it was felt not to be ethically appropriate to do so. The ideal comparator group would be a cohort of Maastricht 4 controlled DCD donors, already certified dead by neurological criteria but where treatment withdrawal is being undertaken as per the family's wishes. However, collecting substantial numbers for such a cohort is unlikely to be feasible. Throughout the twenty month study period there was only one Maastricht 4 category donor despite excess of 75 combined DCD and DBD donors in our centre.

This chapter also demonstrates that the stress hormone cortisol is released in response to the process of dying following withdrawal of treatment. There is suggestion in the literature that patients who have undergone physiological stress prior to death have higher post mortem urinary cortisol levels than controls (Lang). This finding of Hypothalamic-Pituitary-Adrenal axis activation in the proceeding DCD organ donor undergoing circulatory death is a further novel finding.

The implications of activation of the sympathetic nervous system and Hypothalamic-Pituitary-Adrenal axis in the proceeding DCD organ donor offer potential insights into transplanted organ dysfunction and modifiable targets in the potential DCD organ donor and will be discussed in Chapter 10.



## **Chapter 9: The immune response to circulatory death in the DCD donor**

### **9.1 Chapter Overview and Introduction**

The preceding chapters have demonstrated the progressive physiological changes that occur in the DCD organ donor during the withdrawal of life supporting therapy.

Chapters 6 and 7 have demonstrated progressive hypoxia and hypoperfusion occurring in the studied cohort. Hypoxia has been demonstrated to be linked to the development of an immune response (Nizet et al 2009). The link between hypoperfusion and immune system activation is well established, with much of the literature coming from study of intensive care patients suffering from shock. While the typical proceeding DCD donor does not meet criteria for septic or haemorrhagic shock states, they could be considered to meet criteria for cardiogenic shock (Reynolds et al 2008). Chapter 8 demonstrated activation of the Hypothalamic-Pituitary-Adrenal axis in the proceeding DCD organ donor. The link between physiological stress states and an immune response has been the subject of extensive study and is well defined.

As shown in Chapter 1 there is evidence from animal models of DCD organ donation that changes in the immune system may occur during circulatory death. White et al found decreases in IL-6 and TNF $\alpha$  during a 20 minute period between withdrawal of in a porcine model of DCD donation. Whether changes in the immune system occur in the human donor during the withdrawal of life supporting treatment, and the magnitude of any changes that do occur, have not previously been studied in the human population.

The hypothesis for this chapter is that immune system activation will co-exist with the physiological changes that occur in the proceeding DCD organ donor.

This chapter examines the immune response that occurs during circulatory death in the proceeding DCD organ donor. Firstly, it examines the initial state of the immune system in

the DCD donor, followed by an examination of the magnitude and temporal patterns of immune system changes. The final section is an examination of the impact of time to death on the state of immune system activation.

## **9.2 Initial Immune function in patient cohort**

In order to assess the immune function at the start of the withdrawal period, initial cytokine levels for the potential donors recruited into the study were assessed. Using the methodology described in Chapter 4, donors were split into 'typical' and 'non-typical' subgroups, with the 'non-typical' subgroup representing brainstem dead patients where donation was pursued by the route of circulatory death. The rationale behind this initial assessment is to delineate any differences in baseline immune function in patients who had already undergone brainstem death, with its associated immune system activation, as outlined in Chapter 1 section 8.3. Published values of cytokines measured in healthy volunteers (Morris et al 2010), and values of these cytokines in DBD donors are included for comparison. The results of these analyses are presented below in table 9.1.

Donor 6 was removed from analyses in sections 9.2 - 9.4 of this chapter due to consistently grossly elevated cytokine levels out of keeping with the remainder of the cohort under consideration and is considered separately in section 9.5 below.

These data demonstrate that the proceeding DCD donor, whether 'typical' or 'non-typical' exists in a state of acute inflammation prior to withdrawal of life supporting treatment, with levels of IFN- $\gamma$ , IL-6, IL-8 and TNF- $\alpha$  which exceed normal values from healthy volunteers. Table 9.1 also demonstrates the existence of baseline differences in cytokine profiles between the 'typical' and 'non-typical' donor groups, with the level of IFN- $\gamma$  being significantly elevated in the 'typical' DCD donor group relative to the 'Non-typical' group ( $p=0.048$  by Mann-Whitney U test). In contrast, IL-6 was found to be significantly elevated in the 'Non-typical' DCD donor group, with a median level approaching ten-fold that of the 'typical' donor group ( $p=0.039$  by Mann-Whitney U test). Differences between the two groups were not replicated for other cytokines analysed (with the levels remaining unchanged in both groups for IL-1 $\beta$ , IL-10, IL-12p70, IL-2, IL-4, IL-8 and TNF- $\alpha$ ).

Interestingly, although the DBD values in the table below are from another centre, and hence preclude direct statistical comparison, levels of IL-1 $\beta$  and IL-8 were substantially higher in the DBD group compared to those in either the Typical or Non-typical DCD groups in the current study. Levels of IL-6, on the other hand, were higher in both DBD and Not-typical DCD donors than in Typical DCD donors. Finally, IFN- $\gamma$  levels were higher in the Typical DCD group than the Non-typical DCD and DBD groups.

Cytokine	Initial level (pg/ml)		Levels in brainstem death (pg/ml) (Schwarz)	Healthy Volunteer level (pg/ml)	p value <i>Typical vs. non-typical DCD donor</i>
	Typical DCD donor	Non-typical DCD donor			
IFN- $\gamma$	6.8 (3.0-16.0)	2.8 (2.5-2.9)	2.79	(0-4)	0.048
IL-1 $\beta$	0.3 (0.3-0.3)	0.3 (0.3-0.3)	7.11	(0-5)	>0.999
IL-10	0.9 (0.6-1.5)	0.9 (0.9-1.7)	15.73	(0-3)	0.637
IL-12 p70	0.25 (0.2-0.4)	0.4 (0.2-1.2)	ND	NA	0.369
IL-2	0.6 (0.6-1.2)	0.6 (0.6-0.7)	ND	NA	0.418
IL-4	0.1 (0.1-0.1)	0.1 (0.1-0.2)	ND	NA	0.172
IL-6	27.1 (1.6-15.6)	228.5 (132.1-269.4)	223.7	3 (0-4)	0.039
IL-8	13.1 (7.9-31.7)	13.2 (2.5-40.4)	68.24	3 (0-12)	0.809
TNF-a	3 (1.9-5.6)	2.5 (1.4-2.9)	3.28	1 (0-2)	0.196

*Table 9.1: Initial cytokine levels between 'Typical DCD donors' and 'Non-typical DCD donors' during withdrawal of life-supporting treatment. Initial level is the level measured immediately prior to withdrawal of life supporting treatment; P value is by Mann-Whitney U test between groups. Values are shown as median and interquartile range. Healthy volunteer data provided for comparison (Morris et al 2010). NA indicates normal range data not available. Levels in brainstem dead patients are taken from Schwarz et al 2018 published values for cytokine levels at the point of the second set of brainstem criteria testing. ND = no data available. Healthy volunteer and DBD data are shown for visual comparison only as they were not obtained as part of this study. No statistical comparison between these data and the patients included in this study have been undertaken. The presented data represents an initial exploratory analysis – no correction for multiple comparisons has been applied.*

In order to allow for the potential confounder of the disease process underlying the donor clinical condition, an analysis was performed to compare initial cytokine values with underlying donor pathology. Donors were divided into three groups of underlying pathologies: Subarachnoid/Intra-cerebral haemorrhage, Hypoxic brain injury and Traumatic brain injury. The demographics of these groups are shown in Chapter 5. The results of this analysis are shown in table 9.2 below. This revealed a significantly higher IFN- $\gamma$  level in the

hypoxic brain injury group when compared to the Traumatic brain injury group and the Subarachnoid/Intra-cerebral haemorrhage groups ( $p= 0.039$  by Kruskal Wallis test).

Cytokine (pg/ml)	Disease process			P value
	ICH	Hypoxic brain injury	TBI	
IFN- $\gamma$	3.4 (2.8-14.7)	11.9 (6.5-35.7)	2.9 (2.2-5.3)	0.039
IL-10	0.9 (0.7-1.5)	0.7 (0.5-6.2)	0.9 (0.7-2.9)	0.95
IL-6	38.2 (9.9-180.3)	25 (17.8-304.9)	27.1 (24.5-100.6)	0.98
TNF- $\alpha$	2.9 (1.8-5.1)	3.3 (2.7-4.7)	3 (1.7-5.1)	0.937

*Table 9.2: Initial cytokine levels between different disease process groups. ICH = Intracranial haemorrhage, TBI = Traumatic brain injury. Initial level was measured immediately prior to withdrawal of life supporting treatment. p value is by Kruskal-Wallis test between groups. Data derived from 20 proceeding DCD donors, 9 donors in SAH/ICH group, 6 in hypoxic brain injury group, 5 in TBI group. Values are shown as median and interquartile range.*

### 9.3 Temporal changes during the withdrawal period

A key hypothesis to be tested in this chapter is that the progressive physiological changes documented in chapters 5, 6, 7 and 8 which occur during the withdrawal period lead to activation of the immune system in the proceeding DCD organ donor. In order to test this hypothesis, pictorial representations of cytokine levels with time were created for individual donors, these are shown below in Figure 9.1. Visual inspection of these charts show trends in cytokine levels with time to be evident for some donors in the cohort, but are not universally observed.

Analysis of the 20 proceeding donors in the cohort to assess the difference between initial cytokine level at the point of withdrawal of therapy and the peak cytokine level during the withdrawal period demonstrated no significant difference across the range of cytokines investigated, although there was a trend towards an increase in IL-6 ( $p=0.0109$  by Mann-Whitney U test). The results of this analysis are given in table 9.3 below.

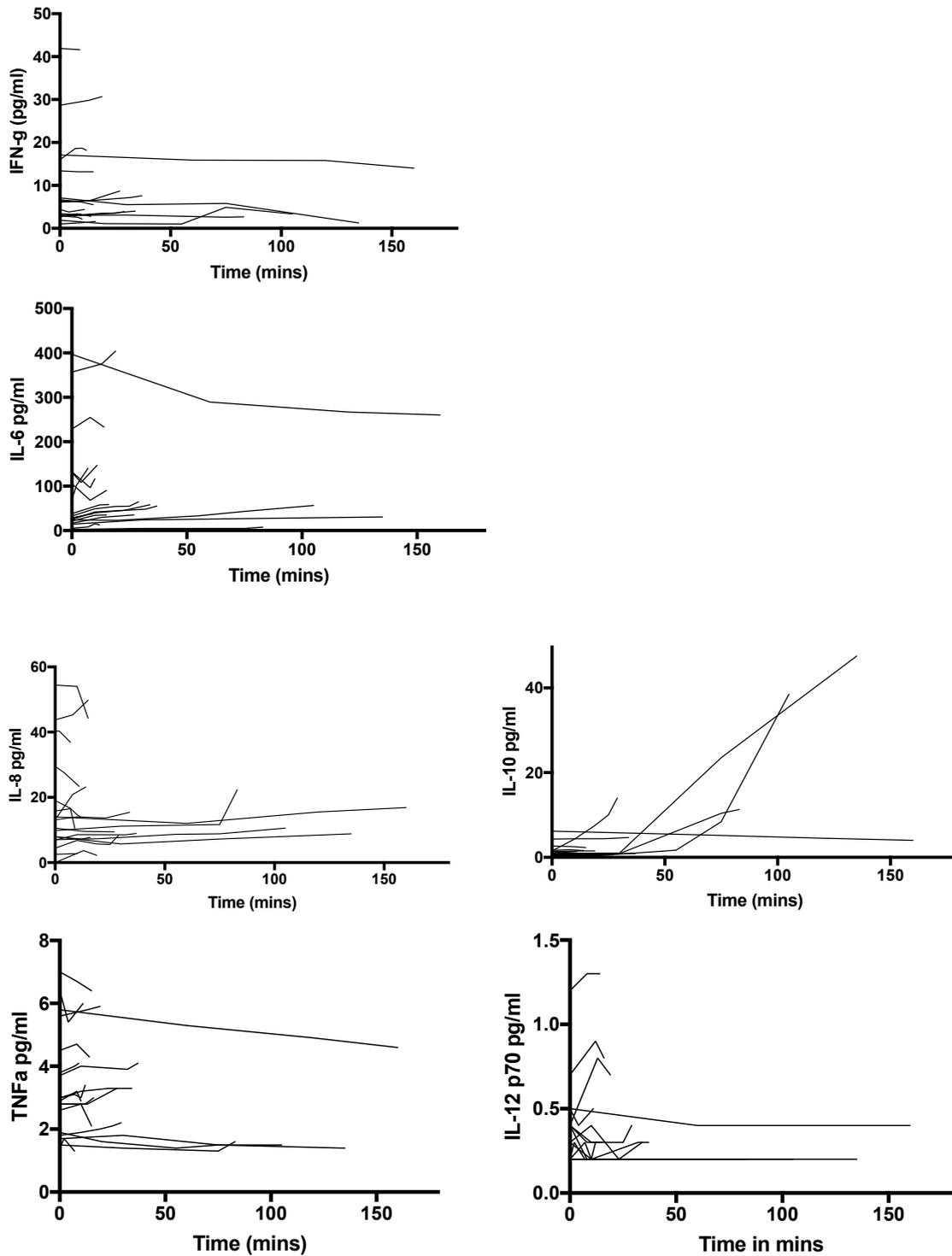


Figure 9.1. Graphical representations of temporal changes in cytokine levels during the withdrawal of life supporting treatment.

Cytokine	Initial level (pg/ml)	Peak level (pg/ml)	p value
IFN- $\gamma$	6.1 (3.0-16.0)	7.1 (3.9-17.1)	0.602
IL-1 $\beta$	0.3 (0.3-0.3)	0.3 (0.3-0.5)	0.287
IL-10	0.9 (0.6-1.5)	1.5 (0.8-11.3)	0.078
IL-12 p70	0.25 (0.2-0.4)	0.3 (0.2-0.5)	0.411
IL-2	0.6 (0.6-1.2)	0.7 (0.6-1.2)	0.699
IL-4	0.1 (0.1-0.1)	0.1 (0.1-0.1)	>0.999
IL-6	27.1 (16.1-105.6)	56.5 (35-105.6)	0.109
IL-8	13.1 (7.9-31.7)	16.9 (8.9-34.3)	0.406
TNF- $\alpha$	3.0 (1.9-5.6)	3.3 (2.2-5.8)	0.630

*Table 9.3: Comparison of Initial cytokine level and peak cytokine level during withdrawal of life-supporting treatment. Initial level is the level measured immediately prior to withdrawal of life supporting treatment. Peak value is highest value recorded during withdrawal period. Data derived from N=20 proceeding donors. p value is by Mann-Whitney U test between groups. Values are shown as median and interquartile range.*

A potential confounding variable for the lack of overall significance seen in table 9.3 is the effect of brainstem death on the immune system, which is well established in the literature (reviewed in Section 1.8.3) and which as demonstrated in table 9.1 had an impact on the state of immune system activation in the donor cohort enrolled into this study. In order to elucidate the effect of brainstem death on the peak cytokine level and change in cytokine level in the cohort, an analysis was performed between the ‘typical’ and ‘non-typical’ donor groups and is summarised below in table 9.4. This analysis demonstrated two findings: a non-significant trend towards a higher peak IFN- $\gamma$  level in the ‘typical DCD donor’ group when compared to the ‘Non-typical’ donor group (p=0.101 by Mann-Whitney U) and a non-significant trend towards higher peak IL-6 levels in the ‘Non-typical donor’ group (p=0.076 by Mann-Whitney U test). These changes reflect the elevated starting values for INF- $\gamma$  and IL-6 in the respective groups that were demonstrated in table 9.1. However, no significant

change in level between ‘typical’ and ‘non-typical’ donors during the withdrawal period was demonstrated across the measured cytokines in these subgroup analyses.

Cytokine	Peak level			Change in level		
	Typical DCD donor	Non-typical DCD donor	p value	Typical DCD donor	Non-typical DCD donor	p value
IFN- $\gamma$	7.1 (3.9-17.1)	3.4 (2.9-3.7)	0.101	0 (-0.9-1.5)	0.1 (-0.7-0.3)	0.803
IL-1 $\beta$	0.3 (0.3-0.5)	0.3 (0.3-0.4)	0.838	0 (0-0.2)	0 (0-0.1)	0.677
IL-10	1.5 (0.8-11.3)	1.0 (1.0-1.8)	0.631	0.1 (-0.1- 7.97)	0.1 (0.1-6.9)	0.616
IL-12 p70	0.3 (0.2-0.5)	0.4 (0.3-1.3)	0.232	0 (0-0.1)	0.1 (-0.2 – 0.1)	0.788
IL-2	0.7 (0.6-1.2)	0.6 (0.6-0.7)	0.355	0 (0-0.3)	0 (-0.2-0)	0.241
IL-4	0.1 (0.1-0.1)	0.2 (0.1-0.2)	0.171	0 (0-0)	0 (-0.1-0)	0.294
IL-6	56.5 (35-105.6)	140.3 (132.1-254.9)	0.076	17.4 (12.7-31.3)	13.2 (-11.6-59.63)	0.645
IL-8	16.9 (8.9-34.3)	23.2 (2.7-40.4)	0.988	2.3 (-0.8-4)	0.2 (-3.5-0.2)	0.985
TNF-a	3.3 (2.2-5.8)	3.2 (1.7-4.7)	0.684	0.2 (-0.4-0.4)	0.3 (0.2-0.3)	0.672

*Table 9.4: Peak cytokine levels and change in cytokine levels between ‘Typical DCD donors’ and ‘Non-typical DCD donors’ during withdrawal of life-supporting treatment. Peak level is the highest recorded level during withdrawal period. Change in level is the difference between level at time of withdrawal and level at time of death. Data derived from 20 proceeding DCD donors, N=16 in typical DCD group, N=4 in non-typical DCD group. p value is by Mann-Whitney U test between groups. Values are shown as median and interquartile range.*

## 9.4 Impact of time to death on immune system activation

Although section 9.2 demonstrates no significant differences between peak cytokine levels or change in cytokine levels during the withdrawal period, it remains a consideration that the length of time between withdrawal of therapy and death in the proceeding DCD donor may influence the magnitude of immune system changes demonstrated. This could be considered as a latent response to the deleterious physiology previously described in this thesis. In order to consider the presence of a dose response effect, Typical DCD donors were

subdivided into those surviving over 60 minutes after withdrawal of therapy and those surviving for a shorter period. 60 minutes was chosen as a cut off after literature review suggesting that reliable measurements of levels of preformed cytokines released from storage by exocytosis could be made at '30-60minutes' (Morris et al 2010). It is clear that the magnitude of response may be greater further out from the initial stimulus but given that the stimulus in the case of the proceeding organ donor is the prolonged and progressive period of deranged physiology, and the fact that only 4 patients from the 20 patient cohort survived in excess of 60 minutes, a 60 minute cut off point was chosen.

The results of these analyses are shown in table 9.5 below. In donors surviving under 60 minutes there were significantly lower peak levels of IL-10 and significantly higher peak levels of TNF- $\alpha$  than when compared to those donors surviving over 60 minutes ( $p=0.036$  and  $p=0.037$  respectively by Mann-Whitney U test). Peak levels in other measured cytokines showed no significant difference based upon length of donor survival. Analysis of cytokine level changes during the withdrawal period in donors surviving for different time periods are given below in table 9.5. Small but significant elevations in IFN- $\gamma$  and TNF- $\alpha$  are demonstrated in donors surviving for less than 60 minutes after treatment withdrawal with corresponding decreases in the subgroup living for in excess of 60minutes ( $p=0.009$  and  $p=0.026$  respectively,  $p$  values by Mann-Whitney U test). Additionally, there is a significant elevation in IL-10 demonstrated in donors living for over 60minutes after treatment withdrawal ( $p=0.003$ )

Cytokine	Peak level			Change in level		
	Typical donor surviving <60min	Typical donor surviving >60min	p value	Typical donor surviving <60min	Typical donor surviving >60min	p value
IFN- $\gamma$	7.6 (4-18.7)	5.1 (2.2-14.6)	0.412	0.55 (-0.225-1.7)	-1.1 (-1.3 to -0.45)	0.009
IL-1 $\beta$	0.3 (0.3-0.5)	0.3 (0.3-0.9)	>0.999	0 (0-0.2)	0 (0-0.45)	>0.999
IL-10	1.0 (0.7-2.7)	22.35 (2.52-45.25)	0.036	0 (-0.1-0.2)	24.3 (9-44.3)	0.003
IL-12 p70	0.3 (0.2-0.5)	0.2 (0.2-0.425)	0.263	0 (0-0.1)	0 (0-0.15)	0.974
IL-2	0.6 (0.6-1.2)	0.95 (0.625-3.525)	0.446	0 (0-0.6)	0.05 (0-0.1)	0.906
IL-4	0.1 (0.1-0.1)	0.1 (0.1-0.25)	0.839	0 (0-0)	0 (0-0)	>0.999
IL-6	58.1 (35.1-105.6)	43.5 (13.4-311.8)	0.489	18.5 (13.7-31.0)	10.5 (-101.2-28.4)	0.412
IL-8	19.0 (8.9-49.8)	13.75 (9.25-20.95)	0.506	2.0 (-3.45-3.25)	3.1 (1.3-10.28)	0.213
TNF- $\alpha$	3.4 (3-5.9)	1.8 (1.63-3.33)	0.037	0.3 (0.2-0.4)	-0.35 (-1.0-0.1)	0.026

Table 9.5: Peak cytokine levels and change in cytokine levels between 'Typical DCD donors surviving <60mins' and 'Typical DCD donors surviving >60mins' during withdrawal of life-supporting treatment. Peak level is the highest recorded level during withdrawal period. Change in level is the difference between level at time of withdrawal and level at time of death. Data derived from 20 proceeding DCD donors, n=16 in surviving < 60minute group, n=4 in surviving >60minute group. p-value is by Mann-Whitney U test between groups. Values are shown as median and interquartile range.

## 9.5 Donor six – a case of immediately post brainstem death?

Donor number six has been excluded from the analyses above due to demonstrating cytokine ranges several orders of magnitude different from those seen in all other proceeding donors in the cohort. However, the contribution of this donor and his family to the study should be noted and merits separate consideration. This donor was a man in his early 20s who had suffered an out of hospital cardiac arrest after an intravenous drug overdose. The patient had received 70 minutes of cardiopulmonary resuscitation prior to restoration of spontaneous circulation. The clinical impression of the treating team was one of brainstem death, but formal brainstem testing was not possible due to high oxygen requirements rendering the apnoea test unfeasible. Immediately prior to treatment

withdrawal the patient developed profound haemodynamic instability, requiring rapid escalation of inotropic and vasopressor support. Cytokine levels are as given in table 9.6 below, with the interquartile range of the study cohort given for comparison. While there are several plausible explanations for the differences seen between donor six and the remaining cohort, these changes may represent the immediate acute inflammatory response to brainstem death. It is equally plausible that the effect seen below is a systemic response related to peripheral ischaemia caused by the catecholamine surge associated with brainstem death.

Cytokine	Peak level (pg/ml)	
	Donor Six	Remainder of cohort
IFN- $\gamma$	71.3	7.1 (3.9-17.1)
IL-1 $\beta$	42.2	0.3 (0.3-0.5)
IL-10	57.8	1.5 (0.8-11.3)
IL-12 p70	12.2	0.3 (0.2-0.5)
IL-2	99.9	0.7 (0.6-1.2)
IL-4	8.5	0.1 (0.1-0.1)
IL-6	1486	56.5 (35-105.6)
IL-8	1154	16.9 (8.9-34.3)
TNF- $\alpha$	22.3	3.3 (2.2-5.8)

*Table 9.6: Comparison of peak cytokine values from Donor six with peak cytokine levels from the remainder of the study cohort. Values for the remaining cohort shown as median values and interquartile range.*

## 9.6 Chapter Summary and Discussion

This chapter provides a description of changes in the levels of a selected group of cytokines during the dying period in 22 proceeding DCD organ donors. These results represent an exploratory analysis of the immune state during human circulatory death and as such no corrections have been made for multiple comparisons

The classical description of the acute inflammatory reaction has two stages, which are not precisely spaced in time. A simplified model of immune response to injury (Floerchinger) assumes that the first stage is the proinflammatory phase, in which inflammatory cytokines are the primary mediators (TNF- $\alpha$ , IL-1, IL-6 and INF- $\gamma$ ). The second stage is an anti-

inflammatory phase, wherein the increased activity of the inflammatory cytokines stimulates the synthesis of anti-inflammatory cytokines (IL-4, IL-10, TGF- $\beta$ ) with a strong immunosuppressive effect. However, recent genetic studies suggest that pro- and anti-inflammatory reactions can proceed in parallel, which is another factor complicating discrimination between the physiological and pathological response to a stimulus (Nizet et al 2009), These conclusions reinforced by work in a mouse model of sepsis which suggests the early response to a stimulus is of a mixed inflammatory pattern, with concurrent release of pro-inflammatory and anti-inflammatory cytokines (Osuchowski et al 2006). This explanation co-existence may go some way to explaining the elevations in pro- and anti-inflammatory cytokines demonstrated in table 9.5 during the withdrawal period.

The work outlined in chapter does not replicate the findings of animal models (White et al) in which decreases in IL-6 and TNF- $\alpha$  were demonstrated during a 20 minute withdrawal period. An explanation for this finding may be drawn from the initial elevation in cytokine levels compared to healthy volunteers which is demonstrated in table 9.1. This demonstrates that the potential DCD donors recruited into the study were already in states of marked acute inflammation prior to treatment withdrawal. Evidence for this statement can be found in the elevated initial levels of TNF $\alpha$  and IL-6 in the cohort, which are presented in table 9.1. This finding is unsurprising in this cohort of critically ill patients, given the nature of the disease processes that have led to ICU admission and the supportive interventions that have been undertaken. Furthermore, this finding emphasises an important shortfall in animal models of DCD donation, which utilise healthy animals with normal immune system until the point of treatment withdrawal. It is possible that 'noise' from the baseline immune activation makes it hard to distinguish the cytokine 'response' to the stimulus of hypoxia and hypotension in the proceeding DCD donor and that any change that is occurring is undetectable. It should also be noted that cytokine levels in the circulation may not reflect tissue cytokine levels (Morris et al 2010). In addition, circulating cytokine levels are a relatively insensitive way of assessing immune system function, but practical constraints and the comprehensive nature of the physiological assessment of patients precluded functional testing of immune cells. It is, however, intriguing that the cytokine with the strongest signal to change was the anti-inflammatory IL-10 and future work could look at immune cell function and dysfunction, and the effect of this on organ outcomes.

A further consideration in the difference between the findings reported above and those of animal models is the variation in the time period between treatment withdrawal and death. The animal model of DCD donation involves an anaesthetised paralysed animal which is terminally extubated and dies within a 15 minute time period. However, as demonstrated in Chapter 5 the range of time to circulatory arrest in donors recruited for this study was from 12 to 235 minutes, with a mean time to arrest after treatment withdrawal of 32 minutes. The majority of donors recruited into this study maintained respiratory drive for a period of time after treatment withdrawal. Consequently, it is possible that the stimulus required to trigger an immune response due to hypotension or hypoxia is not achieved until later in the dying process in the human DCD donor undergoing treatment withdrawal.

An alternative explanation for the lack of significant changes in cytokine levels during the withdrawal period across the entire cohort comes from the subgroup analysis of time to death in the DCD donor undertaken in table 9.5, which demonstrates decreases in TNF- $\alpha$  and INF- $\gamma$  in donors living over 60 minutes, with a co-existent increase in IL-10. This raises the possibility that the effect seen could be considered latency effect, with cytokine levels taking time to be seen in the peripheral blood. This would mean that all proceeding donors may exhibit these cytokine changes were they to survive for long enough after withdrawal of life supporting treatment. Variation in the timeframe of cytokine release after an acute stimulus is poorly understood (Thijs et al 1995). TNF- $\alpha$  release represents an early response to an acute stimulus, with other classic proinflammatory phase cytokines being released later after the stimulus. TNF $\alpha$  release in a mouse model has been shown to occur within 15minutes of a delivered stimulus (Paige et al 2011). In contrast, human studies have demonstrated that IL-6 is slower to rise after stimulation. A study by Nishimoto et al of healthy individuals undergoing elective orthopaedic surgery, IL-6 levels took 1 hour to begin to rise in peripheral blood samples, reaching a peak at 4-6 hours post incision (Nishimoto et al 1989). Consequently, it is possible that the immune system may be being activated, but the timeframes involved between treatment withdrawal and death may not be sufficient for these changes to be seen in terms of soluble mediators secreted by innate immune system cells into the blood.

The elevated initial IFN- $\gamma$  levels in the group of donors with underlying hypoxic brain injury (table 9.2) is an interesting finding but is likely explained by the significantly longer time period between admission to ICU and treatment withdrawal in this donor subgroup. As shown in chapter 5 the hypoxic brain injury group spent a mean of 10.2 days in intensive care prior to treatment withdrawal, compared to 3.4 days for the subarachnoid/ intra-cranial group and 3.6 days for the traumatic brain injury group ( $p=0.046$  by Kruskal-Wallis test). This longer admission can be explained by the requirement for a period of targeted temperature management after out of hospital cardiac arrest, and the subsequent requirement for neuro-prognostication.

The contribution of donor six to the study should be noted, despite not contributing to the analyses set out in sections 9.2 - 9.4 above. While no definitive cause of these elevated cytokine levels can be given, the concurrent rapid onset of haemodynamic instability requiring escalation of inotropic and vasopressor support raises the possibility that samples have been obtained at the point of brainstem death or in a heightened catecholamine environment akin to that seen in brainstem death. Consequently, it is proposed that the elevated cytokine levels described in table 9.6 represent the 'cytokine storm' frequently discussed as occurring at the point of brainstem death.

The lack of significant differences in initial cytokine levels between the typical and non-typical donor group with the exception of an elevated TNF- $\gamma$  in the typical DCD donor group is a surprising finding given that the process of brain death is associated with a substantial release of cytokines. The brainstem dead donors in the 'non-typical' DCD donor group all proceeded to donate organs within 24 hours of confirmation of brainstem death, meaning it is unlikely that an immune response could have occurred and dissipated in this time frame, as animal model work suggests cytokines remain elevated for well beyond this timeframe. Floerchinger et al 2012 demonstrated elevated IFN- $\gamma$  in mouse model of DBD donation which existed for over 72 hours after brainstem death occurred. Consequently, this finding may demonstrate that the immune dysfunction associated with critical illness in the DCD donor cohort may be of a similar magnitude to the immune dysfunction associated with brainstem death and is worthy of further consideration in a larger study.

## **Chapter 10: Discussion Conclusion and Further work chapter**

### **10.1 Introduction**

This chapter will address the points of discussion raised by the results presented in the chapters above. It will draw together the work I have undertaken for this thesis to provide a narrative to the physiological changes that occur in the DCD donor during the withdrawal of life supporting treatment and will provide wider context for the results I have presented above. I will firstly consider the public and patient engagement work undertaken for this study, and the implications of the consent for research rates achieved. I will then move on to consider the physiological changes observed in the proceeding DCD organ donor. Discussion will then focus on the measurement of oxygenation and cardiovascular physiology in the proceeding DCD organ donor and methods of determining the onset of donor warm ischaemia. I will then focus on the implications of the stress response to circulatory death and how the immune system is activated during the dying process. Finally, I will address further areas of work that I have identified as relevant and high priority based upon the results I have presented above.

In summary, this thesis presents the first intensive experimental analysis of the physiological changes that occur during circulatory death in the human DCD organ donor. I demonstrate novel physiological changes based upon the first clinical data from this patient cohort. These findings have substantial scope to alter donor management, positively influence rates of organ retrieval and improve the viability of donated organs.

### **10.2 Public engagement work and consent rates**

The work presented in Chapter 3 showed that there was a high level of public support for a study that recruited potential DCD organ donors prior to death. Results of a survey of 248 members of the public demonstrated that 71% would answer positively if asked to provide consent for a relative to be enrolled in a research study as part of the DCD donation process. This represents a high potential consent rate, with other study specific community consultation surveys suggesting much lower rates of respondents being willing to consent to

involvement in complex studies (Constant et al 2006, Biroš et al 2009). The reasons underlying this relatively high positive outcome rate from the survey may be two-fold: Firstly, previously published survey-based studies looking at consent in circumstances where the patient cannot consent for themselves are generally focussed on whether the individual would consent for *their own* inclusion in a study if they lacked capacity. The focus of our survey was slightly different, looking at whether the participant would consent for *a relative* to be involved in a research study if they lacked capacity to consent. Thus, the outcome is what they would say in the circumstances of being asked to act as a personal consultee, not how they would feel were they to be included in the study. It may be that people are more willing to give consent for others to be involved in research than they are to be involved themselves. Secondly, substantial explanation was given to the survey participants regarding the proposed research project and the process of organ donation. Emphasis was made on the fact that the DCD donor will die at the end of the withdrawal process, although it is not possible to predict how long that process will take. Consequently, there may be that the participant sees no perceived 'risk of harm' for the patient taking part in the study. The outcomes of the focus group meeting with members of donor families gives specific insight into the altruistic nature of the process of consent for organ donation, and the implications that this altruistic outlook has for research in the potential DCD organ donor. One family member stated

*'We agreed to some research studies that the specialist nurse spoke to us about. Dad being on the donor register meant he wanted to help people if he died, and we felt that the research was just an extension of that desire'*

This suggests that, given the research consent will be asked of Personal Consultees who have already made the decision that organ donation is what their relative would have wished for as part of their end of life care, the consent rate in this cohort may be even higher than the 71% suggested by the public survey.

Despite the above discussion, prior to the study start there was skepticism from the regulatory authorities that the study would be successful in achieving an acceptable consent rate. The rationale given to this feeling was two-fold. Firstly, that the study model of researcher consent would be unpopular with families of critically ill patients, due to the introduction of a new person at a time of substantial emotional upheaval (with

conversations regarding withdrawal of treatment and decisions regarding donation already having taken place). Secondly, that the presence of the researcher at the bed-space during the withdrawal of life supporting treatment and subsequent death would be an unattractive and unduly invasive prospect for family members.

However, the positive attitude of the public towards this study, and the suggestion from interviewed members of donor families that research in the potential donor would be welcome, have been borne out by the 92% consent rate achieved by this study, which substantially exceeds the rate predicted by the public engagement work. There are several conclusions that can be drawn from this high consent rate. Firstly, the families of potential DCD donors are highly willing to give research consent for their relatives. This was seen repeatedly during the process of discussing the study with relatives, during which many family members repeatedly used the phrase of *'anything he/she can do that helps'* and *'any good that can come out of this'*. This demonstrates that consent is being sought from a highly selected cohort – a group who have endured an intensive care stay with their relative, considered withdrawal of life supporting treatments, and decided in favour of organ donation. They have made an altruistic decision to help others and appeared to consider consenting to research an extension of that desire. Secondly, despite the reservations expressed by the regulatory bodies described above, researcher led consent has proven to be a successful strategy in this study. Indeed, the introduction of a separate person to discuss research with the donor family has seemed a natural progression from the clinical team and the SNOD and not been questioned by the donor family members. From a personal viewpoint as the researcher, the conversations regarding research consent felt straightforward – the family had already given consent for donation and for other generic NHSBT research studies (QUOD and the use of non-transplantable organs for research) so further discussion of research specific to our institution was a natural progression in conversation. Furthermore, for a senior registrar in anaesthetics with substantial neuro-intensive care experience, 'difficult' and end of life conversations with families are everyday events and represent an area of communication skills with which I felt comfortable and well-practiced. A final, and unanticipated, benefit of meeting the family to discuss the study and gain consent for research was the benefit of having an established relationship prior to meeting in the theatre complex for treatment withdrawal.

### 10.3 Demographic data

Examination of the study recruitment data in figure 5.1 demonstrates that of 28 patients undergoing withdrawal of life supporting treatments 25 proceeded to donate organs while three patients were non-proceeding donors due to prolonged time to asystole. This rate of 8.3% prolonged time to asystole is substantially lower than the national rate of 19.8% from the 2017/18 NHSBT Potential Donor Audit (NHSBT 2018). This significantly lower rate may be attributed to the fact that this study was performed entirely within a tertiary referral hospital, with 24 of 28 study participants coming from a specialist neuro-critical care unit. This unit has substantial experience with neuroprognostication and consequently may well 'filter out' patients who are unlikely to die within the four-hour time frame necessary for organ donation to proceed. The referral process by which patients are admitted to tertiary level hospitals may also have a part to play in the differing rates of prolonged time to asystole seen in this study. In general, patients with clearly devastating intracranial injuries will stay in their district general hospital; however, patients with severe injuries but the potential to benefit from neurosurgical treatment and neurocritical care will be transferred to a tertiary centre with appropriate in-house specialities. Consequently, the demographics between donors from tertiary level intensive care units and those from district general ICUs is likely to be different. An interesting potential confounder to this traditional distribution of patients between district general and tertiary hospitals has been the advent of the 'Major Trauma Network', which bypasses the district general hospital in favour of a tertiary level 'trauma centre' for patients with severe injuries. This has led to a redistribution of patients with severe injuries, and anecdotally has increased the numbers of patients with unsurvivable traumatic intracranial injuries in tertiary units. Work to understand how this process has affected donation rates from district general and tertiary level hospitals would be of interest and may help with resource allocation.

A further interesting finding from assessment of donor demographics was the substantially longer period of time that patients with hypoxic brain injuries spent in intensive care prior to withdrawal of life supporting treatments (Figure 5.2). Patients with hypoxic brain injury spent a median of 7 days in intensive care prior to treatment withdrawal and organ donation

(range 4-19 days) which is substantially longer than patients with intracerebral/subarachnoid haemorrhage (median 2.5 days) or Traumatic Brain Injury (median 3 days). This finding is explained by the fact that the patients for whom underlying pathology had been recorded as hypoxic brain injury had all suffered an out of hospital cardiac arrest from which they had been successfully resuscitated. These patients then require a 48 hour period of targeted temperature management, followed by a period of assessment for neuroprognostication, often involving tests which are time consuming to arrange and perform. Hypothermia is known to impair the immune response and to inhibit the typical inflammatory response seen to stress (Polderman 2009), the implications of which are unknown and unquantified with respect to the organ donor. It is possible that this longer intensive care stay, and the physiological changes that are associated with a period of cardiac arrest and subsequent temperature management may modulate the donor environment and consequently the outcomes for donors and donated organs.

An unexpected finding from assessment of the donor demographic data is the number of brainstem dead donors for whom donation is pursued via the DCD pathway – Maastricht 4b category donors. In the study presented by this thesis, this amounted to 4/25 donors proceeding donors (16%). No NHSBT data is available to record the number of Maastricht 4b donations that take place on an annual basis, but these donors provide an interesting platform for potential study. In patients who have already been certified as dead by brainstem criteria, interventions are permitted to optimise organs for donation prior to withdrawal of life supporting treatment. As will be considered later in this chapter, the work presented in this thesis identifies modifiable targets for intervention in the potential DCD donor. At present, the legal and ethical framework that supports DCD donation in the United Kingdom does not permit interventions in the donor to optimise organ outcomes pre-mortem. The cohort described in this thesis as ‘non-typical’ DCD donors provide an opportunity to trial these interventions in a cohort of patients undergoing treatment withdrawal. However, it must be remembered that these donors have undergone the physiological changes of brainstem death described in chapter 1 section 8.3 and have the demographic differences to standard DCD donors outlined in table 5.3 consequently are unlikely to represent a true comparative group for standard DCD donors.

## 10.4 Physiological data

The successful collection of physiological data in this study demonstrate that intensive patient observation and frequent blood sampling during the period between withdrawal of life supporting treatment and death can lead to the collection of data which are of high quality and reproducible. This can be achieved without interference in the usual end of life care processes that are performed by the SNOD and bedside nurse, and without intrusion for the family who choose to be present at the bedspace during the withdrawal period. This has provided new and unique data which has not before been seen in human subjects. As noted in Chapter 1, section 8.2, the pre-existing work on physiological changes during the process of circulatory death have involved animal models which have substantial shortcomings when applied to the typical DCD organ donor. Studies to understanding the changes that occur in the human DCD donor during circulatory deterioration and subsequent death have been called for in the literature for some time (White et al 2016, Dhanni et al 2014, Sungarlitharim et al 2009)

The study of 28 potential DCD donors undergoing treatment withdrawal has confirmed that the process of circulatory death in the DCD organ donor takes a variable period of time, with proceeding donors in the study surviving for a median of 16 minutes after treatment withdrawal (range 7-175 minutes). Of the proceeding donor cohort, 55% had respiratory activity that continued for some minutes after withdrawal of treatment. The collection of physiological data from all proceeding donors demonstrates that death in the DCD donor invariably follows a final common pathway: a failure of ventilation with concurrent decrease in oxygenation and blood pressure. At an individual level, there is impairment of oxygenation, decreased partial pressure of oxygen in the blood, and an accompanying reduction in arterial oxygen saturation. This leads to decreased delivery of oxygen to the tissues and results in tissue hypoxia. This tissue hypoxia results in anaerobic metabolism, which is evident by elevation of lactate in peripheral blood samples. Measurement of these elevations in plasma lactate may provide a biomarker that allows integrated measurement of hypoxic burden in individual patients. These variables have proven to be easily measurable and recordable using only routine bedside monitoring and point of care analysis.

Of note, in the 25 donors recruited into the study who proceeded to asystole and went on to donate their organs, those that survived for a longer period of time after treatment withdrawal did so with a protracted period of poor oxygenation. This is clearly demonstrated in figure 5.8, where all proceeding donors suffer a precipitous decline in PO<sub>2</sub> after treatment withdrawal such that all donors have an arterial PO<sub>2</sub> below 6kPa by 12 minutes after withdrawal. Those that survived beyond that timeframe did so with a consistently low PaO<sub>2</sub>. It is also worth noting the high initial PaO<sub>2</sub> of many of the proceeding donors, up to 31.6kPa – well above the upper limit of normal. Review of the notes suggests that this finding can be attributed to over oxygenation during transfer from the ICU to the place of treatment withdrawal using portable ventilators. This period of hyperoxygenation did not appear to influence the progression of the donor to death, the rate and trajectory of PaO<sub>2</sub> decrease is similar to those donors with normal oxygenation, and those donors reach a low PaO<sub>2</sub> only minutes after donors undergoing treatment withdrawal with PaO<sub>2</sub> levels in the normal range.

While changes in PaO<sub>2</sub> are rapid and universal in all proceeding donors, changes in PaCO<sub>2</sub> have a more varied pattern as demonstrated in figures 5.9 and 5.10. Donors surviving for a longer period of time after treatment withdrawal did so with levels of PaCO<sub>2</sub> that remained low and climbed slowly during the process. This is markedly different to donors who died rapidly in whom PaCO<sub>2</sub> levels climbed rapidly. A similar pattern is seen in the graphical representations of pH changes after withdrawal of treatment in proceeding donors (Figures 5.13 and 5.14). Those donors who died rapidly demonstrated rapid decreases in pH while those donors who survived for longer periods of time had slower decreases in pH level. Examination of the arterial blood gas results from these donors showed the development of progressive metabolic acidosis, with the development of a mixed pattern of acidosis shortly before death.

A further interesting finding from the physiological data presented in chapter 5 is the observed pattern of change in venous oxygen saturations during the withdrawal process. Measurement of central venous oxygen saturation (ScvO<sub>2</sub>) is known to provide insight into the balance between oxygen supply and tissue demand (Reinhart). The normal range for

SvO<sub>2</sub> is 65 to 75% (Kandel). Low ScvO<sub>2</sub> is predictive of death or poor outcome across multiple cohorts of critically ill patients (Kasnitz). Examination of figures 5.15 and 5.16 demonstrate that ScvO<sub>2</sub> falls rapidly in donors who die rapidly after treatment withdrawal. Of those donors who survived for longer periods after treatment withdrawal, the majority did so with ScvO<sub>2</sub> levels substantially below the lower limit of normal. This would suggest the development of progressive oxygen debt in the longer-lived cohort of patients, with tissue oxygen demand being met only by increased oxygen extraction. Of note, none of the recruited patients had pulmonary artery catheters in situ, so venous oxygen saturations are central venous readings rather than mixed venous oxygen saturations (SvO<sub>2</sub>). The literature is conflicting with regard to how well ScvO<sub>2</sub> correlates with SvO<sub>2</sub> with the larger reviews considering this question suggesting that the correlation may be poor in the case of cardiac failure and states of shock, both of which apply physiologically to the potential DCD donor (Van Beest et al 2010). Consequently, caution should be applied to application of ScvO<sub>2</sub> data to this patient cohort with further studies to assess its implications in the proceeding donor.

The implications of the physiological data examined in chapter 5 (PaO<sub>2</sub>, PaCO<sub>2</sub>, pH and ScvO<sub>2</sub> amongst other parameters) suggests that in those donors surviving for longer time periods, oxygenation may be impaired well before clearance of carbon dioxide becomes prolonged. The development of hypoxaemia appears to be poorly predictive of when death will occur, whereas the development of hypercarbia and acidosis appear to be better associated with imminent cardiorespiratory death. This is the first time that this association has been seen in the potential DCD donor undergoing withdrawal of treatment. It has significant implications for the type and frequency of monitoring that is undertaken during the withdrawal period. Further data collection and analysis to determine if specific markers are indicative of certain progression to death within a short timeframe would be of value to the NORS teams and may aid organ procurement. Further studies incorporating measurements of cerebral perfusion pressure during the process of treatment withdrawal, subsequent cardiorespiratory death has the potential to answer some important ethical issues regarding the use of regional perfusion techniques.

## **10.5 Measurement of arterial oxygen saturation.**

Key markers of donor 'health' during the process of treatment withdrawal are oxygen saturation readings, which are currently obtained by finger probe pulse oximetry. Chapter 6 explores measures of oxygenation in the potential DCD donor undergoing withdrawal of treatment and highlights the inaccuracies of pulse oximetry when used in this cohort of patients. As discussed in section 6.2, pulse oximetry relies upon adequate perfusion of tissues to provide reliable differentiation between light absorption between the pulsatile and non-pulsatile components of the signal so that only the signal from arterial blood is analysed. In situations where there is hypotension and peripheral vasoconstriction, the arterial flow to the peripheries is reduced and the information derived from fingertip pulse oximetry becomes less accurate. These physiological conditions are clearly evident in the proceeding DCD donor, with hypotension being ubiquitous and essential for the progression to asystole and cardiorespiratory determination of death (figures 5.3 and 5.4). Furthermore, as demonstrated in chapter 8 and discussed in further detail below, the process of cardiorespiratory death in the DCD donor has been shown by this study to be associated with catecholamine release which will lead to vasoconstriction and decreased peripheral blood flow. A further source of inaccuracy in pulse oximetry paradoxically arises when used in conditions of severe hypoxaemia. This inaccuracy stems from the fact that the underlying technology involves use of an algorithm to convert the amount of light absorbed at a particular wavelength by oxygenated haemoglobin into a value for percentage oxygen saturation. Algorithms vary by manufacturer based upon their calibration data, but the healthy volunteers used for calibration were not rendered hypoxic to substantial degrees. Consequently, the algorithms produced by calibration data are based upon extrapolation below 85%.

An alternative method of oxygen saturation assessment is through arterial blood gas sampling, where there is direct calculation of oxygen saturation after spectrophotometry techniques have been used to determine the levels of different haemoglobin species. This technique is accurate in conditions of hypoxaemia, hypotension and vasoconstriction. The data presented in this study demonstrates that while there is a correlation between pulse

oximeter and arterial blood gas analysis readings of oxygen saturation (figure 6.1), there is a consistent bias towards higher readings being obtained from ABG samples (Figure 6.2).

These findings have substantial clinical implications for assessment of donor oxygenation during the withdrawal period. This is particularly true for circumstances when oxygen saturations readings are utilised by the NORS teams to make decisions regarding organ retrieval. Our local cardiothoracic retrieval service considers the period of time spent by the donor with oxygen saturations below 50% to be indicative of the hypoxic burden suffered by donor organs. It is worth noting that this is not a universally agreed figure, and other centres use 70% oxygen saturation as a threshold. Inaccuracy in pulse oximeter readings mean that this threshold may not be accurately measured, and consequently organ procurement decisions may be made upon inaccurate information. This is demonstrated in figures 6.6 and 6.7 which shows that when oxygen saturations are measured by ABG analysis in our study cohort, the mean time spent with oxygen saturations above 50% was increased. Figure 6.7 demonstrates that with ABG saturation analysis 9% more surviving patients were in a position to donate at 30 minutes, 5% at 60 minutes and 8% at 120 minutes.

Consequently, ABG derived arterial oxygen saturation thresholds would have resulted in 3 more opportunities for cardiac retrieval (maximum acceptable hypoxic time 30 mins), 2 more opportunities for lung and liver retrieval (maximum acceptable hypoxic time 60 min), should all 25 proceeding patients be capable of donating the above. Section 6.5 considers the implications of this finding for cardiothoracic organ retrievals and identifies that three out of six cardiothoracic retrievals in our cohort were abandoned due to 'prolonged hypoxia'. Figure 6.6 demonstrates that a switch to ABG analysis of oxygen saturation would have led to at least one of the three identified patients proceeding to cardiothoracic donation. A potential switch to using ABG data to identify oxygen saturations would be logistically simple – potential donors have arterial lines in place already, and the ICU staff caring for the patient during treatment withdrawal are familiar with ABG sampling techniques. The costs associated with ABG analysis are small, and the information gained gives other important parameters which will be considered later. Furthermore, in cases where donation of certain organs is stood down due to prolonged periods of low oxygen saturations, cardiothoracic

NORS teams are already in attendance, so the 'cost' of waiting longer for death to occur is small compared to the cost of non-proceeding donation.

Whilst the numbers examined here are small, the opportunity to increase organ retrieval within minimal change in practice is clear and has the potential to increase the numbers of organs available for transplantation, especially cardiothoracic organs.

## **10.6 Measurements of cardiovascular physiology during circulatory death**

Having considered the range of physiological changes associated with circulatory death in the preceding DCD organ donor in chapter 5, chapter 7 goes on to consider specifically the factors that are associated with measurement of arterial and venous oxygen content, oxygen extraction ratios and to examine the utility of lactate measurements as a marker of perfusion during the dying period.

### ***Developing better metrics of critical systemic circulatory failure***

The data presented in Chapter 7 demonstrate that systolic blood pressure provides a poor correlate for the onset of warm ischaemia. Surprisingly, there was a better correlation between lactate and oxygen saturations measured by pulse oximetry than existed when oxygen saturations were measured by ABG analysis. This relationship may be explained by the technology that underpins pulse oximetry, which relies upon the pulsatile component of blood flow to provide a signal for analysis. Low saturations by pulse oximeter can occur due to low blood pressure reducing this pulsatile component, and consequently in this context the pulse oximeter could be considered both a measurement of oxygenation and perfusion.

Calculations of arterial and venous oxygen content showed promising correlations with lactate, and in both cases displayed particularly interesting relationships with lactate at low levels, where there was a large spread in lactate levels. The precise cause for this spread are difficult to determine but may be indicative of individual donor circulatory conditions and would be interesting to study further with cardiac output metrics available. The context of

DCD donation, and the need to avoid burdensome interventions before death, limit the methods that can be used to measure cardiac output in this context. In particular, the insertion of pulmonary artery catheters specifically for the purposes of such a study is likely to be inappropriate. However, several non-invasive techniques are now available, and though these are not uniformly accurate, and have not been specifically tested in the context of circulatory death, they might provide valuable information regarding key physiological parameters in this context (Joosten). It would be particularly valuable to apply the chosen non-invasive cardiac output measurement technique in the occasional patients who do have a pulmonary artery catheter in place, so that we can explore the accuracy of the technique in measuring cardiac output in this specific context.

### ***Modelling the impact of critical circulatory thresholds***

Were cardiac output figures available and reliable for this cohort of patients, modelling work described in figures 7.10 and 7.11 suggests that even in the face of oxygen delivery falling below the critical threshold of 600ml/min suggested by the literature (Lieberman) systolic blood pressure can be maintained. Consequently, systolic blood pressure cannot be relied upon to give a reliable indication of the onset of critical oxygen delivery and hence provide a threshold for anaerobic metabolism. At present, organ retrieval decisions are based upon systolic blood pressure during the withdrawal of treatment. A protracted period of time with a blood pressure below 50mmHg is taken to indicate an unacceptably long period of warm ischaemia, and retrieval plans for specific organs (cardiothoracic and liver) is abandoned. Were the relationships described in the above modelling to be proved correct this would give credibility to the suggestion that the use of a predetermined systolic blood pressure target by the National Organ Retrieval Service (NORS) to determine the onset of warm ischaemia is misleading and incorrect, potentially leading to inaccurate decisions regarding which organs have suffered an unacceptable burden of warm ischaemic injury.

The modelling calculations also suggest that systemic oxygen delivery falls to zero during withdrawal of life supporting treatment, which has important implications when considering the processes of DCD organ donation. Circulatory death is confirmed after five minutes of continuous mechanical asystole. In many cases there is ongoing electrical cardiac activity

visible on the ECG trace at the time of confirmation of death, but that electrical activity is unable to support organised ventricular contraction and has no associated cardiac output. This phenomenon is well recognised in the literature. In an observational study of human DCD donors by Dhanni et al, it was observed that electrical activity (organised complexes present on ECG monitoring) persisted after confirmation of circulatory arrest in the majority of patients. The data presented in figures 7.10 and 7.11 demonstrate that in the presence of electrical activity which does not support cardiac output there is zero oxygen delivery (Figure 7.10 and 7.11). Without cerebral oxygen delivery cerebral oxygenation and hence cerebral activity is impossible, consequently these data confirm that consciousness is impossible under the physiological circumstances described above. Were these findings to be confirmed by a study including cardiac output measurements this would support the current practice of confirmation of circulatory death after a five-minute stand-off period of continuous mechanical asystole. The practice that occurs in some non-UK nations of awaiting complete electrical asystole before commencing a stand off period and subsequently confirming circulatory death is not supported by the data presented here.

### ***The influence of haemoglobin levels on oxygen content and delivery***

An interesting observation that comes from examination of the data presented in chapter 7 is the substantial degree of variation in haemoglobin level in potential DCD donors. Proceeding donors in this study had starting haemoglobin levels ranging from 7.3- 12.9 g/dL). As demonstrated in Chapter 7, calculation of arterial oxygen content is heavily dependent upon haemoglobin level, and this in turn has significant implication for the delivery of oxygen to the tissues, which is calculated as the product of arterial oxygen content and cardiac output. Consequently, for a patient with a low haemoglobin level, the achievement of adequate oxygen delivery will be impaired. The threshold for critical oxygen delivery will vary between individuals, depending upon bodyweight and according to work by Lieberman et al and can be calculated as  $7.3 \pm 1.4 \text{ mlO}_2/\text{kg}/\text{min}$ , this is commonly approximated at 600ml/min for a 75kg individual (Lieberman et al 2009). The data presented in figure 7.11 demonstrates that for some donors, despite arterial oxygen saturations of 100%, oxygen delivery may be below 600ml/min. The implication of this finding is that despite optimised ventilatory strategies achieving maximal oxygen saturation, some donor organs may already operate at the limits of aerobic metabolism prior to withdrawal of life supporting treatments. Normal

haemoglobin levels are typically taken as 13.0 - 17.5 g/dL for men and 12.0 - 15.5 g/dL for women (WHO guideline 2013) consequently, the lowest starting haemoglobin in the study of 7.3 is approaching half the accepted normal range. It is a well-recognised phenomena that the vast majority of critically ill patients become anaemic during their time in critical care (Astin et al 2014, Retter et al 2013). The aetiology of this anaemia is multifactorial and complex, an exhaustive discussion of which extends beyond the scope of this thesis but can be generally attributed to a combination of increased losses through haemorrhage and repeated blood sampling, haemodilution with expanded plasma volume, erythropoietin deficiency and lack of availability of normal haemopoietic nutrients. The rationale behind permissive anaemia in the critical care patient is well established, with avoidance of the morbidity and mortality associated with packed red cell transfusion in the form of volume overload, transfusion-related acute lung injury (TRALI) and anaphylaxis, immunomodulation and increased incidence of nosocomial infection (Marik et al 2008). Transfusion Requirements In Critical Care (TRICC) demonstrated that a restrictive transfusion strategy in the critically ill patient (transfusion trigger of <7g/dL) was associate with a trend towards a lower mortality and a significantly decreased number of PRCs transfused than a liberal transfusion strategy (transfusion trigger <10g/dL) (Hebert et al 1999).

The implications of the above discussion are not clear for the proceeding DCD organ donor. Optimisation of haemoglobin to a value within the normal range will undoubtedly improve systemic oxygen delivery. However, this benefit is likely to be lost as oxygen saturations fall, and as is demonstrated in Figure 6.4, this fall occurs precipitously after treatment is withdrawn. Figures 5.5 and 5.7 demonstrate that those donors who survive for a prolonged period after treatment is withdrawn do so in states of severe hypoxaemia, which has deleterious consequences for systemic oxygen delivery even in the face of an optimised haemoglobin level. Furthermore, examination of the relationship between lactate level and  $\text{CaO}_2$  revealed a marginally worse correlation than the relationship between  $\text{SaO}_2$  and lactate ( $r^2 = 0.41$  vs  $0.44$  respectively) suggesting that incorporation of haemoglobin level into the models has no effect on lactate levels. However, it is conceivable that haemoglobin level in the donor may have an effect on graft survival and further studies incorporating graft outcomes data would be valuable.

Disadvantages of a decision made to optimise haemoglobin include the potential for altering the progression of time to death in the potential DCD organ donor, and the potential side effects of PRC transfusion discussed above, of which the potential for immune modulation would be the most concerning. Research to consider the optimum haemoglobin for the potential DCD organ donor, and the effects of donor haemoglobin level on the condition of organs donated for transplantation would be valuable and is an area previously unexplored.

***Arterial lactate as a metric of oxygen deficit and mechanistic heterogeneity***

The utility of blood lactate level as a marker of the onset of anaerobic metabolism specific to the individual donor is a subject of substantial interest to the donation and transplant community. As demonstrated by the evidence discussed above, current clinical parameters to mark the onset of anaerobic metabolism and warm ischaemia are inaccurate and misleading. This is unsurprising given that this represents an attempt to provide a universal physiological target for individuals of a wide age range suffering from a wide range of pathologies.

As discussed previously, assessment of blood lactate level provides an attractive target for marking the onset warm ischaemia. Blood lactate is readily measurable using point of care technology, and the proceeding DCD organ donor already has the indwelling lines required for samples to be taken in a timely unobtrusive fashion. There is substantial precedent in using blood lactate levels as a marker of tissue hypoxaemia, with elevated blood lactate levels being associated with organ dysfunction in multiple clinical settings (Stacpoole et al 1994), and its magnitude of elevation being correlated with outcomes in critically ill patients (Rivers et al 2001).

The data presented in chapter 7 demonstrates that blood lactate level in the proceeding donor is a readily measurable and accurate marker of tissue hypoperfusion in the potential DCD organ donor. Use of blood lactate level in this fashion has the potential to provide more accurate and individualised information about the onset of warm ischaemia than the current method of reliance on systolic blood pressure. The ability to provide an individualised measurement of the onset of warm ischaemia may lead to the potential for waiting longer periods for asystole to occur after treatment withdrawal, as concerns regarding protracted

periods of warm ischaemia could be accurately addressed. Furthermore, utilisation of blood lactate level assessment during the withdrawal period has the potential to allow for better informed decisions regarding the retrieval of those organs that are traditionally considered sensitive to warm ischaemia, which has the potential to increase retrieval rates and organs available for transplantation.

It is also important to point out that a combination of lactate levels with metrics of oxygen delivery, might allow exploration of pathophysiological heterogeneity in the DCD donor. Inspection of the relationship between lactate and oxygen extraction ratio (Figure 7.8) revealed substantial physiological heterogeneity between patients. Some pairs of data showed normal physiology and others showing elevations in OER which correlated with expected increases in arterial lactate signifying that OER increases were no longer adequate to maintain aerobic metabolism in the face of reduced oxygen delivery. However, the relationship between OER and lactate was poor, even within these data points that broadly conformed to expected classical physiology. Intriguingly, some data points showed complete dissociation between lactate levels and OER – with maintenance of normal lactate despite OER values in the 0.5-0.75 range or elevated lactate levels despite OER values below 0.3. The former presumably represents patients in whom oxygen extraction (and by inference microcirculatory dynamics and mitochondrial oxygen utilisation) was highly efficient in the face of reductions in  $DO_2$ .

The latter findings are less easy to explain, but three broad mechanistic explanations are possible:

First, the variable changes in lactate may still be dominated by impaired oxygen deficiency, but the unavailability of cardiac output measurement makes it impossible to precisely measure the reduction in  $DO_2$ . As discussed earlier, several non-invasive techniques for measuring cardiac output are now available, and though these are not uniformly accurate, they would provide valuable information regarding key physiological parameters in this context (Joosten et al 2017)

Second, the elevation in lactate seen in the context of apparently well preserved  $\text{CaO}_2$  (and  $\text{DO}_2$ , should cardiac output measurement be possible) may reflect mechanisms other than macrovascular ischaemia. Key options in this regard include microvascular ischaemia and mitochondrial dysfunction, both of which have been implicated in organ dysfunction in the context of critical illness and transplantation (Hu et al 2017, Kusza et al 2011, Tan et al 2017, Martins et al 2018). Investigating these options will be challenging given the need to use non-burdensome technologies in this patient cohort, but near-infrared spectroscopy and (possibly) darkfield microscopy provide some interesting options (Butler et al 2017, Lima 2016, Scheeren 2016).

Finally, the elevation of lactate in the face of normal  $\text{DO}_2$  values may be the consequence of a range of unrelated processes that do not affect the transplanted organs themselves. These include liver failure (with impaired lactate clearance), catecholamine driven hyperglycolysis, or sepsis. These processes have been discussed in the relevant chapters, and their identification is important, since providing  $\text{DO}_2$  is maintained, lactate elevation in these cases would not necessarily impact on donor organ transplantability.

## **10.7 The stress response to cardiorespiratory death**

The data presented in chapter 5 demonstrate that circulatory death is a ‘stressful’ process for the body, with progressive hypoxaemia, hypotension and acidosis.

While progressive hypotension during the withdrawal period is an inevitable feature of cardiorespiratory death in the DCD organ donor, and is demonstrated in figures 5.3 and 5.4, there is a notable difference between the ‘Typical’ and ‘Non-typical’ DCD donor groups in terms of the patterns seen in systolic blood pressure after treatment withdrawal. This variability in pattern is clearly seen in figure 5.4, where the non-typical donor group have a rapid and progressive decrease in systolic blood pressure. The Typical donor group display a different pattern – with an elevation in blood pressure occurring prior to subsequent deterioration. This occurs even in the shortest-lived typical donors, who survive for comparable periods of time to the non-typical donor group. Observation of this pattern gives rise to the hypothesis that the typical DCD donor is able to mount a response to the ‘stress’

of cardiorespiratory death that the non-typical donor is unable to do. This proposed stress response is examined in chapter 8 by assessment of circulating adrenaline, noradrenaline and cortisol levels during the process of withdrawal of life supporting care in the proceeding DCD organ donor. The data presented in chapter 8 confirms the presence of a stress response to cardiorespiratory death in humans, with elevation in adrenaline, noradrenaline and cortisol levels during treatment withdrawal clearly demonstrated.

This stress response may come from two potential sources, which will each now be considered in turn. The hypertensive response in the immediate period after treatment withdrawal seen in the typical DCD donor group may be related to the physical processes involved in treatment withdrawal – extubation of the trachea and pharyngeal suctioning – which, in the patient with intact cranial nerve reflexes, is highly stimulating of the gag and cough reflexes, and is known to cause a hypertensive response (Hosseini et al 2012). The lack of this response seen in the non-typical donor group could be considered further confirmation of their status as brainstem dead Maastricht 4 category donors. However, the later hypertensive response seen in the typical DCD donor group cannot be attribute to the reflexes associated with laryngeal manipulation, and instead represents the stress response to progressive hypoxaemia and hypotension.

The data presented in Chapter 8 confirms that in the typical DCD organ donor there is a marked adrenaline and noradrenaline response to cardiorespiratory death. Typical DCD donors exhibited both higher peak adrenaline and noradrenaline concentrations during the withdrawal process than the non-typical DCD donor group, and also demonstrated adrenaline and noradrenaline levels that rose significantly during the withdrawal process. In the same way that the process of brainstem death is associated with a ‘catecholamine storm’ as described in chapter 1 section 8.3, this thesis now provides evidence that cardiorespiratory death is in itself also associated with catecholamine elevation. The magnitude of the catecholamine response to brainstem death has been previously assessed (Perez-Lopez et al 2009) where peak adrenaline and noradrenaline levels during the brainstem death process in their cohort are below the levels described here during cardiorespiratory death. The study by Perez-Lopez et al of 40 donors undergoing brainstem death reports peak values of adrenaline and noradrenaline of 6ng/ml and 3.8ng/ml at the

point of brainstem death. The data from this study gives a median peak adrenaline level at 3.4ng/ml and median peak noradrenaline levels of 12.9 ng/ml (Figures 8.2 and 8.6 respectively).

Thus we present data that demonstrates not only does cardiorespiratory death involve a catecholamine response of its own, but that the magnitude of this response may be comparable to or exceed the magnitude of the response seen during brainstem death. This confirms the unexpected finding of work in the porcine model of the DCD donation by Ali et al which suggests that the catecholamine responses of circulatory death may exceed those of brainstem death (Ali et al 2011). The physiological explanation for this response is likely to be a mechanism to preserve cerebral blood flow at the expense of blood flow to the peripheries and other organs.

The clinical implications of what we could now consider a 'catecholamine storm' during the process of cardiorespiratory death are substantial. The deleterious effects of catecholamine excess on body tissues are well described (Hariskov 2013, Nef et al 2007, Movahed et al 1994) and considered in Chapter 1 section 8.4. The acute structural damage to organs includes myocardial dysfunction (Kassim et al) and worsening lung endothelial damage (Egan et al 2004) and are demonstrated using the models of brainstem death and phaeochromocytoma surgery.

The perception from the thoracic transplant surgery community that the DCD route of organ donation avoids organs being subjected to the catecholamine surge of brainstem has been a contributing factor to the renewed interest in sourcing particularly cardiothoracic organs from DCD donors. The comparable performance of lungs from DCD donors despite the organs sensitivity to warm ischaemic damage has been attributed to the fact that the DCD donation process negates organ exposure to catecholamine excess (Egan et al 2004). Our presented data show that this is clearly not the case, and an alternative explanation for this phenomenon should be sought.

A potential explanation for these observed differences, despite catecholamine levels being at least comparable, is that rather than the peak catecholamine level being the source of

damage, the damage is related to the duration of exposure. In the brainstem dead donor, once brainstem death occurs there then follows the substantial period of time required for formal testing of brainstem reflexes and the logistics of organising donation. This period of time exceeds the several hours of elevation in catecholamine levels that are reported after brainstem death (Perez-Lopez et al 2009). During this period of time cardiac myocytes are exposed to high catecholamine levels and the lung vasculature exposed to the elevated pulmonary vascular resistance that is responsible for the endothelial damage sustained. However, in the DCD donor the now documented catecholamine surge occurs shortly before death occurs and organs are retrieved (Figures 8.1 and 8.5) so were organ damage related to the period of exposure to elevated catecholamines there may not be adequate time for the exposure to occur. Further work to understand the relationship between the magnitude and duration of catecholamine exposure and organ damage would be valuable.

These findings have clear implications for organ retrieval teams and transplant surgeons interested in the assessment of donated organs prior to their transplantation. These findings also give rise to the question of whether sympathetic blockade during the withdrawal period in the DCD organ donor might influence the degree of organ dysfunction seen in donated organs. As described in chapter 3, such an intervention is not currently permitted in human DCD donors in the UK, but it has long been practised in parts of the USA following animal work by Belzer's group in the 1970s (Pryor et al 1971). There are no published data to suggest whether the use of sympathetic blockage would affect the time or progression of the patient to death, although anecdotal data suggests it may. Further work aiming to answer these questions would be of considerable merit.

Furthermore, while the use of platforms that allow for *in situ* assessment of organ function, such as NRP, are clearly of merit in that they allow for detailed organ assessment, the results described above highlight potential disadvantages of the technique. If there is indeed a dose response relationship between the duration of catecholamine exposure and the degree of organ dysfunction, allowing a further two-hour period of organ exposure to catecholamines while NRP is performed may be deleterious. NRP may provide a platform to assess this response through a trial to determine if sympathetic blockade whilst organs are undergoing NRP has an influence on organ outcomes.

Further evidence for the stress response invoked by the process of cardiorespiratory death comes from assessment of cortisol levels in DCD donors. Cortisol represents an endpoint in activation of the hypothalamic-pituitary-adrenal axis and increase in serum levels in periods of acute stress is well recognised (Dobson et al 2007) and the data presented above confirms that cardiorespiratory death is a physiologically stressful experience. Data from the cohort of patients examined in this study has demonstrated that cortisol does indeed rise during treatment withdrawal and cardiorespiratory death. This elevation is particularly apparent in the typical DCD donors living for over 30 minutes, in whom a substantial rise in cortisol from baseline levels is observed (Figure 5.11). The relationship between adrenocortical function and immunity is a complex one, and there is evidence that supra-physiological amounts of cortisol produced during periods of acute stress have an effect on immune function. These immune modulating effects are dependent upon the degree of cortisol secretion and last for three to five days (McEwen et al 1998). Studies by Dhabdhar et al in 1996 suggested that cortisol elevation in acute stress enhances the traffic of lymphocytes and macrophages to the site of acute challenge. The findings presented in chapter 8 of cortisol elevation during treatment withdrawal in the proceeding DCD organ donor are novel findings, not previously demonstrated either in this cohort or during the process of cardiorespiratory death. The influence of this cortisol surge prior to death on both the immune system of the donor and of organs donated for transplantation is unknown and unquantified but would benefit from further study.

## **10.8 The immune response to cardiorespiratory death**

The consequences of the physiological stress responses to cardiorespiratory death upon immune function in the proceeding donor have not been the subject of previous study. This is an unexplored area which has the potential to influence our understanding of the environment in which donated organs function prior to retrieval. Acute stress is known to influence the immune system, and as previously discussed, the activation of other responses to acute stress, such as the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, are known to cause acute-phase responses and dampen cellular immunity (McEwen et al 1997). Chapter 9 presents data from the examination of cytokines during the

process of cardiorespiratory death in a group of proceeding DCD donors and provides novel insight into alterations in immune system activation during the process of cardiorespiratory death.

An initial and important finding of this work is that the potential donors recruited into the study are already in a state of acute inflammation prior to treatment withdrawal. Evidence for this statement can be found in the elevated initial levels of TNF $\alpha$  and IL-6 in the cohort, which are represented in table 9.1. This finding is unsurprising in this cohort of critically ill patients, given the nature of the disease processes that have led to ICU admission and the supportive interventions that have been undertaken, but provide an important insight into the immune conditions in which organs donated for transplantation are functioning prior to retrieval.

A further key finding of the work presented in chapter 9 is the failure to replicate the findings of work in animal models which examined cytokine levels during the process of treatment withdrawal. White et al demonstrated decreases in IL-6 and TNF- $\alpha$  during a 20-minute withdrawal period in a porcine model of DCD donation. Such findings were not replicated in the DCD donor cohort examined in this study, who displayed no changes in TNF- $\alpha$  and IL-6 during the period of treatment withdrawal upon examination of the entire cohort. An explanation for this finding may be drawn from the initial elevation in cytokine levels compared to healthy volunteers which is demonstrated in table 9.1. As previously discussed, this represents a pre-existing state of immune dysfunction related to critical illness and underlying donor pathology. This finding emphasises an important shortfall in animal models of DCD donation, which utilise healthy animals with normal immune system until the point of treatment withdrawal. It is possible that 'noise' from the baseline immune activation makes it hard to distinguish the cytokine 'response' to the stimulus of hypoxia, hypotension and HPA axis activation in the proceeding DCD donor and that any change that is occurring is undetectable. It should also be noted that cytokine levels in the circulation may not reflect tissue cytokine levels (Morris et al 2010). In addition, circulating cytokine levels are a relatively insensitive way of assessing immune system function, but practical constraints and the comprehensive nature of the physiological assessment of patients precluded functional testing of immune cells. It is, however, intriguing that the cytokine with the strongest signal

to change was the anti-inflammatory IL-10. IL-10 is a potent anti-inflammatory cytokine (Moore), which influences the functions of numerous immune cells. It is known to have inhibitory effects on neutrophils (Sun et al 2009), it has been implicated in the pathogenesis of monocyte deactivation (Sfeir et al 2001) and extends its influence into the adaptive immune system by polarising T-cells towards a regulatory phenotype (Langier et al 2010). Indeed, elevated levels of IL-10 have been demonstrated to be predictive of mortality in patients with a variety of critical illness (Simmons et al 2004, Montero et al 2000).

A further consideration in the difference between the findings reported above and those of animal models is the variation in the time period between treatment withdrawal and death. The animal model of DCD donation involves an anaesthetised paralysed animal which is terminally extubated and dies within a 15-minute time period. However, as demonstrated in chapter 5 the range of time to circulatory arrest in donors recruited for this study was from 12 to 235 minutes, with a mean time to arrest after treatment withdrawal of 32 minutes. The majority of donors recruited into this study maintained respiratory drive for a period of time after treatment withdrawal. Consequently, it is possible that the stimulus required to trigger an immune response due to hypotension or hypoxia is not achieved until later in the dying process in the human DCD donor undergoing treatment withdrawal.

An alternative explanation for the lack of significant changes in cytokine levels during the withdrawal period across the entire cohort comes from the subgroup analysis of time to death in the DCD donor undertaken in table 9.5, which demonstrates decreases in TNF- $\alpha$  and INF- $\gamma$  in donors living over 60 minutes, with a co-existent increase in IL-10. This raises the possibility that the effect seen could be considered a 'dose response' effect, with cytokine levels taking time to be seen in the peripheral blood. This would mean that all proceeding donors may exhibit these cytokine changes were they to survive for long enough after withdrawal of life supporting treatment. Variation in the timeframe of cytokine release after an acute stimulus is poorly understood. TNF- $\alpha$  release represents an early response to an acute stimulus, with other classic proinflammatory phase cytokines being released later after the stimulus. TNF $\alpha$  release in a mouse model has been shown to occur within 15minutes of a delivered stimulus (Paige et al 2011). In contrast, human studies have demonstrated that IL-6 is slower to rise after stimulation. A study by Nishimoto et al of

healthy individuals undergoing elective orthopaedic surgery, IL-6 levels took 1 hour to begin to rise in peripheral blood samples, reaching a peak at 4-6 hours post incision (Nishimoto et al 1989). Consequently, it is possible that the immune system may be being activated, but the timeframes involved between treatment withdrawal and death may not be sufficient for these changes to be seen in terms of soluble mediators secreted by innate immune system cells into the blood. This has substantial clinical implications when considering the use of NRP for in-situ organ assessment, as this technique has the potential to prolong organ exposure to the altered immune system and could, in this context, be deleterious.

The elevated initial IFN- $\gamma$  levels in the group of donors with underlying hypoxic brain injury (Figure 9.2) is an interesting finding but is likely explained by the significantly longer time period between admission to ICU and treatment withdrawal in this donor subgroup. As shown in chapter 5 the hypoxic brain injury group spent a mean of 10 days in intensive care prior to treatment withdrawal, compared to 3 days for the subarachnoid/ intra-cranial group and 4 days for the traumatic brain injury group ( $p=0.046$  by Kruskal-Wallis test). This longer admission can be explained by the requirement for a period of targeted temperature management after out of hospital cardiac arrest, and the subsequent requirement for neuro-prognostication.

The lack of significant differences in initial cytokine levels between the typical and non-typical donor group with the exception of an elevated TNF- $\gamma$  in the typical DCD donor group is a surprising finding given that the process of brain death is associated with a substantial release of cytokines. The brainstem dead donors in the 'non-typical' DCD donor group all proceeded to donate organs within 24 hours of conformation of brainstem death, meaning it is unlikely that an immune response could have occurred and dissipated in this time frame, as animal model work suggests cytokines remain elevated for well beyond this timeframe. Floerchinger et al demonstrated elevated IFN- $\gamma$  in mouse model of DBD donation which existed for over 72 hours after brainstem death occurred (Floerchinger et al 2012). Consequently, this finding may demonstrate that the immune dysfunction associated with critical illness in the DCD donor cohort may be of a similar magnitude to the immune dysfunction associated with brainstem death and is worthy of further consideration in a larger study.

Regardless of the specifics of the temporal pattern of the innate immune response in this context, the differences in cytokine levels between Typical DCD, Non-typical DCD and DBD donors are noteworthy in the context of linkage of the acute “alarmin” response to graft dysfunction (Wanderer 2010, Rao et al 2008). In particular, the lower levels of the canonical alarmin, IL-1 $\beta$ , in the DCD donors might suggest that this mechanism of late graft dysfunction is ameliorated when compared to transplantation of organs from DBD donors. Additional investigation of other key alarmin levels implicated in this context (in particular, IL-1 $\alpha$  and high mobility group box 1 [HMGB-1] protein) would be of specific interest (Huang).

## **10.9 Future work**

This work provokes as many questions as it answers and suggests a wide range of avenues for future work. From the study design and researcher led consent model aspect, this study raises the question of whether researcher led consent for complex studies involving the pre-mortem donor should be a universally available option. Opportunities to study and compare different consent models would be valuable and have the potential to increase research consent rates.

Secondly, the relationship developed between the researcher and the family present for withdrawal of life supporting treatment in a study such as the one that forms the basis of this thesis would provide an excellent opportunity to undertake qualitative research with the donor family after donation. This could provide valuable understanding of the donor family experience of the donation process and specifically next of kin experience of research undertaken during the withdrawal process. Such work is of vital importance when considering the design of future pre-mortem studies involving DCD donors and has the potential both to improve the donor family experience of the donation process and improve the perceived acceptability of research during the dying process.

When considering the monitoring utilised during withdrawal of life supporting treatment, this study would suggest that there should be a move away from use of pulse oximetry to assess oxygen saturations and towards regular arterial blood gas analysis during the

withdrawal period. As demonstrated above, this has the potential to increase rates of organ retrieval, particularly cardiothoracic organs. A study of the feasibility of use of ABG samples to collect data and inform decisions would be valuable and has the potential to increase the numbers of donated organs. However, the discussion in Chapter 6 highlighted the possibility that the circulatory and microcirculatory confounds that make pulse oximetry inaccurate in this context may aid its ability to quantify the hypoxic burden in transplanted organs. Consequently, it would be important to objectively assess the prognostic accuracy of both measures of arterial hypoxaemia before picking on one as a source of critical oxygenation thresholds for donation.

Even if pulse oximetry did provide such integrated information, the various processes that contribute to a given pulse oximetry reading are impossible to disambiguate and more precise means of separating oxygenation, circulatory, and microcirculatory deficits would be highly desirable, as this could provide a rational basis for optimising donor organ physiology. As discussed earlier, a weakness of this study is the inability to provide accurate cardiac output data for individuals undergoing withdrawal of life supporting treatment. Modelling work undertaken in Chapter 7 has suggested important relationships between systemic oxygen delivery and systolic blood pressure and lactate that were they able to be confirmed would have substantial implications for organ retrieval practices. A potential avenue for exploration to achieve this aim would be through the use of non-invasive cardiac output monitoring. While non-invasive techniques would be ethically straightforward to achieve and likely agreeable to next of kin, their use comes with the caveat that they are uncalibrated at the extremes of cardiovascular physiology demonstrated in this cohort of patients. Consequently, the information is likely to be of questionable accuracy. Gold standard measurement of cardiac output could be achieved by placement of a pulmonary artery floatation catheter. However, this is an invasive procedure which is unlikely to be considered as being in the best interests of the potential donor.

This work presented in this thesis provides foundations for a more detailed understanding of donor physiology. It is intended that this work provides a platform for further detailed study of the DCD organ donor, with determination of the onset of warm ischaemia of particular interest to the organ donation and transplantation community.

The limitations of the current physiological targets used to identify the onset of warm ischaemia – namely oxygen saturations derived by pulse oximetry and systolic blood pressure – are explored in detail. Evaluation of new thresholds to mark the onset of warm ischaemia using physiological data personalised to the individual donor, rather than predetermined generic thresholds, has the potential to positively influence organ retrieval protocols.

While the data presented here does not support sole measurement of lactate as being an adequate marker for the onset of warm ischaemia, further work to examine in more detail the role that lactate elevation plays in predicting warm ischaemia should be undertaken. Institutional evaluation of the integration of blood lactate levels into clinical decision making algorithms regarding organ retrieval would be a logical subsequent step to evaluate the impact upon organ retrieval rates.

Finally, this study has identified physiological changes that were previously not described in humans as occurring in the human DCD organ donor. These changes include release of adrenaline, noradrenaline, cortisol and modification of the immune system. Further work to understand the implication of these findings on organ function after transplantation would be of considerable importance. Further evaluation of the consequences of these physiological changes allows for potential identification of targets to ‘treat’ donated organs. It is important to explicitly state that the current UK legal and ethical framework that supports DCD organ donation does not permit any targeted intervention in the donor aimed at optimisation of potential organs for transplantation prior to death. This precludes pharmacological interventions to ‘treat’ organ dysfunction. However, it is a legal and professional expectation that the intensive care team will aim to stabilise deteriorating physiology, and to delay withdrawal of life supporting treatment in order to permit adequate exploration of the patient's wishes regarding organ donation. The UK Donation Ethics Committee provides clear guidance that delaying treatment withdrawal in order to facilitate the logistics of organ donation is a professional obligation and is in the best interests of the potential organ donor.

While at present physiological targets could not ethically or legally be modulated in the DCD donor prior to death, the increasing use of NRP provides a potential platform to assess organ

treatments. NRP allows in situ assessment of organs after donor asystole, with restoration of regional blood flow while the cerebral circulation is excluded. This technique allows transplant surgeons to make decisions based upon dynamic markers of abdominal organ function (urine production by the kidneys and bile production from the liver). Studies to assess the utility of these markers for predicting the function of transplanted organs are currently underway.

The nature of the immune system activation described in this thesis requires further clarification, as well as an evaluation of potential therapies to counteract its effects. In order to achieve this objective, samples have been stored for RNA sequencing which will allow for determination of the expression level of approximately 25,000 genes. By comparing and contrasting expression levels throughout the withdrawal we will aim to understand the process of dying at the transcriptional level. In addition, through correlation of the sequencing results with other data collected during the retrieval process we aim to associate specific transcriptional signatures with physiological measurements. This aims to identify patterns associated with poor organ outcomes after transplantation, which would allow for a rational preventative treatment strategy .

Further research involving NHSBT and the UK transplant network would be beneficial to understand how the physiological changes outlined in this thesis can influence our understanding of how the donor environment influences transplanted organ outcomes. Such research would seek to examine which physiological parameters best predict the likely time of circulatory death and permit improved decision making concerning the suitability of an organ for retrieval and transplantation. While we could choose to base decision making on specific physiological thresholds, or more complex modelling based on a variety of different parameters, it is likely that this would need to incorporate functional assessment of organs using techniques such as NRP. This would allow opportunity for functional organ assessment and a platform for organ optimisation prior to transplantation, and may exclude a degree of interindividual variability due to recipient pathophysiology. Any such research should proceed within a framework that ensures patient safety – both for organ donors and potential transplant recipients.

## 10.10 Conclusions

This study is the first work to intensively examine the DCD organ donor during the process of cardiorespiratory death. It has demonstrated that such pre-mortem studies are feasible, agreeable to donors' next of kin, and that newer models of researcher led consent can be highly effective in this cohort.

The collection of physiological data from proceeding DCD donors demonstrates that death in the DCD donor invariably follows a final common pathway: a failure of ventilation with concurrent decrease in oxygenation and blood pressure. At an individual level, there is impairment of oxygenation, decreased saturation of haemoglobin with oxygen and decreased partial pressure of oxygen in the blood. This leads to decreased delivery of oxygen to the tissues, results in tissue hypoxia, and the consequent release of lactate into the bloodstream as a result of anaerobic metabolism. These processes have been shown to be associated with previous undocumented patterns of activation in the sympathetic nervous system activation and hypothalamic pituitary adrenal axis, which are implicated in the development of modifiable organ dysfunction.

The use of arterial blood gas sampling has been shown to be logistically straightforward during the process of cardiorespiratory death and has yielded information that allows more accurate assessment of donor oxygenation which has potential to influence decisions made regarding organ retrieval.

The findings of activation of the sympathetic nervous system and hypothalamic pituitary adrenal axis provide the pathophysiological rationale for identification and exploration of targets to modify outcomes in donor organs.

This study opens the door for further studies in DCD organ donors prior to death and has the potential to both expand the DCD donor pool and to improve both the number and quality of organs donated by DCD organ donors. I suggest that the time has come to break the 'taboo' of studying the dying patient and to modify the DCD donation process to maximise the generous gift made by DCD donors and their families.



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