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Cerebral perfusion markers in full-term neonates as a measure of Hypoxic-Ischemic Encephalopathy during and immediately following Hypothermia Treatment

by

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ABSTRACT

Hypoxic-Ischaemic Encephalopathy (HIE) is a leading cause of neonatal mortality and morbidity. Therapeutic Hypothermia (TH) is the only treatment for HIE, which has reduced the rate of mortality and morbidity although still around fifty per cent of infants affected die or have a neurodevelopmental disability, such as cerebral palsy. Every infant with HIE receives the same TH protocol: 72 hours at 33.5^{o}C, before being rewarmed slowly over 12 hours.

The infants with moderate-severe HIE are at the highest risk of disability or death. Should it be possible to identify those infants at greatest risk during the TH, they could be targeted for modified TH treatment or for additional adjuvant treatments such as allopurinol, melatonin or ethyropeitin. The severity of the HIE, and prognosis on outcome, is challenging while the infant is cooled, as the cooling itself reduces the prognostic power of many of the previously accepted assessment methods. MRI (Magnetic Resonance Imaging) is the gold standard, but moving infants to an MRI whilst cooled is problematic, and the HIE injury to the brain may not be evident for several days.

The objective of this research study was to find a clinical parameter which would identify those infants at highest risk of a poor outcome. HIE is characterised by hyper-perfusion, cerebral blood flow (CBF) exceeding metabolic demand, consequent from a physiological response preferentially redirecting blood flow to the brain during a hypoxic episode. Thus, a marker of cerebral perfusion that could classify HIE infants by the degree of hyper-perfusion was sought.

Initially the Resisitive Index (RI), as determined by measuring the Cerebral Blood Flow Velocities (CBFV's) using Doppler Ultrasound (DUS), was investigated as a possible cerebral perfusion marker. The RI is an accepted measure of the resistance of the cerebral arteries and an RI < 0.55 is considered to be indicative of a previous hypoxic event. In the first part of this study, the RI was evaluated in 80 healthy and 18 HIE infants and found to be unreliable; some infants with HIE and a poor outcome did not have an RI < 0.55, whereas some infants without HIE did. Moreover, DUS is not continuous; it is a one-off measurement requiring a skilled operator.

A pursuit for another cerebral perfusion marker ensued. Frequency-Domain Near Infrared Spectroscopy (FD-NIRS) promised absolute measurements of oxy- and deoxy- Haemoglobin (HbO and HbR) in the brain tissue. Increased CBF was expected to result in a higher than normal concentration of blood and therefore Haemoglobin in the brain tissue. The results from the second part of this research study of 40 healthy and 6 HIE infants suggest that hyper-perfusion is expressed in the concentration of HbO in the brain tissue, and that this is most related to poor outcome. The limited HIE population in this study prevents generalisation from this finding, though suggests that there is justification for further research using FD-NIRS to monitor infants with HIE.

DECLARATION

I hereby declare that all the work included in this thesis is my original work towards the degree of Doctor of Philosophy at the University of Melbourne, except where indicated in the preface.

Due acknowledgement has been made in the text to all other material used.

This thesis is fewer than the maximum word limit of 100,000 words exclusive of tables, figures, bibliographies and appendices.

Signed: Dani Forster

12th February, 2020

PREFACE

The work included in this thesis is original and the outcome of my own investigations. The initial question posed at the commencement of this research was whether the Resistive Index (RI) was a reliable indicator of a hypoxic event in infants with Hypoxic-Ischemic Encephalopathy (HIE)., The research objective evolved to a pursuit to find a reliable prognosticator for HIE that would allow treatment of HIE to be adapted to individual HIE infants.

That evolution was guided by the expertise of my clinical supervisors, Dr James Holberton (Paediatrician, Neonatal Intensive Care Unit) and Dr Virginia Saxton (Dirctor of Medical Imaging) from the Mercy Hospital for Women in Heidelberg, Melbourne where the clinical research study was conducted; whilst Academic support was provided by Dr Emmanuel Koumoundouros and Professor Iven Mareels.

As this was a clinical research study, ethics approvals and registration of the study with the appropriate organisations were required. The ethics approvals were all applied for by myself as the Chief Investigator and granted by The Mercy Hospital for Women's Human Research Ethics Committee (HREC). The research study was registered with the Australian and New Zealand Register Clinical Trials Registry (ANZCTR) and The University of Melbourne's HREC.

This research study required the ability to conduct Cranial Ultrasounds (CUS) and measure the Cerebral Blood Flow Velocity in the cerebral arteries. Mrs Gabrielle Fedai oversaw my CUS training, and kindly gave her time assist with and duplicate Doppler Ultrasound (DUS) measurements in the Healthy infants so that I could verify my DUS measurement technique against that of an expert Sonographer. DUS measurements in the Healthy infants were therefore conducted by both myself and Mrs Fedai. Excepting for formal DUS measurements as part of standard hospital protocol, the DUS measurements in the HIE infants were conducted by myself only. The DUS examinations of the HIE infants were conducted once per day whilst the infants were cooled (no infants were examined prior to cooling), and every 2 hours during the rewarming. The majority of the rewarmings were overnight or in the early hours of the morning, as rewarming must commenced exactly 12 hours from the initiation of the cooling Where clinically indicated, the DUS examinations continued once per day until discharge.

All HIE infants also had full recordings of all standard patient monitoring parameters using ixTrend. Parameters included:

- Arterial oxygen saturation, SaO_2

- Arterial blood pressure (ABP, waveform)
- Electrocardiograph (ECG) waveform, heart rate (HR) and respiratory rate (RR)
- Ventilation parameters: pressure and flow waveforms (where infant was ventilated)
- Transcutaneous measurements of the partical pressures of oxygen and carbon dioxide in the blood: $TcPO_2$ and $TcPCO_2$ respectively (available when clinically indicated)

In addition, aEEG was continually recorded according to standard hospital policy.

The last six infants were also monitored continuously with NIRS from enrolment until they were returned to normal temperature. I conducted the investigation into the type of NIRS device that would be most appropriate, and with the support and funding from The Mercy Hospital for Women, procured the OxiplexTS™ FD-NIRS from ISS Inc. (Illinois, U.S.A). The OxiplexTS™ required constant oversight to ensure sensors remained fixed in position, re-positioning when necessary (usually after standard clinical care was given), and re-setting if sensors were exposed to room light, had become saturated and had subsequently been de-activated (which is an automatic resposne of the software to protect the device from over-saturation). This meant supervision of the OxiplexTS™ for 4-5 days continously. Dr Emmanuel Koumoundouros provided some assistance with this surveillance, most particularly offering an early morning visit and check, to allow me to rest after an overnight shift.

Included as Chapter 3. is the accepted manuscript for the publication "Cerebral blood flow velocities and cerebrovascular resistsance in normal-term neonates in the first 72 hours" (1) which was co-authored by Dr Emmanuel Koumoundouros, Dr Virginia Saxton, Mrs Gabrielle Fedai and Dr James Holberton. Co-author declarations have been submitted with this thesis to verity that this publication was 80% my own, original work. As mentioned earlier, Mrs Gabrielle Fedai taught me to conduct CUS examinations, and assisted with and duplicated the DUS measurements. All co-authors supported me throughout the study and reviewed the manuscript prior to submission. All the remaining chapters are unpublished, and as yet have not been submitted for publication.

Similarly, those same co-authors from the above publication continue to provide support and guidance for the remainder of this research project. In all other respects, including data collection, administration and analysis, and the writing of the published paper and this thesis, has been completed wholly myself. Assistance was provided with revision of this thesis by Dr James Holberton (Paediatrician), Dr Emmanuel Koumoundouros (Clinical Engineering Lecturer, Retired), Paul Junor (Clinical Engineering Lecturer), and Dr Richard Kirsner (Adjunct Professor of Biomedical Engineering).

Note that the U.S spellings of some words, including ischemic in hypoxic-ischemic encephalopathy, were selected for consistency with previously published studies and so that any published papers arising from this research will be found in searches of this topic.

Ethics approval was granted by the Mercy Hospital for Women Human Ethics Committee (MHW HREC). The ethics approval was reviewed and endorsed by the Melbourne Graduate School of Education Human Ethics Advisory Group (MGSE HEAG).

This PhD was undertaken with the financial support of an Australian Postgraduate Award Scholarship.

ACKNOWLEDGMENTS

My first acknowledgement and deepest gratitude must be to my enduring partner Edward Batchelor. Ted, Thank you for your continued support and for sharing me with my PhD, I could not have made it to the end without you!

Without my Principal Supervisor, Dr Emmanuel Koumoundouros, this research project would not have got off the ground; Emmanuel took me on not only as his first student for my Final Year Bachelors of Electrical Engineering Degree project many years ago, but also his first PhD student, and introduced me to the Mercy Hospital for Women. Thank you Mani for your support, guidance and friendship, and for your early morning visits to the hospital to check on me… and the equipment!

Deepest gratitude to my clinical supervisors at The Mercy Hospital for Women, Dr James Holberton and Dr Virginia Saxton for offering clinical expertise and the direction I needed to conduct my first clinical research project, and to write for a clinical audience. Your time at the hospital is precious, so the time you found to spend with me, and to review my writing, was always most appreciated.

The Biomedical Engineering Department of The Mercy Hospital for Women was an invaluable resource for the commencement of this research project, for insight into hospital equipment and staff and protocols. The project would have been infinitely more difficult without the support of the Biomedical Engineers, the two Steves: the late Mr Steve Somerwell and Mr Steven Hatty. Steve, your death was a great loss, and the Mercy Hospital for Women was not the same without you. Steven, thank you to you for all your assistance, most especially in navigating through the myriad of hurdles with equipment and paperwork.

For all the remaining Mercy Hospital for Women staff, thank you for co-operation and support throughout, especially the NICU staff who called when there was an eligible infant, assisted with positioning of sensors, and offered guidance and direction with local clinical protocols. Special thanks also to Donna Hyde in procurement, for facilitating the purchase of the OxiplexTS™ from the U.S.

I am indebted to The University of Melbourne academic and support staff for their guidance and mentorship during this project. I would like to thank in particular: Professor Iven Mareels for your guidance as my part Academic Supervisor and your thought-provoking brain-storming meetings, Professor David Grayden as the Chair of my Committee and for offering me words of wisdom when I needed them (by the time you read this David, I will be able to say the F word!), Professor Andrea O'Connor for accepting the role as Principal Supervisor at a crucial time and to Associate Professor

Leigh Johnston for helping me navigate Graduate School procedures. Also a special acknowledgement to Natasha Baxter just for making things happen!

Great appreciation to Paul Junor for offering unsolicited to read and review this thesis, and arranging for Adjunct Professor Richard Kirsner to do the same. Thank you to you both for getting me across the line. Special thanks also to Dr Rebecca Bailey for your friendship and sharing your own PhD experience, from which you knew just what to say and when, to keep me on track.

Last but certainly not least, my profound gratitude to all the parents and their children for their agreement to participate in this research study. Your ultruistism, in the knowledge that the research would have not direct benefit to you but only to future infants and their families, was inspiring.

Thank you to you all.

Publications and Presentations Arising From This Thesis

Original Articles:

- Forster DE, Koumoundouros E, Saxton V, Fedai G, Holberton J. Cerebral blood flow velocities and cerebrovascular resistance in normal-term neonates in the first 72 hours. J Paediatr Child Health. 2017 08/28.

Conference Abstracts:

- Forster DE, Koumoundouros E. Biomedical Engineer to Clinical Researcher: A clinical case study. *Australian Biomedical Engineering Conference (ABEC).* 20-22 August 2014.

- Forster DE, Koumoundouros E, Saxton V, Fedai G, Holberton J. Monitoring the resistance indices (RI) of cerebral arteries with NIRS – Part II. *Society for Medical and Biological Engineers NSW (SMBE NSW).* 24-26 March 2015

- Forster DE, Holberton J, Saxton V, Fedai G, Koumoundouros E. Modelling the cerebrovascular resistance of neonates with Frequency-Domain Near Infrared Spectrscopy (FD-NIRS) and Doppler Sonography. *Australian Biomedical Engineering Conference (ABEC).* 22-25 November 2015.

- Forster DE, Koumoundouros E, Saxton V, Fedai G, Holberton J. Measuring the resistance of cerebral arteries using NIRS: Part III. *Society for Medical and Biological Engineers NSW (SMBE NSW).* (Bathurst, Australia). 21-23 March 2016.

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- Forster DE, Koumoundouros E, Saxton V, Fedai G, Holberton J. Investigation of FD-NIRS parameters as outcome measure for Hypoxic Ischemic Encepalopathy. *Engineering and Physics in Medicine.* 30 October-1 November 2017.

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- Forster DE, Koumoundouros E, Saxton V, Fedai G, Holberton J. Frequency-Domain NIRS (FD-NIRS) as an indicator of Hypoxic Ischemic Encephalopathy. *Society for Medical and Biological Engineers NSW (SMBE NSW).* 25-28 March 2018.

- Forster DE, Koumoundouros E, Saxton V, Fedai G, Holberton J. Investigation of Frequency Domain Near-Infrared Spectrometry (FD-NIRS) parameters as indicators of severity for Hypoxic Ischemic Encephalopathy (HIE). *Engineering and Physics in Medicine.* 29-31 October 2018.

The following co-authored paper has been submitted for publication:

Sett A, Noble E, Forster D, Collins C. Cerebral Oxygenation in preterm infants receiving noninvasive respiratory support: A prospective cross-over study. Submitted to *BMJ Paediatrics Open* on 13 Dec 2019.

ABBREVIATIONS

aEEG	amplitude-integrated EEG
ABG	Arterial Blood Gases
ACA	Anterior Cerebral Artery
ARTG	Australian Register of Therapeutic Goods
ATP	Adenosine Triphosphate
BD	Base Deficit
BGT	Basal Ganglia/Thalamus
BW	birthweight
bpm	beats per minute
Ca^{++}	calcium ions
CA	Cerebral Autoregulation
CBF	cerebral blood flow
CBFV	cerebral blood flow velocity
CI	confidence interval
cm	centimetre(s)
cm/s	centimetre per second
$CMRO_2$	cerebral oxygen consumption
CO_2	carbon dioxide
CUS	Cranial Ultrasound
CT	Computed Tomography
CW-NIRS	Continuous-wave NIRS
D	Day
DCS	Diffuse Correlation Spectroscopy
DPF	Differential path length factor
DUS	Doppler Ultrasound
ECG	Electro-Cardiogram
EEG	Electro-Encephalography
EDV	End-Diastolic Velocity
FD-NIRS	Frequency-Domain (or Frequency-Resolved) NIRS
GA	Gestational Age
h, hrs	hours
HbO	Oxy-Haemoglobin
HbR	Deoxy-Haemoglobin
HbT	Total Haemoglobin
HC	healthy control population
HI	Hypoxic-Ischaemic
HIE	Hypoxic-Ischaemic Encephalopathy
HR	heart rate
ICP	intracranial pressure
IQR	interquartile range
Lac	lactate
LED	light emitting diode
MABP	mean arterial blood pressure
MCA	Middle Cerebral Artery
MHW	The Mercy Hospital for Women
min	minute(s)
MRI	Magnetic Resonance Imaging
Na^{+}	sodium ions
NE	Neonatal Encephalopathy
NICU	Neonatal Intensive Care Unit
NIRS	Near-Infrared Spectroscopy
NO	Nitric Oxide
normotemp	Normal Temperature

O_2	oxygen
OxCCO	cytochrome oxidase
PA	Perinatal Asphyxia
$PaCO_2$	Partial pressure of Carbon Dioxide (CO_2)
PAE	Post Asphyxial Encephalopathy
PaO_2	Partial pressure of Oxygen (O_2)
PCr	phosphocreatine concentration
PDA	Patent ductus arteriosus
Pi	inorganic orthophosphate
PNA	Post-Natal Age
PSV	peak systolic velocity
PPT	Post-Prandial Time
PI	Pulsatility Index
PSV	Peak Systolic Velocity
PVCL	Periventricular Cystic Leukomalacia
resus	Resuscitation
RI	Resistive Index
$rScO_2$	Regional cerebral Oxygen Saturation
s	seconds
SaO_2	arterial oxygen saturation
SpO_2	peripheral capillary oxygen saturation
SD	Standard Deviation
TAV	Time-averaged Velocity
$TcPO_2$	Transcutaneous measurement of PaO_2
$TcPCO_2$	Transcutaneous measurement of $PaCO_2$
TGA	Therapeutic Goods Administration
TH	Therapeutic Hypothermia
TI	Thermal Index
TIC	TI for Cranial bone
TOF	Time of flight
TOI	Total oxygenation index
US	ultrasound
μ_a	absorption coefficient
μ_s	scattering coefficient
wks	weeks
WS	watershed

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CHAPTER 1. INTRODUCTION

1.1 THE RESEARCH PROBLEM

Hypoxic-Ischemic Encephalopathy (HIE) is one of the most commonly recognised antecedents of death or severe long-term neurologic deficits in children, including Cerebral Palsy (2, 3). HIE is a neurological syndrome consequential to birth (perinatal) asphyxia, which in a World Health Organisation (WHO) report in 2005 (4) was identified as the cause of 8% of deaths of children under 5 years of age. The incidence of HIE ranges from 1 to 8 per 1000 live births, with the highest rates recorded in the developing world (5). Survivors of HIE are at a high risk of neurological deficits, principally motor deficits that may be accompanied by cognitive deficits and seizures, or cerebral palsy (6).

Although there have been advances in perinatal care over the last few decades, the incidence of cerebral palsy attributed to HIE has not declined as much as might have been expected. Despite treatment, a substantial proportion of infants die or are left with disability (7) so research that could contribute to improved treatment strategies is important (3). At present the only accepted treatment of HIE is Therapeutic Hypothermia (TH), wherein the affected neonate is cooled to 33.5^{o}C (8); several long term studies have found that TH, cooling either the head or whole-body for 72 hours, has been found to be beneficial (9-14). Although collective evidence from these clinical trials has confirmed that TH improves outcome, 40-50% of infants so treated still die or suffer a significant neurological disability (7), and the optimal degree and duration of cooling is still unclear (3, 7). Treatment strategies will likely depend on the severity of the encephalopathy (3), but the precise timing, nature and severity of the hypoxic-ischaemic insult is not often clear, and assessment of the severity of the HIE during cooling is problematic (7). There is an urgent need for non-invasive, real-time assessment of the severity of the encephalopathy for optimal application of TH and to indicate the use of adjuvant therapies for individual clinical presentations of HIE (7). Adjuvant treatments currently under investigation include antioxidants: allupurinol (15), erythropeitin (16) and melatonin (17).

Thus the objective of this research project was to identify clinical physiological parameters that could be used to assess the severity of the HIE during TH. Ideally, it would be possible to obtain those parameters non-invasively and continuously at the bedside. HIE is characterised by cerebral hyper-perfusion; the initial physiological response to the hypoxic-ischemic episode is to redirect blood flow to the brain, this is prolonged by vasoparalysis resulting in excess cerebral blood flow

and perfusion. This project therefore focused on cerebral perfusion markers that could detect and classify the severity of that hyper-perfusion; the degree of hyper-perfusion is expected to be related to the severity of the HIE.

The cerebral perfusion markers that were investigated were the Cerebral Blood Flow Velocities (CBFV's) and Resistive Index (RI) determined using Doppler Ultrasound (DUS), and Frequency-Domain Near-Infrared Spectroscopy (FD-NIRS) parameters including Oxy- and Deoxy-Haemoglobin (HbO and HbR), total Haemoglobin (HbT) and the Regional Cerebral Oxygen Saturation ($rScO_2$).

The ultimate aim of this research is to identify a cerebral perfusion marker that reliably predicts those infants at highest risk of a poor outcome, for improved prognostication and to target those infants for modified TH therapy, for additional adjuvant therapies or both.

1.2 HYPOXIC-ISCHEMIC ENCEPHALOPATHY (HIE)

Hypoxic-Ischemic Encephalopathy (HIE) is the neurological syndrome arising from a deprivation of oxygen to the foetus during birth, and thus the term is often used interchangeably with 'birth asphyxia' or 'perinatal asphyxia'. The deprivation of oxygen may be due to hypoxemia, a diminished amount of oxygen in the blood, or ischemia, an inadequate amount of blood perfusing the brain, both of which result in a lack of oxygen at the cells, and damage to the affected brain tissue. Hypoxemia has greater importance as it often leads to ischemia via its negative effect on myocardial function and thus cerebral blood flow (CBF) (6); ischemia results not only in the deprivation of oxygen but also glucose further compromising the production of energy (5). The severity of the HIE is influenced by the duration and degree of the initial hypoxic-ischemic insult, both of which are difficult to determine (6).

Due to the lack of a universally agreed definition, the literature on HIE has reported varying rates of incidence, and Hypoxic-Ischaemic Encepalopathy is often used interchangeably with the terminology of 'Neonatal Encephalopathy' (NE) (5). There is an acute and time-bounded hypoxic-ischaemic cerebral insult during the intrapartum associated with HIE that differentiates it from the broader Neonatal Encephalopathy pathology (5). Nelson and Leviton have described NE as "a clinically defined syndrome of disturbed neurological function in the earliest days of life in the term infant, manifested by difficulty in initiating and maintaining respiration, depression of tone and reflexes, subnormal level of consciousness and often seizures" (18). NE therefore encompasses all conditions which manifest as a state of functional impairment of the brain: HIE is therefore a sub-

group of NE as it is an encephalopathy, which specifically arises from an intrapartum hypoxic-ischemic event.

The HIE sub-group of Neonatal Encephalopathy is most likely around 50% of reported NE cases (6). In the developed world, where information regarding aetiology is more accessible, the prevalence of HIE is estimated at 1.5 per 1000 live births, where the prevalence of NE is approximately 3.0 per 1000 live births (5). The pathogenesis of the neurological condition may have significant implications when assessing the efficacy of treatments and correlation with the severity of the condition, mortality and long-term outcomes (19). For many children that are born in a clinically depressed state (altered physiological state), there are often other factors present before the onset of labour that may have increased their vulnerability to intra-partum events (18). Furthermore, the aetiology of an encephalopathy is not always fully known and is not always attributable to a specific intra-partum hypoxic event, for HIE has a clinical presentation consistent with NE (19). For this reason, there is now ground-swell for the adoption of the more generalised terminology, NE for all cases of encephalopathy[1] (20).

A study of HIE must recognise and consider the effects of the antepartum risk factors and the possible contributors to the pathogenesis of the condition. Therapeutic Hypothermia was instituted for HIE but is also applied to infants who have prior antepartum insults if they present with clinical indicators of HIE (7). The efficacy of TH has been shown to be reduced with commencement of cooling later than 6 hours after the hypoxic-ischaemic insult (9-14); infants who have not had a 'true' intrapartum event may not respond to TH as well an infant where the timing of the hypoxic-ischaemic insult was known to have occurred during the birth (19).

The subsequent neurological deficits of concern resulting from HIE are principally a variety of motor deficits with or without accompanying cognitive deficits and seizures, and are often

[1] Hypoxic-Ischemic Encephalopathy (HIE) is the terminology commonly used at the Mercy Hospital for Women, where this research project was conducted. The first publication arising from this research also used the terminology of HIE, and the infants enrolled in the study had evidence of an intrapartum hypoxic-ischemic event. Thus HIE has been retained for the duration of this project and for this thesis. This author appreciates that the NE could also appropriately describe the infants studied in this research project, and will consider moving to the use of NE in the future. In addition, the United States spelling of Hypoxic-Ischemic Encephalopathy was retained for this research project and for the initial publication to remain consistent with previously published literature.

associated with the complex clinical syndrome of cerebral palsy (6). The World Health Organisation (WHO) reported that from 2000-2003, birth asphyxia accounted for 8% of the 10.6 million yearly deaths in children and was therefore one of the top 6 causes of neonatal mortality (4) and is associated with long-term neurological morbidity (7) . The massive financial and human costs to the infants affected, their parents, the healthcare professionals involved in their care and the wider society (5) make it vital that current treatment protocols are refined or additional treatments are developed (7).

1.2.1 Pathology and pathogenesis of Hypoxic-Ischemic Encephalopathy (HIE)

An understanding of the pathology and pathogenesis of HIE is required to precisely evaluate the intervention and management of the disease. An appreciation of the individual is also necessary, as similar events may have disparate consequences depending on the infant's capacity to respond to various insults (19).

In HIE, the supply of well oxygenated blood flow to the brain is compromised, triggering a cascade of events that can lead to neurological damage or death (6). The hypoxic injury is initiated by a primary ischemic insult with a lack of blood flow to the brain, causing hypoxia and depriving the affected brain of oxygen. The lack of oxygen initially causes the cells to switch to anaerobic prodution of energy temporarily maintaining function. Anaerobic energy production is less efficient and can only be maintained for a limited time; prolonged hypoxia inhibits the cellular function, causing the intial damage to the brain tissue. The subsequent recovery from that hypoxic event triggers a cascade of pathways, including the release of free radicals, that lead to secondary cell death (21). This latter recovery period is often referred to as the "reperfusion phase" of injury (3).

There are two fundamental modes of cell death associated with HIE: necrosis, and apoptosis (6). Necrotic cell death occurs typically after intense, brief insults such as the primary insult and is distinguished from apoptosis by the presence of inflammation caused by cell swelling, membrane disintegration, cell rupture and release of the intracellular contents. By contrast, apoptosis is programmed cell death and is associated with longer, and less intense insults and the delayed cell death during the secondary energy failure. Apoptosis is difficult to detect in tissue as there is no inflammation, and the cell debris is evacuated from the tissue (6). The two processes of cell death have been demonstrated in both animals and humans (6, 22, 23) and the distinction between the two forms of cell death will likely have significance in the development of treatment strategies, as treatments will need to include interventions that target both (3).

Glucose is the predominant fuel for cerebral metabolism in the foetus and developing neonatal brain. Under normal conditions and in the presence of oxygen, glucose is utilised efficiently in the oxidative phosphorylation process to produce Adenosine Triphosphate (ATP), the energy modulator of the cell, and thus the maximal amount of chemical energy (24). This aerobic process is remarkably stable, with ATP remaining constant even under increased energy demands (25), and produces only carbon dioxide (CO_2) and water (24).

Reduction in cerebral blood flow (CBF) and oxygen delivery results in a switch to anaerobic glycolysis (glucose consumption), an energy-inefficient state which requires greater quantities of glucose (25). In the neonate, with lesser glucose reserves than an adult, the glucose levels are depleted quickly with a subsequent rapid consumption of high-energy phosphate reserves including ATP (3) . The ATP provides the necessary energy for the cellular sodium-potassium ion-pump and so the absence of ATP prevents the normal functioning of this ion-pump and results in the intracellular accumulation of sodium (Na^+), calcium (Ca^{++}) and water (cytotoxic oedema) (24). As intracellular concentrations of Na^+ ions increase from the failure of the ion-pump, the ion gradient across the membrane of the cell is reduced and triggers depolarisation of the membrane and the release of excitatory neurotransmitters, specifically glutamate, from the axon terminals. The glutamate in turn activates cell-surface receptors in the post-synaptic neurons which cause a further influx of Na^+ and Ca^{++} into the neurons. In selected neurons, the accumulation of Ca^{++} induces the production of nitric oxide (NO), a free radical. In addition, the accumulation of Ca^{++} also causes the accumulation of free fatty acids in the cytoplasm which then undergo perioxidation by the free radicals. Thus the inability to produce ATP and the subsequent energy failure results in the inability to maintain cellular functions (3), acidosis, glutamate release, intracellular Ca^{++} accumulation, lipid perioxidation and nitric oxide neurotoxicity (22) which ultimately results in neuronal death (25). This primary energy failure resulting in the initial insult to the brain correlates with a decreased ratio of mean cerebral phosphocreatine concentration [PCr]/inorganic orthophosphate [Pi] (26) and in increase in the excitatory amino acids, including gluatmate, measured by phosphorus nuclear magnetic resonance spectroscopy (MRS) (27) and a rise cerebral lactate (Lac) with a fall in acetylaspartate (Naa) as determined by proton MRS (28).

The initial hypoxic-ischemic insult induces hypoxemia, hypercapnia and acidosis which leads to a compensatory cerebral vasodilation, a reduction in the resistance of the cerebral vasculature, thus re-distributing the blood flow preferentially to the brain at the expense of other organs (6, 19). This compensatory mechanism, known as the "brain-sparing effect" (29), initially limits the impact of the insult to the brain, but subsequently cardiac output (CO) decreases. The decrease in CO and

hypotension leads eventually to reduced CBF and impaired cerebral autoregulation (30). The redistribution of cardiac output (CO) as associated with *brain-sparing* and subsequent hyper-perfusion also commonly results in multisystem involvement in severe cases of asphyxia due to the reduced blood flow to other organs. Multisystem involvement may include acute bowel injury, renal failure, hepatic injury, cardiac damage, respiratory complications and haematologic abnormalities (31, 32).

After restoration of blood oxygenation and cerebral perfusion, ATP levels revert to normal and lactate levels return to almost normal within 2-3 hours, suggestive of a full recovery. However, a secondary decline in high-energy phosphates including ATP heralds the onset of a secondary energy failure which becomes apparent after 8-16 hours, and peaks at 24-48 hours. The period between recovery and secondary energy failure, and the severity of this secondary energy failure, varies according to the nature and severity of the initial insult (6). In a piglet model, Lorek et al found that in spite of normal arterial partial pressure of oxygen (PaO_2), mean arterial blood pressure (MABP), blood glucose and intracellular pH during the secondary energy failure 24-48 hours after the primary insult, [PCr]/Pi decreased again, and the decrease was directly related to the extent of energy depletion (26). Penrice et al confirmed a concomitant increase in the lactate (28).

The cause of the secondary energy failure is still debated but a study conducted by Vannucci et al with immature rats suggests that this secondary energy failure is a consequence of the cascade of events set into motion by the primary energy failure and associated cell death (33). This same study observed that the loss of neuronal proteins, an indication of imminent neuronal death, became apparent at 6 hours and was pronounced at 18 hours. As this was prior to the secondary energy failure, the conclusion is that the secondary energy failure is a result of the cellular destruction rather than the cause (6). As the cascade of events leading to the secondary energy failure appears to occur subsequent to the HI insult itself, intervention in this post-insult period prior to the secondary energy failure is likely to be beneficial (6).

The secondary-energy failure is most likely the dominant mechanism causing cell death and subsequent brain injury (34). The secondary energy failure triggers depolarisation of nerve cells and another extracellular increase in neurotransmitters, excitatory amino acids glutamate and aspartate, occurs. The function of the glutamate re-uptake transporters is impaired with the lack of glucose during the secondary energy failure, so glutamate and aspartate accumulates in the extracellular space, particularly in the cortex and basal ganglia. The glutamate perturbs the membrane potential of the nerve cells resulting in depolarisation and an inflow of Ca^{++} ions into the nerve cells. That Ca^{++} influx re-activates the cascade of events that results in neuronal injury. Increases in excitatory

aminoacids have been observed in neonates after HI insults (35, 36) and extent of that increase has been correlated to the severity of the HIE (37, 38). Roth et al found a close correlation between the degree of secondary energy failure and the degree of neurological impairment at 1 and 4 years of age (39).

The initial increase in CBF associated with the "*brain-sparing effect*" is evident within minutes of the insult and may last up to several hours; hypotension, reduced CO and decreased CBF then follows (6). At 12-24 hours, there is a subsequent, delayed increase in CBF causing hyperaemia and this correlates with the timing of the secondary energy failure (6). In late 1960, Lassen labelled this delayed hyperaemia the "Luxury-Perfusion Syndrome" (40, 41). Luxury perfusion suggests that this has a positive effect, whereas this excess perfusion beyond metabolic demand is detrimental. For this reason, the prolonged excess cerebral perfusion associated with HIE will be herein referred to as hyper-perfusion.

Hyper-perfusion may be prolonged, lasting many hours to days (42); during which time cerebral autoregulation (CA), the normal coupling between CBF and metabolic demand, is disturbed. Pryds et al (1990) demonstrated a correlation between the extent of this disruption and the severity of the HIE; infants with the most severe HIE and worst outcomes exhibited the highest CBF that was unresponsive to changes in either blood pressure or the partial pressure of carbon dioxide ($PaCO_2$) (43). Studies conducted by Ilves et al, Meek et al and Levene et al have also confirmed the relationship between the increase in cerebral blood flow and the severity of HIE, and thus poor prognosis using various methods (44-47).

Hyper-perfusion may also cause brain swelling and subsequent tissue necrosis (6). Meek et al have shown that cerebral oedema is evident but the question remains as to whether this is akin to the swelling commonly observed in neonates post-partum or a consequence of the hypoxic event, as it is particularly difficult to quantify (48). Lupton et al correlated increased intracranial pressure (ICP) (>10mmHg) with observed decreased attenuation on Computed Tomography (CT) scans, an indicator of tissue necrosis, and poor prognosis in one study but this occurred in only 22% of asphyxiated infants (49). In contrast, Clancy et al found no correlation between ICP and outcome (50). Brain swelling appears to be a consequence of a hypoxic event but is possibly not causative of brain injury thereafter (6).

1.3 CLINICAL & NEUROLOGICAL MONITORING FOR HIE IN NEONATAL INTENSIVE CARE

1.3.1 Clinical Observation

Neonatal physiology is unique in so far as transition from foetus to neonate is a complex physiological process: as asserted by Pichler (51) it is often at the time of birth that the neonate is at most long-term risk. Dr. Virginia Apgar proposed a method for the evaluation of the newborn infant (52); this Apgar score method of evaluation is still the most widely used and accepted method of evaluation of the newborn in use today. The evaluation is based on the observation of the newborn and includes the assessment and rating of five physiological aspects. Each aspect or objective sign is awarded a 0, 1, or 2, if the sign is poor or absent, fair, or good respectively, with a total possible score is 10. The five physiological signs are heart rate, respiratory effort, reflex irritability, muscle tone, and skin colour. Heart rate (HR) was found to be the most important sign of the five, and skin colour the least. Apgar established from the study of 2096 infants who infants with scores of 2 or less had the worst prognoses.

Visual and observational techniques of clinical assessment of physiological signs such as the Apgar scoring are inherently prone to inter-observer variability and this variability has been found to be amplified when observers score babies in need of resuscitation (53-55).

1.3.2 Electroencephalogram (EEG) and Amplitude-Integrated EEG (aEEG)

Electroencephalography or (EEG) is the monitoring and recording of the electrical signals generated by the brain. This is achieved by recording amplified voltage differences or potentials between electrodes placed on the scalp. Electrode placement was standardised for reproducibility of and comparison between recordings and the generally accepted montage is the International 10-20 System of electrode placement (56) where the distances between adjacent electrodes are either 10% or 20% of the total front-back or right-left distance across the skull, hence the reference to "10" and "20". This 10-20 system is modified for neonates due to the smaller head size; eighteen EEG channels are required for the standard neonatal montage (57). The reduced number of channels allows for fewer electrodes in the fronto-polar region where there is less activity in early life, but enough across the central vertex to capture important EEG patterns such as those associated with seizures. In the neonatal EEG, signal changes resulting from an hypoxic-ischemic event are usually symmetrical and include abnormal voltages, discontinuous patterns and seizure activity (57).

The conventional EEG (cEEG) is limited as it is not suitable for long-term bedside monitoring. Although the cEEG provides a high-resolution picture of the cerebral electrical activity, it was designed for intermittent, brief (45-60 minute) recordings and so has no functionality for continuous trending of that cerebral activity (58). Moreover, particular expertise is required to analyse the EEG in the neonate; this includes the knowledge of maturational changes as well as the effect of pathological processes and medication on brain activity (59). As continuous monitoring with cEEG requires an expert EEG reader it is an impractical task to interpret many hours or days of 16+ channels, especially where multiple patients require simultaneous monitoring. In addition, the availability of cEEG is also often restricted - a consequence of the specialisation and therefore expense - in addition to the practical challenges of correctly placing, securing and maintaining low impedance of eighteen electrodes.

Neonatalogists sought a tool that was practical and easily interpreted for continuous monitoring, and the amplitude-integrated EEG (aEEG) fulfilled that need. The montage for an aEEG consists of either 2 or 4 electrodes which describe either 1 or 2 separate channels. Two channels are preferred, as that improves the diagnostic sensitivity and allows for detection of inter-hemispheric asymmetries (60). In one study, 15% of infants who demonstrated severely abnormal readings on a 2-channel aEEG device supported with the raw EEG appeared to have normal EEG activity on a 1-channel aEEG (61). The electrodes are optimally placed over the central and parietal regions, not only because the frontal regions are relatively underdeveloped electrophysiologically (58) but because this represents the watershed regions in the border zones of arterial blood supply from the cerebral arteries (62).

The conversion from EEG to aEEG essentially translates the amplitude of the EEG into a compact format using time and amplitude compression. The method involves asymmetric filtering in the 2-15 Hz band, to emphasise the relevant signal and reduce noise, rectification and smoothing before time compressing by selecting and displaying the voltage maximum and minimum from each minute of EEG (63, 64). The resulting aEEG advances at just 6 cm/hr; a full minute of EEG is represented in just one millimetre of aEEG (6, 58).

1.3.3 Neonatal Seizures

Seizures are the most common and important sign of acute neonatal encephalopathy. Not only are they a major risk for death or subsequent neurological disability following HIE but may themselves contribute to the adverse neurological outcome (65). Seizures may be identified by visual observation, though myoclonus or rhythmic tremors can be found in normal term newborns and

may be mistaken for neonatal seizures if diagnosed from observation alone (66). Performance of regular routine Electroencephalography (EEG) may assist with monitoring suspicious clinical seizure activity but it is not suitable for continuous bedside monitoring as it requires the connection of eighteen electrodes, and the expertise to interpret the output correctly. Amplitude integrated EEG (aEEG), with only 3 or 5 electrodes and automated seizure recognition allows continuous monitoring and recording of seizure activity (58, 59).

1.3.4 Neurological Assessment

A typical result of HIE is an altered neurological state dependent on the progression of the disease. Neurological assessment and identification of the stages of HIE are therefore important. Sarnat and Sarnat (66) developed the first observation-based neurological assessment of asphyxiated newborns based on the observation of 21 HIE affected neonates, and identified three stages of post-anoxic neonatal encephalopathy. This neurological assessment remains commonly used as a guideline for rating the severity of an encephalopathy; clinical evidence of encephalopathy by this method is the positive identification of one of the three stages, detailed in Table 1-1.

Stage 1 is characterised by hyperalertness, overactive stretch, and Moro reflexes (infantile reflexes normally present in newborns as a response to a sudden loss of head support), and generalised sympathetic autonomic function including pupil dilation and tachycardia lasting less than 24 hours. In contrast, Stage 2 is characterised by lethargy, weak or incomplete Moro reflexes, hypotonia and generalised parasympathetic effects such as constriction of the pupils, and bradycardia. The duration of this stage may be several days, and seizures are common. Seizures were observed in half of the neonates studied by Sarnat and Sartnat and were almost exclusively limited to Stage 2. The recovery from Stage 2 was indicated by the reduction of myoclonus, or involuntary twitching of the muscles, waning of parasympathetic responses, increased alertness, increased sucking reflexes, and the eventual reversion of the EEG to normal. Sarnat and Sarnat suggested that infants who had signs of Stage 2 for less than 5 days appeared normal later in infancy. Infants in Stage 3 were stuporous, flaccid, with suppressed brain stem and autonomic functions, and seizures were rare. These infants also lost body temperature and became hypothermic despite external warming.

Sarnat staging is still the accepted and commonly-used basis for neurological examination of infants with neonatal encephalopathy and is included in many protocols for the identification of infants who would benefit from Therapeutic Hypothermia (TH). It is now less likely to have the same predictive ability with TH and the increased use of mechanical ventilation and sedatives (67).

Table 1-1 Sarnat Staging of post-anoxic encephalopathy in the full-term neonate

The distinguishing features of the three clinical stages of post anoxic encephalopathy in the full-term newborn infant as reproduced from Sarnat & Sarnat (1976) "Neonatal Encephalopathy Following Foetal Distress: A Clinical and Electroencephalographic Study" (66).

Category	**Stage 1** **Mild Encephalopathy**	**Stage 2** **Moderate Encephalopathy**	**Stage 3** **Severe Encephalopathy**
Level of Consciousness	Hyperalert	Lethargic or obtunded	Stuporous
Neuromuscular control			
Muscle Tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch Reflexes	Overactive	Overactive	Decreased or absent
Segmental Myoclonus	Present	Present	Absent
Complex Reflexes			
Suck	Weak	Weak or absent	Absent
Moro	Strong; low threshold	Weak; incomplete; high threshold	Absent
Oculovestibular	Normal	Overactive	Weak or Absent
Tonic neck	Slight	Strong	Absent
Autonomic Function	Generalised sympathetic	Generalised parasympathetic	Both systems depressed
Pupils	Mydriasis	Miosis	Variable; often unequal; poor light reflex
Heart Rate	Tachycardia	Bradycardia	Variable
Bronchial and salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal or decreased	Increased; diarrhea	Variable
Seizures	None	Common; focal or multifocal	Uncommon (excluding decerebration)
EEG findings	Normal (awake)	Early: low-voltage continuous delta & theta. Later: periodic pattern (awake) Seizures focal 1-to 1.5 Hz spike-and-wave	Early: periodic pattern with isopotential phases. Later: totally isopotential.
Duration	Less than 24 hr	2-14 days	Hours to weeks

1.3.5 Cranial Ultrasound (CUS) & Doppler Ultrasound (DUS)

Ultrasound is a diagnostic tool frequently used in the clinical management of the neonate to interrogate the physiological structure of the brain, inspecting for physiological abnormalities and haemorrhages that can be prevalent in extremely premature and critically ill infants (68). Cranial ultrasound (CUS) in neonates is possible due to the fontanelles and sutures of the skull still being open, providing 'acoustic windows' through which the brain can be imaged (69). Furthermore, ultrasound can be performed at the bedside and repeated as often as required, does not require sedation of the neonate, is free of ionising radiation, and allows functional colour Doppler sonography (DUS) for the visualisation and measurement of blood flow, as well as anatomical grey-scale imaging (70).

Pinto et al (70) confirmed in a neonate suffering from an encephalopathy there is an increased echogenicity in the parietal white matter in contrast to the grey matter. The subtlety of this effect and its dependence on the operator, the probe and frequency, the adjustment of gain and focal set points, is of limited value, but Doppler sonography allows the measurement of blood velocities in the cerebral arteries, providing an objective and quantitative indication of the cerebral perfusion. Volpe, a key contributor to the study of the neurology of neonates, suggests the use of the anterior fontanelle as a suitable site to measure the cerebral blood flow velocity through Doppler Ultrasound (71).

Doppler sonography exploits the Doppler effect, based on the principle that the frequency of the sound waves reflected from a moving object is shifted by an amount proportional to the velocity of that object. In the case of the measurement of blood flow velocity, the moving objects are the red blood cells, and the ultrasound waves emitted by the transducer are reflected by those red blood cells and the frequency is shifted by that interaction. Ultrasound machines can thus calculate the velocity of the red blood cells, and estimate the velocity of the blood flow in the insonated vessel using the following well-known Doppler equation:

$$v_r = \frac{c\,(f_r - f_i)}{2\,f_i \cos\theta}$$

Equation 1-1 Doppler Equation

where f_i is the frequency of the incident sound wave, f_r is the frequency of the reflected wave, $f_r - f_i$ is then the Doppler shift, c is the propagation speed of the wave, and θ is the angle of insonation or the angle from which the measurement was taken in relation to the direction of the

red blood cells (72). The inclusion of $\cos\theta$ accounts for the component of the velocity vector directed towards the transducer being reduced from the velocity vector along the axis by the cosine of the angle. This means that the insonation angle has a significant impact on the measurement of blood velocity, and the measurement should preferentially be made with the direction of the blood flow parallel to the ultrasound probe.

Yamamoto et al (73) studied the errors introduced in the measurement of blood flow velocities using Doppler Ultrasound. Modern ultrasound machines will "angle-correct" to take account for the angle of insonation. Even so, the angle correction relies on the operator to manually correct the position of the angle guide in the vessel of interest, which may still introduce error. The error that is introduced as a function of the assigned angle of insonation is:

$$\frac{v_m}{v} = \frac{\cos\theta}{\cos(\theta + e)}$$

Equation 1-2 Error introduced to Doppler velocity measurement by error in the angle of insonation.

Where $\boldsymbol{v_m}$ is the measured velocity, $\boldsymbol{v}$ is the true velocity, $\boldsymbol{\theta}$ is the angle determined by the operator and $\boldsymbol{e}$ is the error or the difference angle between the actual direction of blood flow and $\boldsymbol{\theta}$. The percentage error introduced as a function of insonation angle is demonstrated in Figure 1-1:

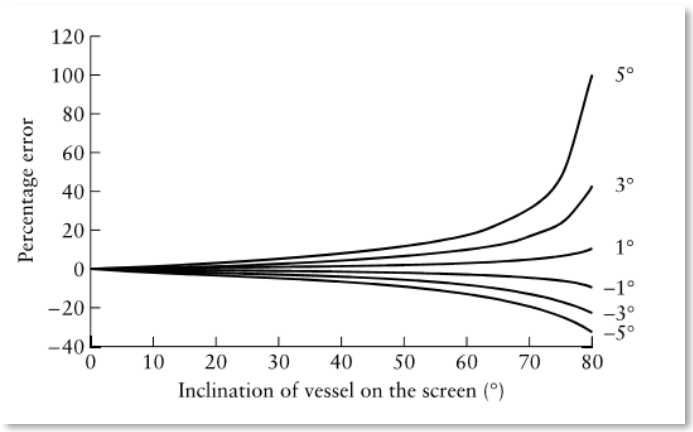


Figure 1-1 The percentage error introduced as a function of the insonation angle

Angle of insonation as determined by the operator versus the percentage error introduced for introduced errors of 1 degree, 3 degrees and 5 degrees. Figure courtesy of Yamamoto, M. et al "Error introduced into Velocity Measurements by Inappropriate Doppler angle assignment" (73).

The larger the angle of insonation, the greater the error that may be introduced: an error of 5° at an insonation angle of 80° can introduce up to 100% error on the measured velocity. Yamamoto et al suggested that to obtain the most accurate blood velocity measurements, the image of the vessel is oriented in the most vertical position possible, and that the angle-correct feature is used to account for the (now) small angle of insonation. This will introduce the least error into the measurement. Also, the angle of insonation and the error margin for this angle should be reported where blood velocity measurements made by Doppler are used.

In practice, it can be particularly difficult to ascertain the correct angle where the vessel changes direction or the image is insufficiently clear to allow for accurate interpretation of blood flow. Additionally, measuring blood flow near a bend in a vessel may inadvertently provide a blood velocity faster than that in straight parts of the vessel. This is due to the Venturi Effect whereby red blood cells that are changing direction, or are moving through a part of the vessel which is small in diameter than those blood cells, will move faster than elsewhere in the vessel. Doppler flow measurements require precise estimates of the flow velocity as well as the vessel's area in a transverse cross-section (74).

Cerebrovascular Resistance Indices

Two indices involving ratios of systolic and diastolic velocities were proposed to remove the dependence on the angle of incidence. These are Pourcelot's Resistance (or Resistive) Index (RI) and the Pulsatility Index of Gosling (PI). The RI is calculated by the following equation (67, 68):

$$Resistance\ Index\ (RI) = \frac{Systolic\ Velocity - Diastolic\ Velocity}{Systolic\ Velocity}$$

Equation 1-3 Calculation of the Resistive Index (RI)

The PI is calculated similarly using the following equation (6):

$$Pulsatility\ Index\ (PI) = \frac{Systolic\ Velocity - Diastolic\ Velocity}{Mean\ Velocity}$$

Equation 1-4 Calculation of the Pulsatility Index (PI)

In practice the RI is commonly used when examining the cerebrovascular resistance of the neonate, whereas the PI is more commonly determined for the evaluation of cerebrovascular resistance of the foetus in utero. Bada et al (75) recommended the use of the RI, as it has a reduced data spread to the PI.

Doppler indices such as the RI and PI, as measured from the Doppler flow velocity waveforms have been long established as indicators of downstream vascular resistance (76) providing a non-invasive means of monitoring the neonatal circulation. Due to the removal of placental circulation, inflation of the lungs, and subsequent changes to the blood gas composition, birth itself brings about profound changes in circulation (76). In term neonates, the RI was found to decrease initially in the first 24 hours, with little change thereafter (77, 78). The Connors et al study reported an initial increase in RI in the antenatal (20 hours before delivery) and 8-hour post-natal periods, corresponding to a decrease in CBF, which may be attributed to the CA being affected by an increase in blood oxygenation (76). The RI then subsequently decreased during the period of 8-24 hours after birth, and was unchanged between 24 and 48 hours (76).

It had been proposed that the initial increase in RI does not reflect the vascular resistance at all, that instead it is caused by a reduction in diastolic flow (or "diastolic run off") due to left to right shunting of the Patent Ductus Arteriosus (PDA); a delayed closure of the ductus arteriosus allows oxygenated blood to continue to flow from the aorta to the pulmonary artery after birth. The subsequent closure of the PDA results in the RI returning to prenatal levels (76). Perlman et al reported that the closure of clinically significant PDA correlated with a decrease in RI (79) but Connors et al found the same alteration in the RI both in infants without symptoms of ductal patency and closed ductus arteriosus (76). Thus, the haemodynamics of the PDA cannot fully account for the changes in the RI observed in healthy newborn infants.

Cerebral metabolic activity results in an increase in oxygen consumption and therefore CBF to supply that demand (80). Where cerebral autoregulation (CA) is intact, the increase in CBF is achieved by lowering the vascular resistance. Although this may explain the decrease in RI between 8-24 hours, Connors et al took care to measure the RI in the same behavioural state (quiet sleep or quiet awake) for each participant, suggesting the alteration was not due to an increase in cerebral metabolic rate (76).

Impedance matching between the resistance of the cerebral feeder vessels (the arteries) and the small cerebral 'capacitance' (storage) vessels (the arterioles and capillaries) might be a better explanation. The arterial oxygen content increases perinatally, as was demonstrated by a higher PaO_2 in the 8-hour old newborn versus that in the umbilical artery at birth (76). This initial change in blood gases results in an increase in RI as the CBF is reduced. The subsequent reduction in RI cannot be explained by changes in blood gases as $PaCO_2$ and PaO_2 as they remained unchanged in the period following, 8 and 24 hours after birth. Instead, the increase in oxygenation of the blood causes constriction of the capacitance vessels and an increase in the vascular resistance of the

arteries, ultimately causing an impedance mismatch and increased velocity wave reflections (81). Cerebral arteries may change diameter and alter vascular tone to match the new flow rate over time, and minimise this impedance mismatch and thus velocity wave reflections (82). This vascular remodelling may account for the observed reduction in RI between 8-24 hours in normal neonates.

1.3.6 Near-Infrared Spectroscopy (NIRS)

Near-Infrared Spectroscopy (NIRS) is a recent development in non-invasive cerebral oxygenation monitoring. NIRS is the process of transmitting light in the infrared spectrum into human tissue and estimating the perfusion of that tissue based on the proportion of light absorbed. NIRS is only possible because in the near-infrared red region between 650 and 900nm there is a range of wavelengths in which photons can penetrate human tissue to interrogate deeper structures such as the cerebral cortex (83, 84). The depth to which the tissue is penetrated by the photons depends on the physiological parameters of the tissue being interrogated: human tissue is composed of substances which in turn consist of chromophores, pigmented compounds that absorb the near-infrared light as it passes through the tissue. The chromophores give those substances well-known and well-defined absorption spectra or characteristics that can, as is the case of haemoglobin, depend on their oxygenation status (85).

NIRS is achieved with a set of near-infrared light emitters and a corresponding set of light detectors. The amount of light which is reflected and not absorbed relates to the concentrations of certain compounds including oxygenated Haemoglobin (oxy-haemoglobin, HbO) and reduced, de-oxygenated Haemoglobin (deoxy-haemoglobin, HbR) in the tissue through which the light has travelled. A NIRS device can estimate the concentrations of HbO and HbR (and thus also the total haemoglobin HbT, which correlates to CBV) from the amount of light received at the detectors. Some NIRS devices present the actual concentrations of HbO and HbR, some provide information regarding the change in HbO and HbR and still others offer an index or percentage of the oxygenation of the tissue based on the amount of light absorbed. All NIRS devices use sources which emit light in the near-infrared spectrum and detectors which detect light in the near-infrared spectrum and have algorithms based on the Beer-Lambert Law, which relates the absorption of light to the properties of the material through which the light is travelling (86).

The Beer-Lambert law states that the concentration (c), of a substance in a sample can be determined from measuring the attenuation of the light as it passes through the sample if the extinction coefficient (α), the rate of light asorbance for the substance at that wavelenth, and the

thickness (d) of the sample is known. This Beer-Lambert Law for one substance is given by Equation 1-5 where $\frac{I_0}{I}$ is the attenuation; the ratio of incident light to emergent light (87):

$$\frac{I_0}{I} = \alpha \cdot c \cdot d$$

Equation 1-5 Beer-Lambert Law for one substance.

Moreover the Beer-Lambert Law is linear; each component substance absorbs independently of all other component substances and the total amount of light absorbed is therefore the addition of the absorbance of all the constituent components.

In the practice of using NIRS to measure the perfusion of human tissue, the measured attenuation of the light is dependent on a complex interaction of factors including detector and transmitter geometry, the shape of the surface on which those photodiodes are placed, and the scattering and absorption properties of the components of the tissue (83). Human tissue is a highly scattering medium, approximately 80% of the total attenuation of near-infrared light in tissue is due to scattering and only 20% due to absorption (88), and therefore the absolute attenuation of light in tissue due to absorption cannot be determined given the majority of the light is lost due to scattering (86).

The Beer-Lambert law may be modified to take into account the complexities of human tissue. Due to the scattering, the photons travel much further than the direct geometrical path length; the Differential Pathlength Factor (DPF), a scaling factor, is introduced to account for this. It is a function of tissue type, wavelength and the geometry of transmitting and receiving photodiodes (88).

$$Attenuation = \log\left(\frac{I_0}{I}\right) = \alpha \cdot c \cdot d \cdot DPF + G \quad (83)$$

Equation 1-6 Modified Beer Lambert Law

G is a constant representing the loss of attenuation due to other factors, and relates to the geometry and scattering of the tissue under investigation (88). As G is unknown and dependent on the geometry of the photodiode placement, it is does not allow an absolute calculation of the attenuation and thus the concentration of a chromophore. However, if a system of equations can be constructed with one equation for each chromophore considered, and G is assumed constant for all chromophores in the medium, then G may be ignored (88). Moreover, as the DPF and geometrical distance (d) remain constant during a measuring period, changes in the concentration

of a chromophore may be monitored by observing the changes in light attenuation without determination of the DPF (88).

There are several methods of using Near-Infrared Spectroscopy to measure perfusion. Most commercial NIRS monitors are *Continuous-Wave (CW)* instruments that use continuous light of several wavelengths and the modified Beer-Lambert law, thereby formulating and solving a system of equations, one equation for each chromophore, to measure changes in concentration of the substances under investigation. NIRS devices utilising up to 5 separate wavelengths,such as the ForeSight Elite (CAS Medical Systems Inc., Branford, Connecticut, U.S.A) are now commercially available. Theoretically, the use of more wavelengths should result in increased accuracy, due to the ability to account for chromophores other than just HbO and HbR being present in the sample. CW spectroscopy has a fast response time but only allows changes in concentration to be measured because it estimates, rather than calculates, the DPF.

Spatially Resolved Spectroscopy (SRS) is a sub-group of the CW spectrometry; light is still emitted from the photodiode continuously but the detectors are located at multiple, varying distances from the transmitting photodiode (89). There are two main methods of approaching this multi-distance spectroscopy. The first method is to have the detectors in close proximity to each other but at a distance of several centimetres from the emitter. As the inter-detector distance and intra-detector attenuation are known, the DPF may be estimated from the Beer-Lambert equations using mathematical regression. This method relies on the assumption that the tissue is homogenous, and for an accurate measurement to be made the distance between the light source and sensors must be maximised so that this assumption becomes proximally true; the distance is large enough that the scattering distribution becomes isotropic and the loss due to scatter is the same at all the sensors (85). As this distance may be greater than 3cm, this is not always feasible in the measurements made on neonates (85). This is the method implemented by the devices manufactured by Hamamatsu, the NIRO-200NX™ and NIRO-300™ where the distance between the emitter and the two detectors is between 3-4 cm (the two detectors are separated by 8mm) (90). Both Hamamatsus use the estimation of the *DPF* to calculate HbO and HbR concentrations and display this as a Total Oxygenation Index (*TOI*) where:

$$TOI\ \%\ (= rScO_2\%) = \frac{HbO}{HbT}\ x\ 100\ \%$$

Equation 1-7 Total Oxygenation Index (TOI) or Regional Cerebral Oxygen Saturation $rScO_2$

The INVOS™(Somanetics, Troy, Michigan, U.S.A) uses a simplifed form of SRS (91); two detectors are located in close proximity (1cm), with an emitter placed at a proportionally larger distance from those detectors (3 & 4 cm in the adult sensor) . The INVOS™ utilises their own proprietary algorithm, calibrated on the assumption that the there is a 75:25 split of venous and arterial blood in the cerebral tissue(92). A comparison study of the INVOS™ 5100, NIRO-200NX™, NIRO-300™ demonstrated that their respective "absolute" mean values could be very different, and the corresponding reproducibility of the measurements was between 4-5% for all three devices (90).

The second method of approaching the multi-distance spatially resolved spectrometry is that used by the ForeSight Elite (CAS Medical Systems Inc., Branford, Connecticut, U.S.A).Two detectors are separated by several centimetres, with one in close proximity to the emitter, the other at a distance of several centimetres. The light measured at the detector nearest to the emitter has only traversed shallower regions, such as the skin and skull in the case of the head, whereas the distal detector receives light that has penetrated deeper into tissue; subtraction of signal from the proximal detector from the signal from the more distal detector theoretically provides a measurement of the regional saturation of the deeper tissue due to the linearity of the Beer-Lambert Laws (93).

Time-of-Flight (TOF) or Time-Domain Near-Infrared Spectrometry (TD-NIRS) instruments emit a pulse of very short duration, of the magnitude of pico-seconds, into the tissue and detect the reflection of that pulse (88). As the method requires a pulsed laser capable of emitting a pulse of such short duration and a detection system sensitive enough to detect the photons reflected from that pulse, coupled with the temporal resolution to be able to calculate the delay between the incident and reflected pulses, the equipment required for *TOF/TD-NIRS* measurements can be bulky and expensive, inhibiting its use in many situations.

In *Frequency Domain Near-Infrared Spectrometry (FD-NIRS)* the emitted light is modulated, therefore the AC and DC attenuation and the phase shift between the incident and reflected light is used to calculate the absorption and scattering coefficients, and so the DPF can be calculated directly. As the incident and subsequent emergent light is not of as short duration as in the *TOF* spectrometry, the equipment is smaller and less expensive and therefore more accessible. FD-NIRS allows the

absolute quantification of HbO,HbR and HbT, and thus also the ratio of HbO to HbT the regional cerebral oxygen saturation ($rScO_2$[1]) (94).

NIRS relies on either a calculation of the Differential Pathlength Factor (*DPF*), a measurement of or an estimation of it. *TOF* and *FD-NIRS* spectrometers calculate the *DPF* directly making absolute measurements of chromophore concentrations possible. Standard *CW* instruments will use proprietary or published data; several studies have measured the *DPF* in both neonatal and human subjects using *TOF* or *FD-NIRS* methods (83, 95-97). The *DPF* depends on both the composition of the tissue and the wavelength used; the *DPF* is approximately 4.99 in the neonatal brain at 807nm (87). The DPF is influenced by alterations in the tissue such as changes in water concentration and changes in geometry, so real-time measurements of DPF are preferable.

Calculation of absolute values of Cerebral Blood Volume (CBV) and Cerebral Blood Flow (CBF) can be made using NIRS, by manipulation of the subject's fractional inspired oxygen (FiO_2), monitoring the change in concentration and using the Fick principle (88). Manipulation of inspired oxygen is feasible only when/if the infant is ventilated, and this may not always be the case. Not only does the inspired oxygen need to be altered, but the CBF and the cerebral metabolic rate of oxygen must remain constant during the measurement, and this will not be the case if the neonate is unstable. Alternatively, the CBF may be calculated using the Fick principle and NIRS, by the injection of indocyanine green, but as this is an invasive technique which is undesirable (88).

Near-Infrared Spectroscopy (NIRS) devices are able to monitor the cerebral oxygenation of neonates using light in the near-infrared spectrum: and has been employed to indicate the cerebral oxygenation neonates, since the mid-80's (98). Normal measurements of $rScO_2$ using NIRS in neonates without any known pathology is between 65 and 80% (99). In a study of preterm (GA < 32 wk) neonates, $rScO_2$ had a parabolic relationship with general anaesthesia from 0 to 72 hours, peaking at 36 hours; female participants had a lower $rScSO_2$ compared to their male counterparts (100). A longer study of very preterm (GA <32 wk), preterm (GA: 32-37 wk) and term (GA ≥ 37 wk) infants reported a decrease of $rScO_2$ from Day 1 to Day 7 and no difference between left and right, frontal or occipital region, suggesting uniform cerebral oxygen saturation in normal neonates (101). $rScO_2$ is influenced by arterial saturation, CBF, CBV and cerebral oxygen consumption (99).

[1] NIRS monitors use different terminology to indicate the ratio of HbO to HbT in the tissue which includes TOI, rSO_2 and $rScO_2$. The latter is adopted to describe the regional cerebral oxygen saturation for the remainder of this thesis for consistency, and ease of comparison of results independent of the NIRS device used to obtain those results.

Large variations in $rScO_2$ measurements (101), differences between NIRS devices and sensors have been previously reported (91, 102-106).

Brazy et al (107) initially recommended the use of NIRS monitoring in 1985 after observing alterations in the $rScO_2$, and cytochrome oxidase (OxCCO), measured using NIRS, during and after hypoxic episodes in sick pretrum neonates. Wyatt et al subsequently confirmed Brazy et al's findings in 1986 (108). Livera et al (109) realised that spontaneous or induced falls in oxygen saturation (SaO_2) of 5-10% resulted not only in a fall in HbO but also a rise in the HbT in a study of 25 neonates in 1991: a correlation to an increase in CBV, demonstrating compensatory vasodilation.

Since the early 90's, *CW-NIRS* has become commonplace and there are several commercial units available for normal clinical use. Numerous studies demonstrate their ability to indicate alterations in brain perfusion. In a study of neonates of greater than 34 weeks' gestation delivered by Caesarean section, Pichler et al proved that monitoring of cerebral oxygenation was possible during transition and immediately postpartum (110). In doing so they reported that neonates in need of respiratory support at this time exhibited lower $rScO_2$ in the first 10 minutes than those neonates who had not required such support (110).

1.3.7 Diffuse Correlation Spectroscopy (DCS)

Diffuse Correlation Spectroscopy (DCS) is a new technique developed for the measurement of blood flow without needing to introduce any contrast. DCS provides a measure of tissue perfusion based on the movement of blood cells inside the tissue (111, 112) and has been proven to provide a reliable, non-invasive assessment of CBF (113, 114).

1.3.8 Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) can be used to detect hypoxic-ischemic injury to the brain. MRI for neonates was developed in the mid-1980's (67). Significant difficulties had to be overcome to accommodate the neonate. The neonatal brain has a very high water content and so neonatal-specific coils and sequences for optimal neonatal imaging is required (115). Also, protocols had to be established to allow neonates to be placed safely in a scanner to allow the relatively long scan time (30mins-1hr). Even now, neonates are not usually scanned if they are in a critically ill state, mechanically ventilated or cooled. This limits the MRI diagnostic and predictive capability to post-recovery periods, where there is little to no influence over the outcome.

1.4 CURRENT CLINICAL INDICATORS OF HIE

Although birth asphyxia was acknowledged by Little in 1862 as a condition that may result in cerebral palsy, diagnoses and prediction of outcome was restricted to observation-based neurological assessment and preliminary electroencephalogram studies until the 1980's (116). Since then the EEG study has emerged as a field in its own right, as new methods for both the diagnosis and prediction of outcome of infants suffering from HIE continues to develop extensively, the ultimate goal being able to pinpoint the aetiology of the disease and predict long-term outcome accurately.

The primary risk factor for HIE is a clinically recognised sentinel event such as antenatal haemorrhage (often placental abruption), uterine rupture, or cord prolapse which has compromised oxygen supply (6, 117). Although an event offers a clear explanation of the encephalopathy, sentinel events are identified in only 8-15% of infants diagnosed with NE (117-120).

Volpe describes a myriad of destructive processes including excitotoxicity, neuroinflammation, apoptosis, free radical production, seizure activity, blood-brain barrier disruption, blood vessel leakage, cerebral thermo-pooling amongst numerous others causing damage to brain tissue in an ischemic-reperfusion episode (121). These processes result in a neurological syndrome of encephalopathy which is manifests itself clinically in many ways including stupor, coma, seizures, Apgar scores of less than 3 at 5 minutes, umbilical cord blood gas acidaemia and/or foetal bradycardia (65, 66, 122).

Nelson and Ellenberg considered foetal bradycardia of 60 bpm or less, a five-minute Apgar score of 3 or less, or a first cry more than five minutes after birth as clinical indicators suggestive of asphyxia (18). Criteria proposed by the American College of Obstetricians and Gynaecologists that are indicative of an asphyxial insult intra- or near intra-partum and are non-specific for an asphyxial insult include (123):

- a sentinel hypoxic event occurring immediately before or during labour, including but not limited to ruptured uterus, placental abruption, umbilical cord prolapse, maternal cardiopulmonary arrest and foetal exsanguination from either vasoprevia or massive fetomaternal haemorrhage
- a sudden and sustained foetal bradycardia or the absence of foetal heart rate variability
- Apgar scores of 0-3 beyond 5 minutes

- Onset of multisystem involvement within 72 hours of birth; the clinical manifestation of the redistribution of CO

The American College of Obstetricians and Gynaecologists and the International Cerebral Palsy Task Force put forward four prerequisites for the identification of an intra-partum hypoxic event sufficient in severity to cause cerebral palsy (123), these being:

- Evidence of a metabolic acidosis in foetal and umbilical and arterial blood obtained at delivery (pH less than 7 and base deficit of at least 12 mmol/L)
- Early onset of severe or moderate encephalopathy in infants born at 34 or more weeks of gestation, where NE is a clinically defined syndrome of disturbed neurological function determined by neurological examination and characterised by abnormalities in:
    - cortical function characterised by lethargy, stupor, or coma
    - brainstem function (pupillary and cranial nerve abnormalities)
    - tone (hypotonia)
    - reflexes (absent, hyperreflexia)
- Cerebral palsy must be of the spastic quadriplegic or dyskinetic type as these are the only types associated with acute intrapartum events
- Exclusion of other identifiable aetiologies

1.5 THERAPEUTIC HYPOTHERMIA (TH) FOR HIE

Therapeutic Hypothermia (TH) is currently the only post-insult treatment clinically approved to treat HIE (8). Several prominent studies including the Cool-cap and TOBY studies (9, 10) have demonstrated that TH treatment, where the neonate is cooled by 3-5^{o}C following an hypoxic-ischemic event, may reduce mortality and improve long-term outcome.

Gluckman et al, known as the CoolCap study group, conducted the CoolCap trial, a multicentre randomised clinical trial with participants randomly assigned to either head cooling for 72 hours within 6 hours of birth with rectal temperature maintained at 34-35^{o} Celsius, or conventional care. The 234 term infants enrolled in the study were diagnosed with moderate to severe NE, and primary outcome was death or severe disability at 18 months. There were follow-up data for 218 of the 234 infants; 66% of those receiving conventional care had died or had severe disability compared with 55% of those receiving head cooling. Predefined sub-group analysis suggested that head cooling had no effect on infants with the most severe aEEG changes. The CoolCap study

group concluded that TH could safely improve survival without severe neurodevelopmental disability in infants who had exhibited less-severe aEEG changes (10).

The TOBY trial conducted by the group led by Azzopardi et al was similarly a multicentre randomised trial and compared whole-body cooling to conventional care (9). Results from the TOBY study group demonstrated that the positive predictive value (PPV: the proportion of positive results which are true positives) of a severely abnormal aEEG assessed by the voltage and pattern methods was reduced from 0.63 and 0.59 respectively in the non-cooled group, to 0.55 and 0.51 in the cooled group of infants (124). Although this reduction was not significant, it was consistent with observational studies showing that cooled infants have a higher statistical chance of a good outcome than do their non-cooled counterparts.

Shankaran et al conducted a non-randomised trial of 108 infants and similarly reported a reduced PPV associated with death or severe neurological disability in the cooled versus non-cooled group, with a PPV of 0.51 reported in the cooled group of infants versus a PPV of 0.62 in the non-cooled group (125).

Recently, Gane et al considered the effect of TH both on the DNA and neurodevelopmental outcome. The cell death in the phase of secondary energy failure is thought to be due in part to oxidative stress caused by the free radicals and this should be observable as damage also to the DNA. The results of the study found that the levels of 8-hydroxy2-deoxyguanosine (8-OHdG), the most important by-product of oxidative DNA damage, were significantly higher in the control group than in the group receiving TH indicating greater DNA damage in the control group. The control group also had significantly lower motor and mental development quotients at 12 months (126).

Thoresen et al continuously monitored neonates with HIE during cooling, while cooled, and during rewarming, and discovered that the mean arterial blood pressure (MABP) increased by a median of 10 mmHg during cooling, and fell by a median of 8 mmHg on rewarming (127). They also found that the heart rate decreased by a median of 34 beats per minute on cooling and increased by a median of 32 beats per minute on rewarming.

1.6 OUTCOME PREDICTORS FOR HIE

1.6.1 EEG/aEEG

In 1972 Monod et al first reported the correlation between the EEG in the first days of life and the long-term outcome (128). In a study including 160 full term neonates, poor prognosis

corresponded to inactive or paroxysmal EEG's or EEG's of low voltage with theta rhythms within the first week, or abnormal activity such as slowness or low voltage or abnormal superimposed activity such as abnormal anterior activity or fast spikes occurring beyond the second week.

Sarnat and Sarnat (66) also monitored the neurological status of the hypoxic neonate. They recorded the EEG as soon as possible following admission, again at 24 hours and then every 3-5 days subsequently, and where possible, samples were taken both in a wakeful state and during sleep. The EEG during Stage 1 was interpreted as normal in wakefulness, Stage 2 was characterised by voltage depressions of 5-25μV in the low-theta (4-6Hz) down to delta (0.5-4Hz) frequency ranges, and limited activity in the upper theta (6-8Hz) to alpha (8-13Hz) frequency ranges. High amplitude bursts or periodic activity of 1-3 seconds alternating with low amplitude delta and theta (4-8Hz) phases of 3-6 seconds was also observed in Stage 2. These bursts were of lower voltage and less frequent in Stage 3, with delta and theta phases increasing to 6-12s. The delta and theta phases were depressed to the point of isopotential or flat trace in some cases. Further progression into Stage 3 saw the EEG depressed to the point of consistent isopotential or complete absence of electrical activity. Focal motor seizures presented as 1-1.5Hz periodic sharp and slow wave complexes of one hemisphere; the focus sometimes shifted to the other hemisphere a few minutes later. Recovery from Stage 2 was indicated by the commencement or return of continuous non-periodic cortical activity during wakefulness. Those infants whose recovery was delayed more than 5 days exhibited low-voltage continuous delta and theta activity afterwards. In some cases where an isopotential EEG had been observed, this persisted during sleep even after recovery.

Later studies include Tharp's study (1989) of 81 newborns of low birthweight where 41 neonates had EEG's with burst suppression patterns and of those 85% had a very poor outcome, including 8 deaths (129). Menache et al reported from a study of 43 infants who had excessive discontinuous brain activity patterns, that a predominant interburst interval of more than 30s heralded either death or severe neurological disability in all cases, and 86% of those surviving subsequently developed epilepsy (130). Of the 7 severely depressed infants with abnormal EEG's in the study conducted by Pressler et al, the 3 in which the EEG recovered within 12-24 hours had normal outcomes, whereas the 4 whose EEG's deteriorated to severe background abnormalities either died (n=2) or developed major neurological sequelae (n=2) (131).

The EEG may also be utilised to measure the brainstem-evoked responses of the neonate. Evoked responses may be elicited by auditory, visual or somatosensory stimulation. Abnormalities of the evoked response are expected after an hypoxic ischemic insult due to the neuropathological involvement of the cochlear nuclei, the inferior colliculus and other brain stem nuclei, and the

cochlear itself (6). The visual evoked potential (VEP) has accurately predicated abnormal outcome (100%) in both a study of 25 term infants by Whyte et al (132) and in a study by Muttitt et al (133) of 36 post-asphyxial infants.

The 18 electrode EEG recording is useful for identifying abnormal brain activity and also in confirming brain death in infants where electro-cerebral silence persists for more than 72 hours (134). It also provides some benefit to the prediction of long-term outcome in neonates with encephalopathy, but although a normal EEG or the recovery to normal of the EEG to a normal pattern within 24 hours is associated with a good outcome, the converse does not always hold true. In a study by Monod et al, 17 infants with absent electrical activity died but 2 survived, 1 who was considered normal at 6 years of age, and the other had epilepsy which could be considered as only a minor neurological sequelae (135). Ancona et al found that out of 10 neonates where the EEG was abnormal at 24 hours of life, only 4 developed a long-term adverse outcome whereas the 2 with normal EEG's developed normally (136). Thus, a normal EEG has a good negative predictive value, but as an abnormal EEG may subsequently have no significant neurological sequelae, abnormal EEG has lower positive predictive value of a poor prognosis.

During a study of 46 neonates monitored with aEEG during and immediately following Caesarean section, Pichler et al reported that the V_{min} (the mean minimum voltage amplitude measured for each minute) for the 3rd minute, and the V_{max} (the maximum voltage amplitude determined for each minute) for the 3rd and 4th minutes were higher compared to the 10th minute post-partum in neonates who required respiratory support (110). This was the first study to use aEEG and NIRS to monitor cerebral activity and oxygenation of neonates during the transition and immediately post-partum, and in doing so proved that this was possible.

Toet et al studied 68 asphyxiated infants for the period of 3-6 hours after birth and found that 16 who had continuous low voltage, burst suppression or a flat trace either died (n=9) or developed a major handicap. One infant whose pattern changed from continuous low voltage to flat trace and another whose pattern altered from discontinuous low voltage to burst-suppression also had major handicaps at follow up. Their study concluded that it would be feasible to use the aEEG in the first 6 hours of life to identify the infants who would benefit from intervention (137).

HIE studies now commonly define the severity of the HIE by the aEEG. Wintermark et al categorised their HIE subjects based on a moderately or severely abnormal aEEG: in their study, none of the babies with an initially moderately abnormal aEEG developed significant hypoxic-ischemic HI brain injury (21). TH treatment affects the aEEG trace and thus also predictive value

of the aEEG. In one randomised trial of hypothermia treatment by Thoresen et al, seventy-four neonates were monitored with aEEG for 72 hours (127). The time for the aEEG trace to return to normal in the hypothermic group was on average longer than that for the normothermic cohort where a normal trace was established.

1.6.2 Doppler Ultrasound (DUS)

One of the earliest investigations into the alterations to cerebrovascular resistance using Doppler Ultrasound in asphyxiated infants was conducted by Bada et al in 1979 (75) in which they interrogated the Anterior Cerebral Artery (ACA). The RI was significantly lower in the ACA in the asphyxiated infants compared to the normal control group, mostly due to a higher end-diastolic blood velocity. This indicated a lower resistance to cerebral blood flow, or vasodilation. This phenomenon is the manifestation of the "luxury perfusion" syndrome referred to herein as hyper-perfusion (29). Furthermore, Bada et al noted in one case that the reduced RI persisted beyond the period of one week and the resolution of Respiratory Distress Syndrome (RDS). They concluded that the continuing vasodilation could be the result of impaired autoregulation, originally hypothesized by Lou et al (138).

The relationship between HIE and hyper-perfusion has been confirmed in several early studies. Hyper-perfusion, as determined by an RI of <0.55, was observed in 11 of 12 infants developing severe encephalopathy in the study conducted by Eken et al (1995), but this lowered RI was not evident until after 6 hours of age; the high diastolic flow associated with a low RI developed between 12-24 hours. Thus the RI was not predictive between 2-6 hours, but the positive predictive value (PPV) had increased by the 12th hour. The PPV of the RI pooled from all published studies of HIE at normothermia is 84% (95% CI 73%, 91%) (42).

Hypothermia treatment reduces the PPV of a low RI. The PPV in a study conducted by Elstad et al during hypothermia was 60% (95% CI 45%, 74%), less than the PPV from pooled normothermic studies. Vasoconstriction associated with hypothermia or the pharmacological effects of drugs given during TH may be the cause of this diminished predictive value or perhaps the hypothermia has no effect on the RI per se, but the neuroprotective effect of TH improves outcomes for those infants who would have had poor prognosis previously and so lessens the predictive power of the RI (42).

An RI of less than 0.55 is considered abnormal (42, 139) and although the RI may not be useful as a predictor in the first 6 hours of life, the subsequent alterations may, and a low RI at birth may

indicate a prior hypoxic injury, an injury acquired pre- rather than intra-partum, given the delayed decrease in RI in the Eken et al study (42).

There are several confounding factors when utilising the RI as an indicator of the cerebral perfusion. A patent-ductus arteriosus (PDA) will increase RI as end-diastolic velocity could be zero or even negative (42). Breast-feeding has been shown to reduce the RI and PI, and increase mean velocity (140) which may be the consequence of an increase in metabolic demand (141) or the increased cardiac output (CO) associated with sucking effort (142-144). No studies have been found that examine this relationship in HIE infants undergoing TH. This may be because they receive limited enteral nourishment, and thus of no significance, but should be considered when comparing the measurements with a normal cohort.

Moreover, the value of the RI and PI is not universally accepted; Couture et al insist that the blood velocities should be constantly preferred to the RI (or PI) as the RI alone is of poor value, as only measurements of velocities identify a low cerebral blood flow which may be associated with a normal RI (68). Other investigators also have found that the high cerebral blood velocity to be a better prognostic marker (44-47). Care must be taken when estimating CBF using cerebral blood velocities determined by Doppler, because in periods of hyper- or hypo-perfusion, CBF may be overestimated, as demonstrated in a piglet model by Thoresen et al (127). In addition, there may be errors introduced by the operator and the Ultrasound device, as described previously, that may affect the measurement of blood velocity, more so than the calculation of the RI or PI which uses a ratio of those velocities (71).

1.6.3 Near-Infrared Spectroscopy (NIRS)

In a recent study by Ancora et al (136) of 12 HIE neonates who underwent selective brain cooling, the infants with adverse outcomes exhibited much higher $rScO_2$ at 6, 12 and 24 hours of age suggesting that NIRS could be used as an early predictor of outcome. Similarly, Goeral et al also reported the corollary to the Ancora et al study, observing lower $rScO_2$ in infants with normal MRI's (145). In contrast, Shellhaas et al did not find that cerebral $rScO_2$ contributed to a predictive model: unexpectedly reduced systemic or peripheral $rScO_2$ variability was instead the predictor of adverse outcome (6). This finding might not be entirely unexpected: the $rScO_2$ was measured with different devices, differences between sensors and devices has been previously reported (91, 102-106).

Furthermore, Nicklin suggested in 2003 that the lack of quantification of the brain oxygenation using CW-NIRS was inhibiting the widespread introduction of NIRS as a clinical tool (146).

Monitoring aims to keep parameters within set limits, but a parameter not static between individuals does not allow the estimation of standard values, nor the definition of upper and lower limits. This lack of quantification also precludes proper comparison between at-risk neonates and normal neonates (98).

However, Benaron et al reported in 1992 that they could resolve the location of an intracranial bleed in a critically ill neonate to within 7mm using *TOF* spectroscopy (147). Quantification is therefore possible, but has not been feasible due to the complexity of TOF (98). This may have changed with the development and introduction of an FD-NIRS system as reported at the Proceedings of the SPIE, the International Society for Optical Engineering, by Franceschini in 1995 (148). The quantitative capability and accuracy was confirmed by Zhao et al in 2005 who concluded that FD-NIRS was able to provide a quantitative optical measurement of the infant brain (149). This was followed closely by Franceschini et al in 2007, whose results showed a close agreement between FD-NIRS and PET and SPECT measures while studying changes in $rScO_2$ and cerebral oxygen metabolism ($CMRO_2$) over the first year of life (94). Although PET and SPECT may be considered the gold-standard, FD-NIRS has the advantage that it does not involve ionising radiation and measurements may be made at the bedside.

More recently, the same group used FD-NIRS combined with DCS to study the changes in the cerebral oxygen metabolism ($CMRO_2$), CBF, CBV and cerebral oxygen saturation ($rScO_2$). Results showed that their indices of $CMRO_2$ ($CMRO_{2i}$) and of CBF (CBF_i) were significantly lower, and CBV and $rScO_2$ were significantly higher in neonates during TH than post-TH and against age-matched control values (150). Also, post-TH CBV was significantly decreased compared with values during TH, whereas the $rScO_2$ remained unchanged, conclusively showing that FD-NIRS provided information regarding the haemodynamics over and above that ascertained by measuring $rScO_2$ alone (150).

Therefore, the evidence suggests that to be able to measure and monitor cerebral haemodynamics quantitatively in neonates, a device that provides absolute values of haemoglobin content is required. CW-NIRS is the most common NIRS in clinical use today and the oxygen saturation measure (TOI, rSO_2, SO_2) which is presented by CW-NIRS devices is useful in the clinical monitoring of trends of oxygenation of the cerebral tissue. Nevertheless, the 'absolute' oxygen saturation measures as provided by SRS devices such as the Hamamatsu NIRO-200NX and INVOS 5100 are not reproducible, and have been shown to have wide variability between patients (90). In specific studies with neonates affected by HIE, the TOI has not been shown conclusively to provide useful information regarding the status of the cerebral perfusion. In contrast, Dehaes et

al demonstrated that the FD-NIRS combined with DCS can offer a more complete description of the cerebral haemodynamics at play (150).

1.6.4 Cerebral Blood Flow (CBF) and Cerebral Autoregulation (CA)

In non-asphyxiated newborns in the first 24 hours of life, there is a three-fold decrease in cerebral blood flow (CBF) at birth due to an increase in the oxygen content of the blood (151). In a normal neonate, CBF is coupled to brain function and metabolism, and so thereafter CBF will increase proportionally with cerebral metabolic rates in line with increased energy demands in the growing brain (151).

Studies have identified at least 3 cerebral autoregulation mechanisms at play to control CBF (152): cerebral pressure autoregulation which maintains constant CBF despite fluctuating cerebral perfusion pressures (CPP) and thus mean arterial blood pressure (MABP), flow metabolism coupling which varies CBF to match metabolic rate, and neurogenic regulation via perivascular nerves (152). Cerebral autoregulation is achieved by the vasodilation and vasoconstriction of the cerebral arteries (153, 154). The autoregulatory range of MABP for normal neonates is approximately 25-50mmHg, but this depends on several factors including gestational and postnatal age (6).

Brain-sparing invoked by a perinatal asphyxial insult to the foetus leads to neurogenic vasodilation and thus impairs normal cerebral autoregualtion (CA) (6, 99). The degree of impairment depends on the timing, severity, pattern and duration of the insult (6). Several neonatal animal studies have confirmed impaired CA with hypoxia and associated hypercarbia and acidosis (155-157), and a linear relationship between CBF and systolic blood pressure was established in asphyxiated neonates using Xenon clearance studies (138). Also, compromised reactivity of the CBF to the partial pressure of carbon dioxide ($PaCO_2$) has also been observed in asphyxiated full-term infants (6).

In a study of adults, two indices demonstrating the correlation between MABP and CBFV, and MABP and $rScO_2$ respectively were both sensitive to weaker autoregulation as a result of vasodilation associated with lower MABP or increased $PaCO_2$ (158). Impaired cerebral autoregulation as defined by the lack of reactivity to MABP and/or $PaCO_2$ has also been linked to the severity of the brain injury and poorer outcome in preterm neonates (6, 43, 159). Although increased CBF and subsequent increased CBV is reported in HIE neonates (47) there are no known published data focussing on the impairment of CA specifically in HIE neonates.

Therapeutic hypothermia reduces CBF by approximately 40% (160). Rapid rewarming has been associated with increases in cerebral metabolism that may be unmatched by CBF (161-163), seizures (163), increased glutamate and lactate levels (162) and reduced cerebrovascular responsiveness which may contribute to further neuronal cell damage (164). Although it is widely accepted that a rewarming rate of 0.5 degrees Centigrade per hour is safe, it may still result in an uncoupling of CBF and cerebral metabolism, thus loss of CA, and neonatal seizures (99).

Changes in CBFV as measured by DUS reflect relative changes to CBF where the insonated vessel diameters remain constant, thus monitoring the CBFV during spontaneous changes to MABP provides a measure of CA (165). CA may be assessed in the frequency domain by such methods as Transfer Function Analysis which quantifies by CA by the transfer function gain, or the phase coherence between MABP and CBFV (63, 165-168), or in the time domain using correlation analysis between MABP and CBFV (168).

There are significant challenges to obtaining a reliable estimate of CBV from the mean CBFV or TAV (Time-Averaged Velocity). Firstly a robust CBFV waveform must be obtained from a single, straight non-dividing cerebral artery with no angle deviation or with a known angle of insonation; the CBFV waveform must accurately represent the flow profile in the recorded frequency spectrum. Secondly, there must be an ability to remove the frequency shifts due to the slow moving vessel wall to allow an accurate calculation of mean flow velocity or TAV. Thirdly, the arterial cross-section area must be measured, and the arterial cross-sectional area in relation to the volume of brain tissue supplied by the artery must be determined (169). Only then can the CBF and CBV be estimated from the CBFV's.

There is growing interest in the use of NIRS as an alternative to measure CBF and CA (170-173). The cerebral intravascular oxygenation, as defined by Tsuji et al, is the difference between the concentration of Oxy-Haemoglobin (HbO) and Deoxy-Haemoglobin (HbR) as measured by NIRS, and is represented by HbD where HbD=HbO-HbR.. HbD was found to be well correlated with CBF determined by radioactive microspheres in a study of neonatal piglets (174). Tsjui et al also proposed the coherence between HbD and MABP as a measure of CA (175). Use of HbD relies on absolute measurements of Haemoglobin, which is only possible with frequency-domain or time-resolved near-infrared spectroscopy (89).

The regional cerebral oxygen saturation ($rScO_2$), the proportion of HbO to HbT, has been investigated as an alternative to HbD as a surrogate for CBF, and the use of a ratio eliminates the need for absolute Hb measurements (176). $rScO_2$ allows CBF to be estimated, provided that arterial

oxygen saturation is stable (177) and changes in HbT reflect relative changes to CBV (178). Wong et al confirmed that $rScO_2$ determined by a spatially-resolved NIRS correlated with CBF in frequencies <0.1Hz in newborns (179), and $rScO_2$ was also found to be highly correlated with an MRI measure of CBF (180). The coherence between $rScO_2$ and the MABP is an index of CA, with a high coherence indicating a pressure-passive relationship and impaired CA (6, 177). Good agreement has been reported between the CA as determined by CBFV via Doppler ultrasound and NIRS (158, 173, 181). A high gain between MABP and $rScO_2$, suggesting impaired CA, has been correlated with worse outcomes in premature neonates (170, 171, 175).

1.6.5 Cerebral Fractional Oxygen / Tissue Oxygen Extraction (cFOE / cFTOE)

The balance of cerebral oxygen delivery (CD_{O2}) versus cerebral oxygen consumption ($CMRO_2$) can also provide an estimation of the cerebral oxygenation, and is referred to as the Fractional Oxygen Extraction (cFOE) (182). CD_{O2} is the oxygen delivery to the brain so equals the arterial blood oxygen content (CaO_2) x CBF. Cerebral oxygen content consumption is the how much oxygen is consumed, so it is the difference in the arterial to venous blood oxygen content multiplied by the CBF: (CaO_2-CvO_2) x CBF. By making substitutions for CD_{O2} and $CMRO_2$, and then eliminating CBF, the formula for cFOE is given by Equation 1-8:

$$\text{cFOE} = \frac{\text{CMRO}_2}{\text{CD}_{O2}} = \frac{\text{CBF x (CaO}_2\text{−CvO}_2\text{)}}{\text{CBF x (CaO}_2\text{)}} = \frac{\text{CaO}_2\text{−CvO}_2}{\text{CaO}_2} \quad (183)$$

Equation 1-8 Cerebral Fractional Oxygen Extraction (cFOE): the ratio of cerebral oxygen consumption ($CMRO_2$) to cerebral oxygen delivery (CD_{O2})

Calculation of cerebral oxygen metabolism and cFOE have traditionally involved invasive methods. The measurement of the jugular venous oxygen saturation can be achieved via direct cannulation of the jugular bulb (184, 185). Measurements of CBF and subsequently $CMRO_2$ can be obtained via Xenon-133 clearance measurements (186) or Positron Emission Tomography (PET) (187). More recent, non-invasive measurements of $CMRO_2$ and cFOE in neonates involve partial jugular venous occlusion (188, 189).

Cerebral Fractional Tissue Oxygen Extraction (cFTOE) has been proposed as an alternative index of brain tissue oxygen utilisation which is determined non-invasively by the ratio of the difference in the arterial and cerebral oxygen saturation and the arterial oxygen saturation as shown in Equation 1-9 (178). cFTOE was found to correlate well with cFOE ($R=0.4$ and $p=0.016$) in a validation study in piglets (182) suggesting cFTOE may be a useful indicator of cerebral

metabolism. A normal range of cFTOE in newborns with intact CA has been reported as 0.2 to 0.3 (178).

$$\text{cFTOE} = \frac{\text{SaO}_2 - \text{rScO}_2}{\text{SaO}_2} \quad (182, 190, 191)$$

Equation 1-9 Cerebral Fractional Tissue Oxygen Extraction (cFTOE) determined by NIRS-derived $rScO_2$

A normal cFTOE indicates that CBF is satisfying and not exceeding brain metabolic needs, whereas a higher than normal cFTOE suggests reduced cerebral oxygen saturation, with cFTOE increasing to compensate for less available oxygen. In contrast, if cFTOE is lower than normal while CBF is maintained, this suggests a decreased oxygen extraction which means a lower cerebral metabolism and possibly cell death (188, 189). cFTOE was found to decrease until 36 hours prior to increasing again (100), and continue to increase to day 7, indicative of an increase cerebral metabolic demands (101).

1.6.6 Magnetic Resonance Imaging

The patterns of injury in neonates have not been linked definitively to the timing or severity of the injury as has been shown in primates. In an attempt to determine this relationship, Miller et al found that injury to the basal ganglia/thalamus, known as the BGT pattern of injury, was most associated with the more severe neonatal signs including intensive resuscitation required at birth, and severe encephalopathy and seizures. The BGT pattern was also associated with more impaired cognitive and motor outcomes at 30 months of age (192).

Hypoxic-ischemic injury may not be evident on MRI up to 4 days after the injury (193) and may change then change considerably during the first 17 days (194). In a study of 73 term neonates evaluated after 1 year of age, all infants with abnormal magnetic resonance signal intensity in the posterior limb of the internal capsule developed neurological impairments, although in 4 infants the injury was not apparent until 4 days after birth (193). If the MRI was conducted prior to 7-days post-injury, T1-weighted and first-echo T2 weighted sequences correlated best with 12-month neuromotor and mental developmental outcomes, whereas if the MRI was conducted after the 7th day, the first and second echo of the T2-weighted sequences were better correlated (194); this may be due to the oedema resulting in T2 prolongation.

1.7 OUTCOME MEASURES FOR HIE

1.7.1 MRI

The gold standard for determining hypoxic-ischemic (HI) injury to the brain is MRI. The pattern or specific regional distribution of HI injury is associated with different durations and severities of ischemia. In two studies in primates, Myers et al reported that partial asphyxia causes cerebral white matter injury whereas acute and prolonged asphyxia produces deep grey-matter nuclei injury primarily in the basal ganglia and thalamus (195, 196). Similarly in the term newborn there are two major patterns of injury consistent with HI injury detectable with MRI: the watershed (WS) pattern involving the white matter so called for its localisation in the vascular watershed region of the brain between the anterior and middle cerebral arteries, and the Basal Ganglia/Thalamus (BGT) pattern involving the deep grey matter in the basal ganglia and/or thalamus (6, 194, 197, 198). The WS pattern may extend to the cortical grey matter when severe, whereas the BGT injury may extend to the cortex. These patterns of injury consistent with HIE can be recognised with MRI, and lesions in the basal ganglia and thalami are better visualised with an MRI inversion recovery sequence than with CT (116).

1.7.2 Cognitive, Behavioural and Motor Development: The Bayley Scales of Infant Development

The Bayley Scales of Infant Development were developed by Nancy Bayley to measure development objectively, specifically in the areas of cognition, motor skills, and behaviour in infants (199). The Bayley Scales are frequently used as an indicator of long term outcome in studies of HIE (6, 150, 200-202). There are three editions known as the BSID (Bayley Scales of Infant Development, 1969) (203), the BSID-II (Bayley Scales of Infant Development-Second Edition, 1993) (204) and the Bayley-III (Bayley Scales of Infant Development-Third Edition, 2006) (205).

The BSID was designed for infants from 2-30 months of age, and consisted of three complementary scales: mental and motor scales, and infant behaviour record. The main purpose of the BSID was to establish normative data for the rapid developmental changes in this period. The mental scale measured cognitive abilities by assessing response to visual and auditory stimulation, perceptual discrimination, imitation and social communication, object permanence, memory, hand-eye co-ordination, problem-solving, classification, vocal ability and language skills. The motor scales assess gross motor co-ordination and fine motor skills. Behaviour during the tasks would be recorded as an infant behaviour record (199).

The BSID-II expanded the age range to 1-42 months and although it maintained the three scales, the mental and motor scales were extended, and the infant behaviour record was replaced with the behaviour rating scale. The BSID-II update reflected the change in purpose of the infant development scales from providing normative data, to a scale for measuring the development of high-risk infants, and so the BSID-II was considered appropriate for diagnosing development delay. A disadvantage of the BSID-II was that the normative sample consisted of very few low-scoring infants especially at the higher age ranges, skewing the scales (199).

In an attempt to be more representative of the range of functioning in the general population of infants and toddlers, the Bayley-III standardisation sample included more children with various risk conditions. The Bayley-III was also revised to include 5 scales: cognitive, language, motor, socio-emotional and adaptive behaviour. The first 3 are task-based items administered to the child, the behavioural elements are in the form of a questionnaire completed by the caregiver. The separate cognitive and language scales address the issues with poor language scores affecting the Mental Development Index (MDI) in the BSID-II, but there is no direct equivalent composite score in the Bayley-III. The scores are often presented as a standardised score or percentiles allowing better comparison between scales and populations.

1.8 MODELS OF CEREBRAL CIRCULATION AND HAEMODYNAMICS IN NEONATES

Quantitative modelling can assist us with a better understanding of complex physiological systems. There are several models of the adult cardiovascular system (206-210), where variables such as arterial and venous pressures, blood flow and blood gas concentrations or blood volumes are relatively accessible, but there are fewer models specific to neonatal cerebral circulation and cerebral autoregulation where these are less accessible (165) and the system is complex: cerebral circulation is influenced by many stimuli, both chemical and physical with varying timescales and multiple pathways (211).

The only known published model of neonatal cerebral circulation is by Greisen et al who, while investigating the difficulty of estimating CBF from Doppler Ultrasound, developed a simple electrical model of the haemodynamics of the newborn (169) by modifying the model systemic circulation of Spencer et al (212). Their model included three parallel coupled *RC* circuits, with *R* representing the resistance of the blood vessels and the capacitance *C* designating the arterial compliance. Two *RC* circuits represent the peripheral and cerebral circulations with a 3rd *RC* circuit representing the compliance of the ductus arteriosis and the aorta and arteries proximal to the DUS

measurement. Two *LC* circuits (Inductor-Capacitor in series) were included between the Ductal/Aortic circulation and the cerebral and peripheral circuits to model the impedances to arterial blood flow to the respective circulations. Greisen et al reported good correspondence between the drop in RI measured during 2 seizure episodes in an infant with HIE with their calculated CVR. They concluded that their computer simulated haemodynamic model supported the assertion that RI truly reflected changes in cerebrovascular resistance and the compliance of cerebral arteries and the aorta influence the RI significantly (212).

Banaji et al have proposed a general model of brain circulation and metabolism based on the previous mathematical models developed by Ursino et al for the study of cerebral haemodynamics and intra-cranial pressure (ICP) in adult patients with brain injury (213, 214). The cerebral circulation model of Banaji et al includes two sub-models, one for cerebral circulation the other for the mitochondrial metabolism, with the two structures linked by oxygen transport and consumption (215, 216). Inclusion of the metabolic processes allows for the metabolic demand for oxygen which influences the circulatory model through oxygen demand, and to allow closer study of the mitochondrial metabolism. The study of the cerebral circulation includes PaO_2 and $PaCO_2$ as inputs influencing CBF, with SaO_2 and the $rScO_2$ as outputs. Functional activation influences CBF and also the mitochondrial metabolism in the metabolic sub-model, which has cerebral metabolism ($CMRO_2$) and the change in cytochrome oxidase (ΔoxCCO) as outputs. Banaji et al offer their computational model of the cerebral circulation in an open-source modelling environment (217). Moroz utilised this modelling environment to construct a neonatal piglet model of brain energy metabolism and circulation during oxygen deprivation to aid interpretation of NIRS and magnetic resonance data (218-221).

The brain circulation and metabolism models of Banaji et al and Moroz et al were developed using a physiological approach, examining the underlying physiology and then attempting to represent it mathematically (215). Other mathematical approaches such as linear regression (222, 223) or transfer function analysis (165, 224, 225), commonly used for the analysis of cerebral autoregulation, may be able to reproduce specific experimental results but do not provide an explanation of the non-linear physiological processes producing the effect (215).

1.9 FORMULATION OF HYPOTHESES

The Summary Proceedings from the Neurology Group on HIE concluded that treatment strategies for HIE will likely depend on the severity of the findings during the initial examination (3). The initial examination though does not take into account the ensuing cascade of excitatory and oxidative mechanisms, the evolution of the encephalopathy and hyper-perfusion. The HIE related hyper-perfusion has been linked to the severity of the HIE and prognosis (43). This research project aimed to determine markers of that increased cerebral perfusion and determine their reliability in predicting a poor outcome in HIE infants. The identification of a cerebral perfusion maker that could reliably predict outcome would allow those infants with a poor prognosis to be targeted for modified TH treatment or adjuvant therapies.

Amplitude-integrated EEG is the best predictor of neurological outcome following HIE in term neonates (124), many studies already use the aEEG as a way of classifying the severity of HIE in the first 6 hours. Although a correlation between the aEEG and outcome has been shown in neonates who are not cooled (124), it has also been seen that the induction of hypothermia disturbs the normal physiological patterns and in doing so also affects the electrical activity of the brain and the aEEG, reducing not only its predictive but also its diagnostic value.

Wintermark et al observed that infants developing brain injury despite being treated with TH displayed initial hypoperfusion on day one of life followed by prolonged hyper-perfusion on Day Two and Day Three (21). The hyper-perfusion, following the initial period of hypoperfusion, may last several hours or days. CBFV's have been used as surrogate measure of CBF and increased CBFV's have been correlated to the severity of the HIE (44-46). The Resistive Index (RI) as determined from the cerebral blood flow velocities (CBFV's) is an accepted indicator of the resistance of the cerebral arteries and an RI < 0.55 is suggestive of a prior hypoxic-ischemic event.

The first proposed hypothesis of this research project is that the RI is a reliable marker of altered cerebral perfusion marker and indicates the severity of HIE during TH. The alternative hypothesis is that, by calculating the RI as the ratio, information from the CBFV's is lost and derangements in the cerebral blood flow velocities (CBFV's), rather than the RI, more reliably predicts poor outcome in HIE infants. Regardless, there are draw-backs to using DUS measurements as cerebral perfusion markers as they are one-off, non-continuous measurements and require an expert, skilled operator to perform.

Near-Infrared Spectrometry (NIRS) monitors cerebral oxygenation, how well delivery of oxygen via CBF is matching cerebral oxygen demand. Changes to CBF and CBV have been indicated by

changes to NIRS parameters in infants previously (150). Whereas determination of the cerebral vascular resistance using ultrasound requires a skilled operator, and is thus restricted to one-off measurements, NIRS holds the promise of *continuous* monitoring of cerebral oxygenation, which is an indication of whether cerebral perfusion is matching demand. If NIRS is able to offer surrogate measures of CBF and CBV, this could offer reliable continuous markers of increased cerebral perfusion sought.

NIRS measures the concentrations of HbO and HbR in the interrogated brain tissue. The ratio of HbO to HbT (HbT=HbO+HbR), is known as the regional cerebral oxygen saturation $rScO_2$. Infants with HIE are treated with hypothermia treatment, which slows the metabolism. This will likely result in an increased $rScO_2$ ratio regardless of whether there is hyper-perfusion or not. Hyper-perfusion resultant from HIE occurs despite the cooling, which would be reflected in a higher than normal CBF and hence higher than normal HbO concentration. Thus it is the HbO, rather than than the $rScO_2$ which is expected to have a greater correlation with the increased perfusion, and the severity of the HIE. HbO could therefore be a marker for the increased cerebral perfusion. This forms the basis for the second hypothesis for this study; that HbO will be the NIRS parameter that most correlates with the degree of hyper-perfusion, and therefore the severity of the HIE. Cerebral perfusion markers for HIE are sought so that the infants at the highest risk of neurological disability or death can be identified during the TH treatment. If these infants with the the poorest prognosis can be identifed, then TH treatment could be adapted so that the obtain maximal benefit from the treatment, or they could be targeted for adjuvant treatments to improve long-term outcome.

1.10 HYPOTHESES

1.10.1 Hypothesis 1

The Resistive Index (RI) is a reliable marker of altered cerebral perfusion marker and indicates the severity of Hypoxic-Ischaemic Encephalopathy (HIE) during TH.

1.10.2 Alternative Hypothesis 1

Derangements in the cerebral blood flow velocities (CBFV's), rather than the RI, are more reliably able to predict the poor outcome in HIE.

1.10.3 Hypothesis 2

Oxy-Haemoglobin (HbO) as measured by Frequency-Domain NIRS (FD-NIRS) is a marker of cerebral hyper-perfusion in infants with Hypoxic-Ischemic Encephalopathy (HIE) during therapeutic hypothermia (TH).

1.10.4 Alternative Hypothesis 2

Oxy-Haemoglobin (HbO) in infants with Hypoxic-Ischemic Encephalopathy (HIE) undergoing therapeutic hypothermia (TH) is a consequence of the hypothermia and not correlated to hyper-perfusion related to the HIE.

CHAPTER 2. MEASURING CEREBRAL BLOOD FLOW VELOCITY AND CEREBROVASCULAR RESISTANCE

Cerebral haemodynamics are expected to be disturbed in newborn infants with Hypoxic-Ischemic Encephalopathy (HIE) due to the physiological response to hypoxia, the brain-sparing effect and subsequent hyper-perfusion. To be able to quantify the disturbance in the HIE infants, normal cerebral haemodynamics in term infants needed to be established. Two cohorts of full-term neonates with no known pathology were examined using ultrasound to investigate the cerebral perfusion biomarkers that can be determined by Doppler Ultrasound in a normal population. The results of these two studies are presented in Chapter 3. Chapter 4.

The velocity of the blood in an artery can be measured using Doppler Ultrasound; when it is measured in a cerebral artery it referred to as a Cerebral Blood Flow Velocity (CBFV). CBFV may be measured in any of the cerebral arteries, although access to the Anterior Cerebral Artery (ACA) and Middle Cerebral Artery (MCA) though the anterior fontanelle and the transcranial window respectively make them ideally suited for blood flow measurements. The blood flow velocity is a continuous waveform with each cycle corresponding to a cardiac cycle, the highest point of the cycle is referred to as the Peak Systolic Velocity (PSV), whereas the lowest velocity at the end of the period of relaxation is referred to as the End Diastolic Velocity (EDV). The average velocity over the cardiac cycle is the Time-Averaged Velocity (TAV). The CBFV's are direct measurements of the velocity by Doppler Ultrasound and so may be prone to error if care is not taken to correct for the angle of insonation (74).

The indices of vascular resistance remove the angle dependency as they take a ratio of CBFV's. The Resistive Index RI (Equation 1-3) is the difference in PSV-EDV divided by the PSV. The Pulsatility Index, PI (Equation 1-4) also has the pulse pressure as the numerator but the denominator is the TAV. These indices are accepted indicators of downstream arterial vascular resistance and are currently used to confirm evidence of hyperaemia following an hypoxic event (71).

2.1 PREPARATION FOR UNDERTAKING CRANIAL ULTRASOUNDS

The first phase of the research project was to measure Cerebral Blood Flow Velocities (CBFV's), and establish a baseline for the Doppler indices of Resistive Index (RI) and Pulsatility Index (PI) in a normal cohort of neonates. Measurement of CBFV's using Doppler Ultrasound requires an

operator with appropriate knowledge, skills and experience to ensure accurate and reliable measurements. The gold standard for an operator would be a sonographer with two years of training and several years of experience. Although there are such appropriately trained people at the Mercy Hospital for Women in Heidelberg, resourcing restrictions would not allow for a sonographer to be available at call when the research was being conducted. Thus it became necessary for this researcher to become competent to measure CBFV's using DUS in neonates.

This presented the first challenge as there was no such organised or accredited course available for a non-medical, non-clinical person to train how to undertake cranial ultrasounds of neonates. An ultrasound training centre, known to the sonographers at the Mercy Hospital for Women (MHW), was based in Ivanhoe, not far from the hospital. The training centre, Ultrasound Training Solutions, ran a 2-day program specifically designed to provide instruction on how to conduct cranial ultrasounds (CUS) and accurately measure CBFV's using DUS. The program included practical sessions with an infant of 6 months of age. The 6 month old neonate proved challenging as the infant was mobile and the fontanelle was narrowing, but thus the infant served as very good practice.

The practice continued at MHW where the protocol at the Neonatal Intensive Care Unit (NICU) required that all infants of < 32 weeks undergo cranial ultrasounds at 3 days, 14 and 42 days. The author accompanied the sonographers from the Medical Imaging Department of MHW to assist with these cranial ultrasounds. The cranial ultrasounds would normally consist of 6 images coronally and five images sagitally to provide a detailed picture of the brain. Doppler would not normally be included in the examination unless there was an indication, for example, a suspicion of an episode of hypoxia. If a period of hypoxia was suspected then CBFV's would be measured in the ACA and the RI calculated. The sonographers' rounds of the NICU provided some scope for observation of clinical practice of the cranial ultrasound, and hands-on practice when CBFV and RI measurements were required.

The cranial ultrasounds in the control studies would be undertaken by the author and the Head Sonographer of the Medical Imaging Department of MHW, Mrs Gabrielle Fedai. This both provided an additional forum for instruction and an opportunity to examine and review the agreement between the two sets of measurements recorded by both operators; to scrutinise the accuracy and reliability of the measurements and to verify this researchers's technique and competency.

2.2 SAFETY AND ETHICAL ASPECTS OF THE RESEARCH STUDY

The safety aspects of the use of ultrasound to interrogate the new-born brain must be considered and particular care taken during neonatal cranial ultrasound (CUS) as the developing brain is sensitive (226-228) and the energy imparted by ultrasound may induce thermal and mechanical effects in human tissue (229, 230). Pulsed DUS especially, used to measure the velocity of red blood cells in blood vessels, uses higher power than diagnostic ultrasound and so there is the potential for adverse biological effects (231).

Output display standards (ODS) have been adopted in preference to rigid exposure limits to allow sonographers greater flexibility to utilise higher frequencies and greater penetration for better resolution (232). ODS dictate that modern ultrasound systems display the Mechanical Index (MI) and Thermal Index (TI) to provide a real-time indicator to the operator of the relative potential for bio-effects whilst scanning (231, 232). The MI indicates the potential for a non-thermal, mechanical bio-effect whereas the TI is the indicator for the potential of tissue heating with TIS used for the thermal index for soft tissue, TIB for bone at depth and TIC for bone at the surface (228).

Guidelines recommend that examination times are kept as short as is necessary and outputs as low as is reasonably achievable (the ALARA principle) whilst still achieving a useful diagnostic result (228, 231). The British Medical Ultrasound Society provides detailed guidelines for neonatal cranial investigations which suggest MI < 0.7 and TIC < 3.0 is acceptable although TIC values between 0.7 and 3.0 should be time restricted as follows (228):

Table 2-1 Time restriction guidelines for neonatal cranial ultrasound investigations

	Thermal Index Value (TI)					
	0.0-0.7	0.7-1.0	1.0-1.5	1.5-2.0	2.0-2.5	2.5-3.0
Time Restriction	None	60 min	30 min	15 min	4 min	1 min

At the commencement of this research project, the ultrasound device employed for neonatal CUS examination in the NICU of MHW was a Philips HD11 XE Ultrasound System with a C8-5; 5-8 MHz Curved Array Transducer probe suitable for neonatal cephalic use. During a routine round to undertake the neonatal CUS in the NICU of MHW, employing this ultrasound machine with the settings as would be used for the research project, it was found that during the structural images the TIC (Thermal Index for Cranial bone) as displayed by the Ultrasound was 0.4 and during

Doppler measurements the TIC was 0.9. A CUS examination completed by a trained sonographer takes less than 5 minutes. Any additional DUS measurements, usually 1 set of 3 DUS measurements at one site was completed in less than 2 minutes by a trained sonographer.

In this research study, a structural CUS examination was completed by the head sonographer, Mrs Gabrielle Fedai, to preclude any pathology and took less than 5 minutes. There were 2 sets of 3 DUS measurements at each site: ACA and MCA undertaken by both this author/researcher and Mrs Gabrielle Fedai. The DUS measurements were completed in 5 minutes. Thus the full ultrasound examination for the research study was less than 10 minutes duration; this complies with the British Medical Ultrasound Society Guidelines, thus ensuring the safety of the study participants.

2.3 DOPPLER ULTRASOUND PROTOCOL

2.3.1 Anterior Cerebral Artery (ACA)

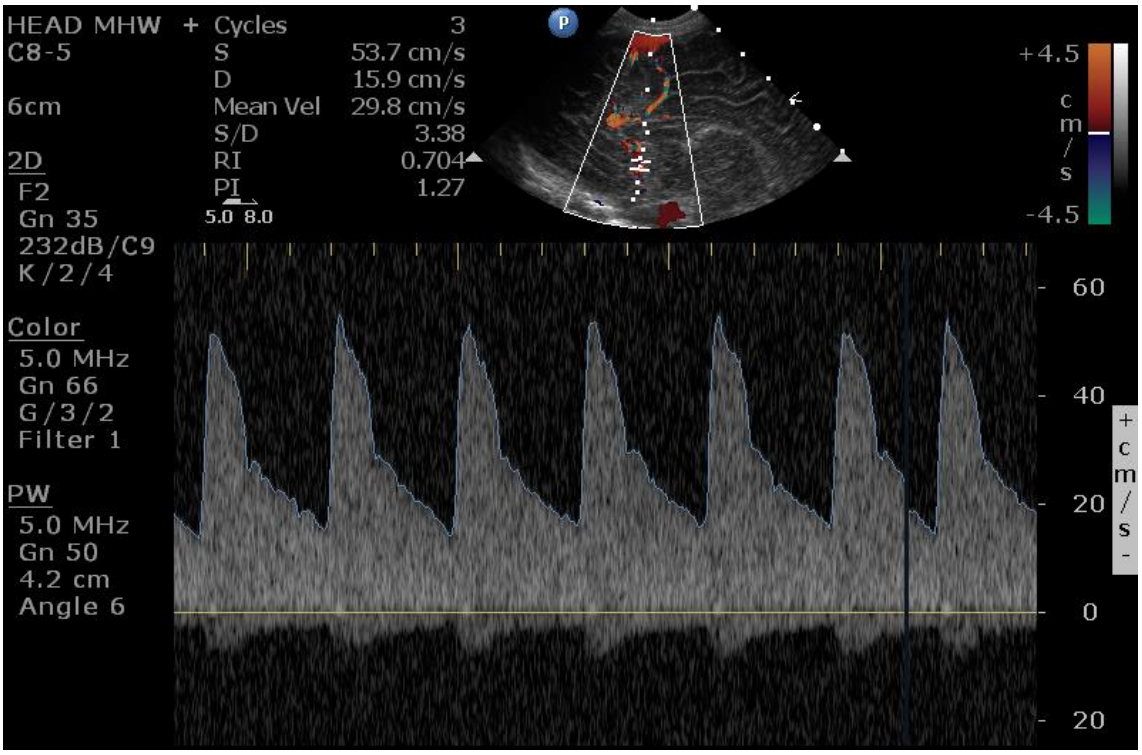
To obtain the CBFV's in the Anterior Cerebral Artery (ACA), the transducer was positioned in the anterior fontanelle of the infant in the sagittal orientation, the placement was then adjusted using the B-Mode imaging function. The midline was found by orientation with the midline structures; the corpus callosum and ventricles. The Doppler function is turned on whilst in sagittal orientation and the left and right branches of the ACA can be discovered by angling the probe slightly off midline. Measurements were made on the vertical (A2 segment) of the ACA to minimise the angle of insonation. Separation and identification of left and right branches was found to be difficult to distinguish and so identification and recording of the interrogated branch of the ACA was therefore not made.

2.3.2 Middle Cerebral Artery (MCA)

The Middle Cerebral Artery (MCA) was located through the temporal window. The MCA was interrogated proximal to its departure from the Circle of Willis and antecedent to any subsequent branching of the artery. The angle of insonation was minimised as much as possible by placing the Doppler gate on a section of the MCA with an angle as close to 0^{o} to angle of the probe as possible.

Examples of measurements of the CBFV's, RI and PI in the ACA and MCA are presented in Figure 2-1.

a)



b)

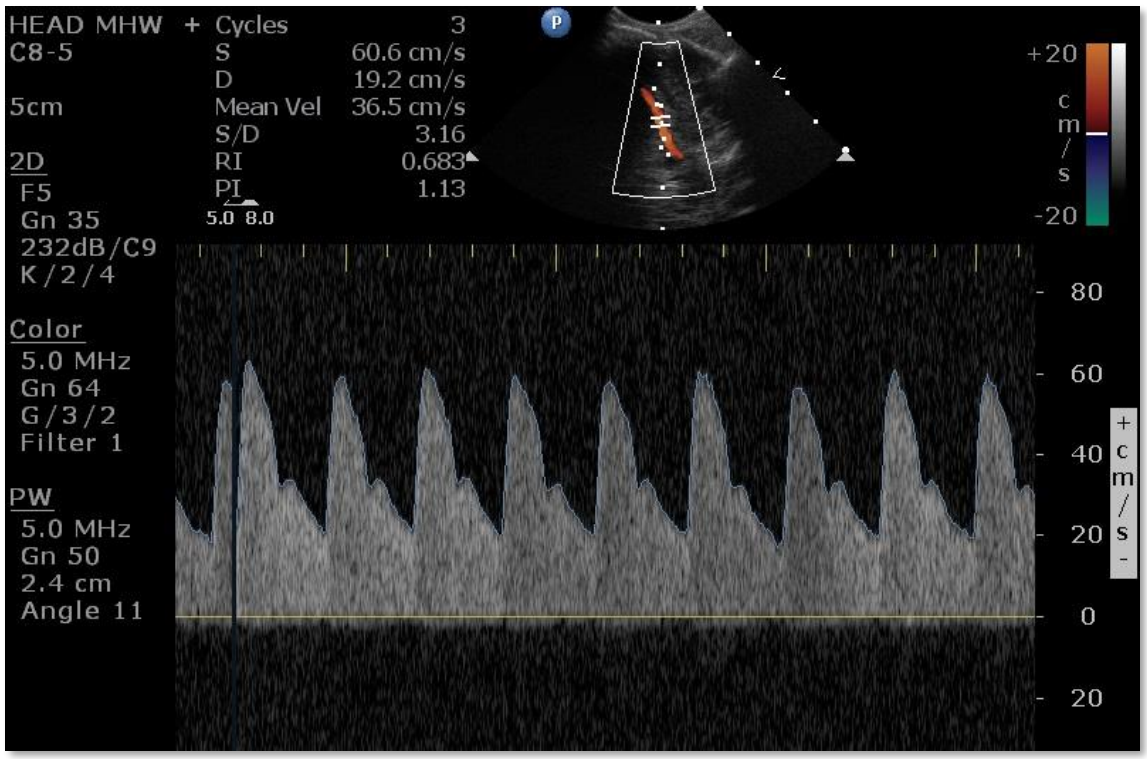


Figure 2-1 Demonstration of the measurement of CBFV's, RI and PI in the ACA and MCA.

Images from the interrogation with colour Doppler of the Anterior Cerebral Artery (ACA) (Top - Figure 2.1a)) and the Middle Cerebral Artery (MCA) (Bottom - Figure 2.1b)) spectral traces demonstrate several cardiac cycles. 3 cardiac cycles were used to derive RI and PI. S: Peak Systolic Velocity. D: End Diastolic Velocity. TAV: Time-Averaged Velocity. S/D: Ratio of Peak Systolic Velocity to End Diastolic Velocity. RI and PI as defined in the text.

2.3.3 Measurement of the CBFV's

Once the probe positioning was verified then the Doppler function on the ultrasound machine was engaged. The Doppler "gate" would be placed by eye in the centre of the vessel with the angle of insonation adjusted to most closely represent the direction of the artery. The Doppler gate specifies the volume from which the ultrasound signal is interrogated and analysed. It is from this volume that the ultrasound machine determines the velocity of the moving red blood cells in the artery.

The movement of the red blood cells is pulsatile and so the velocity changes over time. The peak velocity is termed the Peak Systolic Velocity (PSV) as it coincides with the Systolic portion of the Cardiac cycle. The End Diastolic Velocity (EDV) is the velocity at the end of the Diastolic part of the Cardiac Cycle. The Time Averaged Velocity (TAV) is the average velocity over the period of one cardiac cycle.

The blood flow velocity waveform was observed over several cardiovascular cycles and the trace was paused and saved when the spectral waveform was stable and consistent. A minimum of three traces of more than 5 cycles were obtained on each artery. The Auto-Trace function of the HD11 was engaged at the same time and the parameters that were automatically calculated over 3 cycles were recorded. Using calipers, parameters were also calculated manually from the mean of 3 consistent cycles of the same spectral trace. The initial sets of measurements were immediately repeated by a different operator.

The method was designed in line with accepted protocols in studies which obtained Doppler traces in both the foetus (233-235) and the neonate (76, 77, 235) in which repeated observations are made and the mean taken from at least three consecutive, consistent cardiac cycles. The recorded parameters were the PSV, EDV, TAV, Resistive Index (RI) and Pulsatility Index (PI).

2.4 CONCLUSION

The RI is determined from the CBFV's that are measured using Doppler Ultrasound. The technique for measuring CBFV's was researched to promote accurate measurements for this study. This author attended training to learn the skill of undertaking cranial ultrasounds and measurement CBFV's, supplemented with 6-12 months of assisting with cranial ultrasounds in the NICU at the Mercy Hospital for Women. Although this training was of shorter duration and with less experience than that of a registered Sonographer, it was limited to the scope of cranial ultrasounds. A trained Sonographer's knowledge and experience encompassess all physiology, for all ages and both

genders, and all types of Ultrasounds scans. The shorter duration training undertaken by this author was found to be sufficient, as following the training and practice, this author's CBFV measurements were interrogated and found to correlate well to those of a trained Sonographer.

The safety and ethical aspects of conducting the study with Ultrasound, and particularly Doppler Ultrasound, were considered. Cranial ultrasounds including Doppler Ultrasound are undertaken as part of standard protocol in Neontal Intensive Care Units, and are deemed to be safe if conducted within The British Medical Ultrasound Society guidelines. The Cranial Ultrasounds incorporating Doppler Ultrasound in this study would be undertaken using the standard hospital protocols, and remain well within the safe limits set by The British Medical Ultrasound Society.

CBFV's can be measured in any of the cerebral arteries. The accuracy of the measurement is reduced when the angle of insonation is minimised. The ACA can be interrogated via the anterior fontanelle gaining a line of sight with limited angle of insontaion on the vertical (A2 segment) of the ACA. A line of sight with limited angle of insonation can also be achieved to interrogate the MCA through the temporal window. Thus it was decided that the ACA and MCA were the most appropriate cerebral arteries in which to measure the CBFV's, and determine the RI. It was also resolved that 3 recordings of the CBFV's in both cerebral arteries would be recorded for 3 consistent cardiac cycles. This was method is in line with previously published studies of the CBFV's and RI in neonates.

The method of measuring of CBFV's was researched and the technique of undertaking the measurements was learnt to enable this author to independently obtain CBFV measurements in neonates as part of this study.

CHAPTER 3. CEREBRAL BLOOD FLOW VELOCITIES AND CEREBROVASCULAR RESISTANCE IN HEALTHY FULL-TERM NEONATES

3.1 INTRODUCTION

The first phase of the research project was to determine the Cerebral Blood Flow Velocities (CBFV's), Resistive Index (RI) and Pulsatility Index (PI) in a cohort of normal, healthy full-term neonates; neonates without any known pathology. The CBFV's, the RI and PI in healthy controls would form a baseline against which the same parameters in infants with HIE could be compared. This comparison would then allow an evaluation of these parameters as markers of altered cerebral perfusion in the infants with Hypoxic-Ischemic Encephalopathy (HIE).

The results from the measurement of the CBFV's and cerebrovascular resistance in the first cohort of full-term neonates was published as *"Cerebral Blood Flow Velocities and Cerebrovascular Resistance in Normal Term Neonates in the first 72 hours"* in the Journal of Paediatrics and Child Health, January 2018 (Vol. 54 Issue 1) (1). The accepted manuscript follows, forming the remainder of this Chapter.

3.2 CEREBRAL BLOOD FLOW VELOCITIES AND CEREBROVASCULAR RESISTANCE IN NORMAL TERM NEONATES IN THE FIRST 72 HOURS

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What is already known on this topic:

- The Resistive Index (RI) is an accepted indicator of cerebrovascular resistance.
- In previous studies the normal RI in term neonates has been established as greater than 0.55.
- An RI lower than 0.55 may be observed after periods of hypoxia.

What this paper adds:

- It is preferable to measure cerebral blood flows in the Anterior Cerebral Artery (ACA) as they exhibit less variance and are therefore more reproducible than Middle Cerebral Artery (MCA) measurements.
- Low RI's which may be considered clinically significant (RI < 0.55) were observed in 2 normal infants without a known pathological cause.
- The RI and PI can remain unchanged if component velocities decrease or increase together.

3.2.1 Abstract

Aim

The objective was to determine the range of cerebral blood flow velocities and Doppler indices of cerebrovascular resistance in normal term neonates as a baseline for a study of Hypoxic-Ischemic Encephalopathy.

*Method*s

The Cerebral Blood Flow Velocities (CBFV's), Resistive Index (RI) and Pulsatility Index (PI) were measured in the Anterior and Middle Cerebral Arteries (ACA and MCA) of 38 normal neonates.

Results

The mean Peak Systolic, End Diastolic and Time-Averaged Velocities (PSV, EDV and TAV) were 36.3±6.6, 12.4±3.9 and 22.0±4.0 cm/s (ACA) and 41.4±13.2, 13.0±5.5 and 25.8±7.9 cm/s (MCA). All CBFV's in the ACA correlated with gestation; only EDV was correlated to post-natal age.

The RI in the ACA (0.67±0.06) and MCA (0.68±0.07) were correlated ($r=0.72$, $p<0.001$); RI correlated to post-natal age. Two infants with RI < 0.55 were fed within 25 minutes of the study; a correlation was detected with post-prandial time as a dichotomous variable (pivot 25 mins).

The mean PI was 1.11±0.18 (ACA) and 1.17±0.23 (MCA). Correlations were with post-natal age and post-prandial time (dichotomous).

The average angle of insonation was greater in the ACA than in the MCA (5º vs 18º).

Conclusions

Results corresponded with previous published studies. Although CBFV's increase with advancing gestation, component velocities all increase so there is no correlation between Doppler indices and gestation. The smaller angle of insonation in the ACA led to less variation and smaller standard deviation in CBFV's and better reliability. Low RI's (<0.55) were measured in 2 neonates without a pathological cause warranting further study.

3.2.2 Introduction

The Doppler indices, the Resistive Index (RI) and the Pulsatility Index (PI), are considered a measure of downstream Cerebrovascular Resistance (CVR) and provide a non-invasive means of investigating the neonatal circulation (76). These indices are calculated from the cerebral arterial velocities measured using Doppler in the neonate through the "acoustic windows", the fontanelles and sutures of the skull (236). Doppler Sonography exploits the Doppler Effect in order to measure the velocity of the red blood cells and estimate the blood flow velocity in a blood vessel (71). As Doppler indices are ratios they reduce the error of the velocity measurement associated with the angle of insonation (237).

Pourcelot's Resistive (Resistance) index (RI) is the ratio of the difference in pulsatile flow velocities, Peak Systolic Velocity (PSV) – End Diastolic Velocity (EDV), divided by the Peak Systolic Velocity (PSV) (75, 238):

$$(ii)\ Resistive\ Index\ (RI) = \frac{PSV - EDV}{PSV}$$

The Pulsatility Index of Gosling (PI) is a ratio of the difference in pulsatile flow velocities divided by Time-Averaged Mean (TAV) (6):

$$(ii)\ Pulsatility\ Index\ (PI) = \frac{PSV - EDV}{TAV}$$

The RI is more often referred to when studying the CVR of the neonate, whereas the PI is more commonly determined for the evaluation of CVR of the foetus in utero. Bada et al suggested that the PI provided a greater "data spread" and for this reason should be preferred to the RI for

neonates (75). Alternatively, Couture et al recommend cerebral blood flow velocities in preference to the RI, as the RI alone is of poor prognostic value and may be the source of severe errors (68).

A normal RI is greater than 0.55 and is often lower than this in infants who have suffered a hypoxic-ischemic event (237). Initially due to the "brain-sparing" effect (75, 138, 239), a compensatory reduction of CVR in order to maintain or restore O_2 delivery to the brain (68), and may persist when there is an impairment to autoregulation resulting in vasoparalysis (138).

3.2.3 Aim

To establish a baseline of cerebral blood flow velocities, Resistive and Pulsatility indices (RI & PI) in normal neonates for comparison against infants with hypoxic-ischemic encephalopathy (HIE) in future studies.

3.2.4 Patients and Methods

Neonates experiencing normal births with Apgar scores of >7 at 5 mins and >36 weeks gestation and <72 hours postnatal age were recruited from the maternity ward of the Mercy Hospital for Women in Heidelberg (Victoria, Australia). Patient records including details of antenatal care, the birth and subsequent feeding regime were available to the study.

Cranial Ultrasounds were performed on each neonate with a Philips HD11 XE Ultrasound System with a C8-5; 5-8 MHz Curved Array Transducer probe suitable for neonatal cephalic use. A complete diagnostic cranial ultrasound was conducted, and all subjects presented with normal cranial anatomy.

Three Doppler traces of 3 cardiac cycles were obtained in the Anterior Cerebral Artery (ACA) and the Middle Cerebral Artery (MCA) by methods previously described (76, 77, 233-235). Angle correct was used. Figure 2-1 demonstrates the views used to visualise and interrogate the ACA and the MCA respectively. The recorded parameters were the Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), and Time-Averaged Velocity (TAV) from which the Resistive Index (RI) and Pulsatility Index (PI) were derived. Three observations were obtained by two operators each during the same examination (this Principal researcher and the Head Sonographer of the Medical Imaging Department at the Mercy Hospital for Women). The three most consistent observations for each participant are included in the results.

Data was analysed with MATLAB 2015a (The MathWorks, Inc., Natick, Massachusetts, U.S.A). The Pearson's linear correlation coefficient and p-value is presented for comparison of continuous variables and the point biserial correlation coefficient for dichotomous variables. The similarity

between paired sets of measurements was analysed using the paired sample t-test for means. The agreement between the ACA and MCA was analysed and presented by a Bland-Altman plot. Multivariate analysis was conducted using multivariate linear regression. A p-value of <0.05 was considered significant.

Ethics approval was obtained from the Mercy Health Human Ethics Committee prior to commencement and the study conforms to the provisions of the Declaration of Helsinki (as revised in Brazil 2013). Full informed, written consent was obtained from at least one parent before an infant was admitted into the study.

3.2.5 Results

Thirty-eight singleton neonates were recruited with mean post-natal age of 33 hours (range 5-71), mean gestation of $39^{+1/7}$ weeks (range $36^{+4/7}$-$41^{+6/7}$) comprising: $36^{+4/7}$ (n=1), Early Term ($37^{+0/7}$-$38^{6/7}$ wks, n=15), Full Term ($39^{+0/7}$-$40^{+6/7}$ wks, n=19), Late Term (≥41 wks, n=3)(240). Every participant in the study received standard antenatal surveillance as per hospital protocol, no antenatal corticosteroids were given, and all were born in good condition (mean Apgar score at 5 mins of 9.42 (range 9-10)). 23 of 38 (61%) were female, 15 were male (39%). No correlations were detected with mode of delivery, Apgar scores. There was a mean post-prandial time of 101 min (15-300).

The birthweight range was 2420-4310g. All birthweights, bar one, were ≥ 10^{th} percentile according to the "Australian national birthweight percentiles by sex and gestational age, 1998-2007"(241). The infant with the lowest birthweight (2420g) was a female of 38^{+0} wks gestation who was only 20g less than the 10^{th} percentile. A The measured parameters for this infant were within the 95% confidence level of the group mean for all parameters and so included in the study. Birthweight correlated to gestation (0.57, $p<0.001$) and sex (-0.36, $p<0.05$).

Results are displayed in Table 3-1.

Table 3-1 The Resistive Index (RI), Pulsatility Index (PI) and Cerebral Blood Flow Velocities (CBFV's) in the ACA and MCA

The Resistive Index (RI), Pulsatility Index (PI) and Cerebral Blood Flow Velocities (CBFV's) in the Anterior and Middle Cerebral Arteries (ACA and MCA respectively) as determined in this study and the corresponding significant correlations with Postnatal Age (PN), Postprandial Time (PP), Gestation (G) and Birthweight (B)[2]. (Results shown as mean ± SD).

		Correlations					Correlations			
Index	**ACA**	**PN**	**PP**	**G**	**B**	**MCA**	**PN**	**PP**	**G**	**B**
Resistive Index (RI)	0.67 ± 0.06	-0.33*	-0.51***			0.68 ± 0.07	-0.39*	-0.41*		
Pulsatility Index (PI)	1.11 ± 0.18	-0.33*	-0.46**			1.17 ± 0.23		-0.36*		
Peak Systolic Velocity (PSV)[1]	36.3 ± 6.6			0.35*	0.41**	41.4 ± 13.2				-0.33*
End Diastolic Velocity (EDV)	12.4 ± 3.9	0.42*	0.38*	0.44**	0.35*	13.0 ± 5.5		0.44*		
Time-Averaged Velocity (TAV)[1]	22.0 ± 4.0			0.42*	0.48**	25.8 ± 7.9				

[1]PSV and TAV differed significantly between the ACA and MCA

[2]Although all cross correlations were calculated, only the R-values for significant correlations are displayed. Levels of significance are indicated as follows:

- * indicates significant correlation with p-value <0.05
- ** indicates significant correlation with p-value <0.01
- *** indicates significant correlation with p-value <0.001

Cerebral Blood Flow Velocities (CBFV's)

The CBFV's were of approximate normal distribution as confirmed by Quantile-Quantile (Q-Q) normality tests. Between the ACA and MCA, the mean Peak Systolic Velocity (PSV) and Time-Averaged Velocity (TAV) were statistically different as determined by t-test (t=2.03, p=0.015 and t=2.04, p=0.013 respectively); the EDV was statistically similar (t=2.03, p=0.48). The boxplots of the CBFV's in the ACA and MCA are displayed in Figure 3-1.

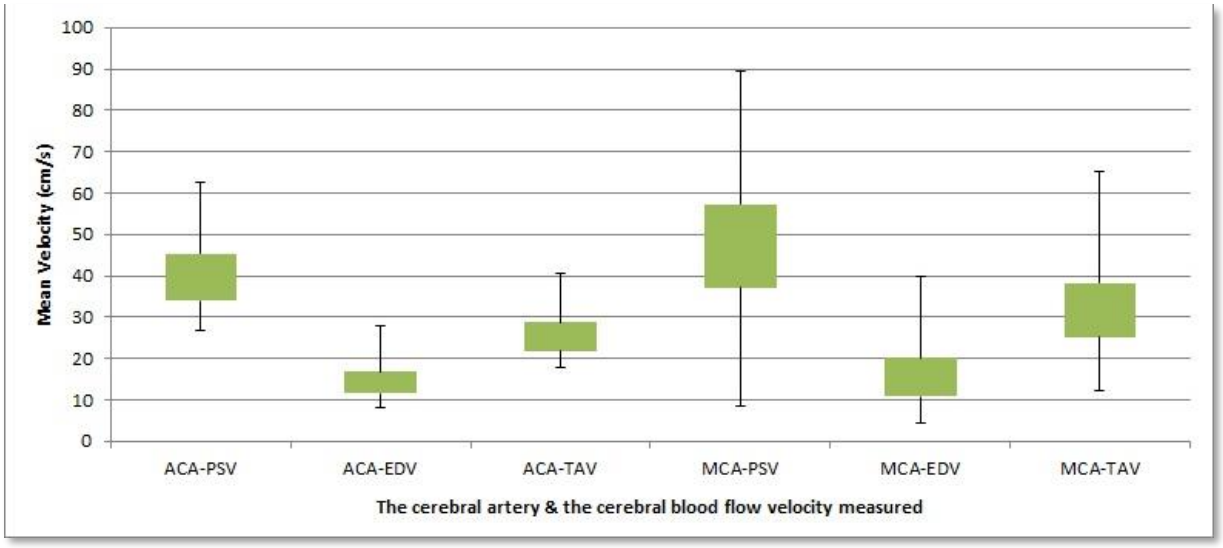


Figure 3-1 Boxplots of Cerebral Blood Flow Velocities in the ACA and MCA.

Cerebral Blood Flow Velocity (CBFV) measurements in the Anterior Cerebral Artery (ACA) and Middle Cerebral Artery (MCA). Boxes indicate the lower (25%) to upper (75%) quartiles and whiskers extend to minimum and maximum measurements.

In the ACA, the average angle of insonation was 7° (range 0°-20°) with a median of 5°; the angle altered between measurements in only 6 cases (21%) and there was at least one measurement with an angle > 10° in only 10 cases (25%). In comparison, the average angle of insonation in the MCA was greater at 18° (range 0°-42°) with median 17°; the angle differed between measurements in 9 cases (23%) and was > 10° in 29 cases (74%). The CBFV's mostly increase with post-natal age, though a statistically significant correlation was only detected between the EDV and the post-natal age in the ACA (r=0.42, p=0.009). Correlations were observed between all CBFV's and gestation in the ACA; consequently, birthweight was also correlated to the ACA CBFV's and to the PSV in the MCA. Sex was correlated to the ACA PSV only.

Multivariate linear regression for the CBFV's was undertaken to determine the relative influence of individual independent variables. Initially all recorded variables were considered for inclusion

as predictors and analysis determined the most optimal model was a 3-dimensional model of the most significant predictors; post-natal age, the dichotomous post-prandial time and birthweight. Models fit best to the ACA measurements as R_Square values were higher. The ACA EDV was the CBFV best represented by a linear regression model; with coefficients of determination were $\beta=2.94$ ($p<0.001$) for post-natal age (Days), $\beta=7.71$ ($p<0.001$) for the dichotomous post-prandial time ($\beta=7.71$ $p<0.001$) and $\beta=4.05$ ($p<0.01$) for birthweight (kg) ($\beta=4.05$ $p<0.01$) and an overall model fit of R_Square=0.51.

Resistive Index (RI)

The overall mean RI (± SD) in the ACA was 0.67±0.06 and 0.68±0.07 in the MCA. The ACA and MCA RI measurements were correlated ($r=0.72$, $p<0.001$); the Bland-Altman plot (Figure 3-2) demonstrates this agreement, albeit with a small positive bias (0.016) in the MCA measurements. This relationship was also confirmed with a paired sample t-test ($t=2.0$, $p=0.06$). The interobserver correlation was $r=0.69$ $p<0.001$.

A correlation between RI and post-natal age was observed (ACA $r=-0.33$ $p=0.045$, MCA $r=-0.39$, $p=0.016$), with the highest RI measurement observed in the youngest infant at a post-natal age of 5 hours, although only 2 infants less than 12 hours of age were included in the study. There was no correlation detected between the RI and the gestation.

The two lowest RI measurements (both 0.54 in the ACA) occurred in two infants most recently fed (15 and 23 min) with postnatal ages of 24 and 39 hours respectively. Therefore, although there was no correlation between RI and post-prandial time if taken as a continuous variable, if the post-prandial time was converted to a dichotomous variable with 25 minutes as the pivot there was a statistically significant correlation with the RI. This was the result of a decrease in the EDV not the PSV.

Multivariate linear regression for the RI determined that only the post-natal age (Days) ($\beta_{ACA}=-0.03$, $\beta_{MCA}=-0.05$, $p<0.01$) and dichotomous post-prandial time ($\beta_{ACA}=-0.15$, $\beta_{MCA}=-0.15$, $P<0.01$) were significant predictors of the RI in both the ACA and MCA. Although birthweight was a significant predictor for CBFV's, it was not a significant predictor for the RI in either the ACA or MCA.

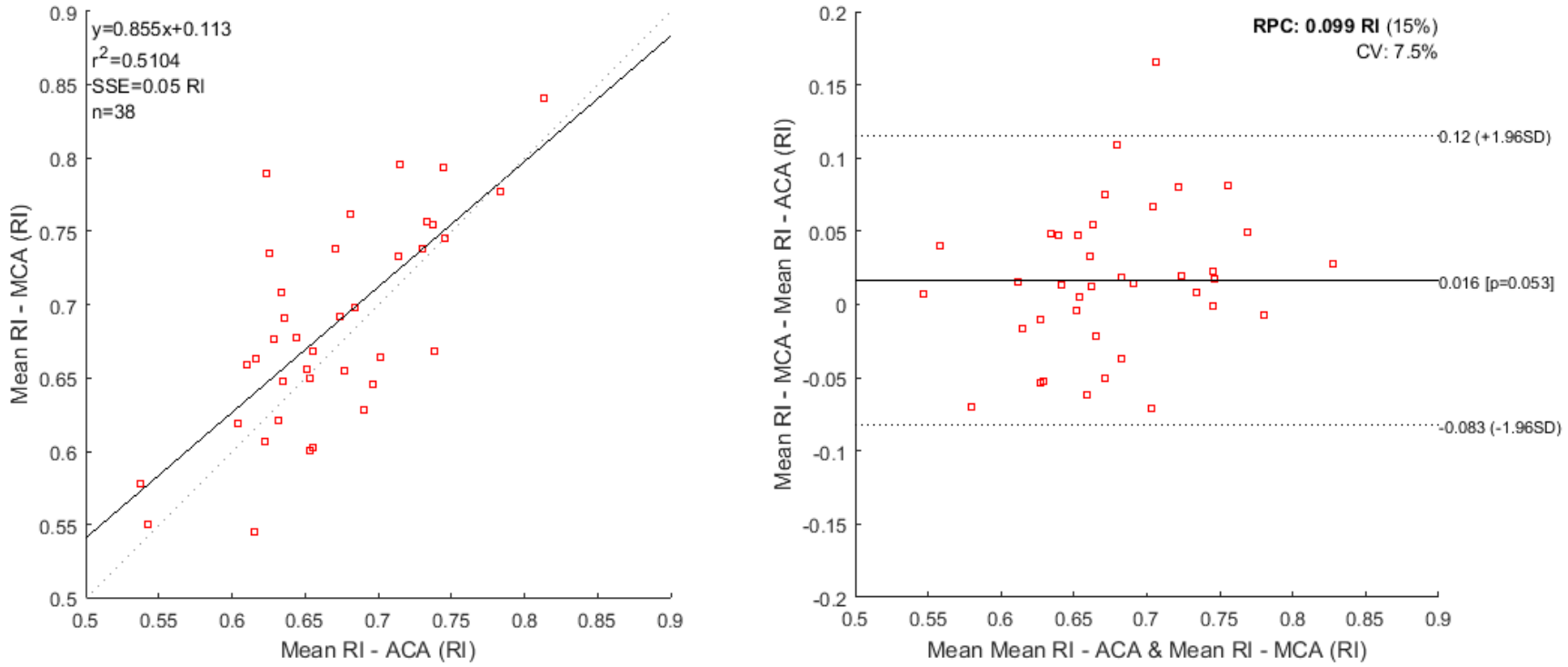


Figure 3-2 Bland-Altman Plot of the Resistive Index (RI) in the ACA versus the MCA.

Bland-Altman plot demonstrating the correlation of the Resistive Index (RI) between the Anterior Cerebral Artery (ACA) and the Middle Cerebral Artery (MCA).

Pulsatility Index (PI)

The mean PI ± SD was 1.11±0.18 in the ACA and 1.17±0.23 in the MCA and the measurements in the ACA and MCA were strongly correlated ($r=0.68$, $p<0.001$). There was a correlation between the PI in the ACA and the post-natal age ($r=-0.32$, $p=0.049$); this correlation in the MCA was not statistically significant. There was no statistically significant correlation between the PI and the gestational age (or birthweight). Like the RI, there was a correlation between the PI and the dichotomous post-prandial time (ACA $r=-0.46$, $p=0.005$; MCA $r=-0.36$, $p=0.038$).

Multivariate linear regression for the PI determined the post-natal age ($\beta_{ACA}=-0.09$ $p<0.05$) and dichotomous post-prandial time ($\beta=-0.37$, $p<0.01$) to be the most significant predictors of PI in the ACA (R_Square=0.44). Only the dichotomous post-prandial time was a significant predictor in the MCA ($\beta_{MCA}=-0.35$ $p<0.05$). Birthweight was not a significant predictor for the PI in either the ACA or MCA.

3.2.6 Discussion

All results were in agreement with previous published studies (68, 76, 77, 140, 242-247). The mean RI in determined in this study corresponds to those studies with similar post-natal age and gestation (refer to APPENDIX A for an overview of relevant studies) (68, 76, 77, 242, 244-249). Studies with earlier post-natal ages reported higher RI's; RI's of greater than 0.74 were reported in the 10 hours after birth in the Ando et al, Wright et al, Connors et al and Pezzati et al studies (76, 77, 242, 244). This study detected a weak inverse correlation between post-natal age and RI, and this may be due to the limited number of infants studied within the first 12 hours. One infant studied within the first 5 hours demonstrated the highest RI of 0.81 (ACA) and 0.84 (MCA). This may be a result of a reduced diastolic velocity due to the presence of ductal flow known as the "steal" phenomenon (75, 77, 250) although studies by Connors et al (76) and Batton et al (251) suggest the higher RI associated with post-natal age due to cerebrovascular re-modelling for improved impedance matching rather than ductal steal (76).

Several studies have suggested that the RI is inversely proportional to the gestational age (243, 245, 247), and so differences in gestational ages of the research population may account for further variations in reported RI. The mean RI in the Seibert et al study was 0.75 ± 0.1 with a mean gestational age of 34 weeks but this consisted of two groups of < 33 weeks and > 34 weeks that had RI's of 0.77 ± 0.09 and 0.71 ± 0.07 respectively, demonstrating that a higher RI is associated with lower gestation (245). No correlation was observed between RI and gestational age in this study, which may have been due to the narrow gestational age range ($36^{+4/7}$-$41^{+6/7}$ weeks).

Couture et al suggests that cerebral blood flow velocities should be constantly preferred to the RI, as the RI alone is of poor prognostic value and may be the source of severe errors (68). APPENDIX B presents an overview of the reported CBFV's for this and other studies (68, 77, 242, 243, 248, 249, 252, 253). Higher Cerebral Blood Flow Velocities (CBFV's) in the MCA have been previously reported (254) and we found the PSV in the MCA to be statistically greater than in the ACA. Our results correlate well to the Aranha et al (243) study where the gestational and post-natal ages are most similar.

Correlations were observed between all the cerebral blood flow velocities in the ACA and the gestation which suggests velocities increase with gestation. Birthweight, which correlated to gestation, was similarly correlated to ACA CBFV's. Correlations of the RI and PI with gestation and birthweight were not statistically significant, most likely because a matched increase in contributing velocities to a ratio may result in that ratio being less or un-affected. Thus, information gained with individual values may be hidden when those values are combined in a ratio. This supports the assertion by Couture et al that velocities should be preferred to the RI and suggests that changes to CBFV's may not always be transmitted to a calculated index. Although the RI's and CBFV's established in this study are slightly lower than reported by Couture et al (68) and Deeg et al (249), this is thought to be a result of the study method. Both these studies use corrected gestation age rather than separately reporting the contribution of gestation and post-natal age. The time-course of the increase in RI and CBFV's may well be altered by the gestation at birth thereby obviating a direct comparison between studies with corrected gestation versus actual gestation with post-natal age included.

The correlations observed between the post-natal age and the post-prandial time with the RI in both the ACA and MCA can be largely explained by the variation in the EDV, as the EDV and RI are significantly and strongly inversely correlated (ACA $r=-0.67$, $p<0.001$; MCA $r=-0.54$, $p<0.001$). Similarly, Couture et al observed that in all 41 cases of Perinatal Asphyxia studied there was a decrease in the RI to below 0.55 as a direct result of an increase in the diastolic amplitude of the cerebral blood flow velocity (237), although this is the only other known reference to this phenomenon.

The PI determined in this study correlates well with Aranha et al who reported normal PI's of 1.12 ± 0.21 in the ACA and $1.25\text{-}1.29 \pm 0.23\text{-}0.27$ in the MCA (L-R) (243). The only other known study of the PI in neonates was that of Couture et al which reported a Pulsatility Index (PI) of 1.34 (68). The higher PI in the Couture et al study aligns with their higher RI and may again be explained by the differing contributions to the corrected gestational age. The same correlations observed with the post-natal age and post-prandial time and the RI in the ACA were also observed with the PI in the ACA. As the Pulsatility Index (PI), used primarily in obstetrics, demonstrated the same correlations as the RI it could be considered as an alternative for neonates.

An interesting observation in this study was a lower than normal RI in 2 infants. These 2 infants had been the most recently fed of the cohort and thus if the post-prandial time was converted to a dichotomous variable with 25 minutes as the pivot there is a correlation with RI, and this corresponds to a concurrent increase in EDV. This observation is supported by the Monteiro et al study which reported a lower RI in feeding infants than those at rest before or after feeding (140). As only 2 infants were measured soon after feeding in this study further investigation is required before any definitive conclusion may be drawn but indicates that a lower than normal RI (<0.55) may be observed in normal neonates without any known pathological cause.

The angle of insonation was much lower in the ACA than in the MCA, and the angle differed in only 20% (ACA) and 23% (MCA) of cases suggestive that the angle is dependent on the anatomy. A smaller angle of insonation would be expected to result in less error in the velocity measurements (74) and should translate to greater reliability and reproducibility. The boxplot (Fig 2) indicates that there was less variability in the CBFV measurements in the ACA versus the MCA. In addition, the Bland-Altman plot (Fig 3) of the RI measurements in the ACA versus the MCA indicated a bias in the MCA measurements and the p-value for the paired sample t-test was suggestive of an almost significant variance between ACA and MCA measurements. The bias and the variance in the ACA and MCA measurements may be attributed to the greater variance in the MCA.

The ACA is easier to interrogate given its proximity to the transducer in the anterior fontanelle and its trajectory towards the probe. The smaller temporal window used for interrogation of the MCA, and the increased angle onto the MCA from this direction is reflected in the greater variance in the MCA measurements in comparison to the ACA and was especially prevalent in the PSV measurements. Correct measurement of the peak systolic velocity relies on an axisymmetric velocity profile with the interrogated sample volume consisting of the maximum velocity, otherwise the maximum velocity may be significantly underestimated (255). The observation that a significant correlation observed between the CBFV's and gestation in the ACA was not detected with the MCA measurements supports the reduced reliability hypothesis. The ratio in the calculation of the RI cancels out this error, and was found to be significantly similar in both the ACA and MCA regardless of the angle of insonation and so confirms that there is value in using an index such as the RI especially where access to the interrogated vessel may be difficult.

3.2.7 Conclusion

The cerebral blood flow velocities and resistive indices determined in this study agree with previously published studies and provide a baseline against which comparisons may be made with other

populations such as infants with HIE as was the objective. The smaller angle of insonation required for ACA measurements corresponded to greater reliability and reproducibility when compared to measurements in the MCA. Lower than normal RI's were observed in two normal neonates without any known pathological cause which suggests a degree of caution needs to be utilised when relying on a low RI to diagnose pathology. Further study of this phenomenon with serial measurements before, during and after feeding on the same infants may assist in answering this question.

CHAPTER 4. FACTORS AFFECTING CEREBRAL BLOOD FLOW VELOCITIES AND CEREBROVASCULAR RESISTANCE IN HEALTHY FULL-TERM NEONATES

4.1 INTRODUCTION

Baseline Cerebral Blood Flow Velocities (CBFV's), the Resistive Index (RI) and the Pulsatility Index (PI) were established in the first phase of the research study. The mean RI in a normal population of neonates, those without any known pathology, was found to be 0.67 ± 0.06 in the Anterior Cerebral Artery (ACA). The mean RI in a normal population supports the clinically accepted approach to consider an RI of > 0.55 as a normal finding. There were however, two infants in the first normal cohort for which the RI was determined to be < 0.55. It was also discovered that these 2 infants had been fed immediately prior to the CUS examination.

A second cohort of 42 normal full-term neonates of < 100 hours post-natal age (PNA) were recruited for a second research study to confirm the baseline of CBFV's and RI in the normal population. The second study focused on measurements of CBFV and cerebrovascular resistance in the ACA as it was established in the first study at that the CBFV measurements were more reliable and reproducible in the ACA. This second study presented an opportunity to control for post-prandial time and improve the understanding of the factors that affect the CBFV and the cerebrovascular resistances as measured by Doppler Ultrasound (DUS).

4.2 ABSTRACT

4.2.1 Aim

The objective of the study was to investigate the Resistive Index (RI) in normal neonates in the first four days to confirm the previously established baseline, and explore the possible reasons for observing pathologically low RI's in normal neonates in our previous study.

4.2.2 Methods

The Cerebral Blood Flow Velocities (CBFV's), Resistive Index (RI) and Pulsatility Index (PI) were measured in the Anterior Cerebral Artery (ACA). As an objective of the study was to identify possible causes for lower than the accepted normal RI, the post-prandial time, behavioural state and heart rate were recorded. The results were then compared against those reported in the previous study.

4.2.3 Results

The mean (±SD) Peak Systolic, End Diastolic and Time-Averaged Velocities (PSV, EDV and TAV) were 38 ± 8 cm/s, 11 ± 4 cm/s and 22 ± 6 cm/s respectively. The mean RI was 0.71 ± 0.07, and mean PI was 1.29 ± 0.25; these were higher than in the previous study. Low positive correlations (256) were observed between post-natal age (PNA) and the PSV, EDV and TAV (all $r=0.4$, $p<0.01$). These correlations translated to low negative correlations with PNA and the RI ($r=-0.4$, $p<0.05$) and PI ($r=-0.4$, $p<0.05$). Stronger correlations were also discovered between the behavioural state and the CBFV's (PSV: $r=0.4$, $p < 0.01$, EDV: $r=0.5$, $p < 0.001$, TAV: $r=0.5$, $p < 0.001$) and the RI ($r=-0.5$, $p < 0.001$) and PI (-0.5, $p < 0.001$).

4.2.4 Conclusions

The mean RI in this study was higher than in the previous study as the mean PNA of the population was lower. The RI was also higher in infants in a calm state with post-prandial time > 30 mins.

4.3 BACKGROUND

The RI is an accepted indication of the cerebrovascular resistance in neonates and an RI > 0.55 is accepted in current practice as normal whereas an RI < 0.55 may suggest a prior hypoxic event. In a previous study of a normal cohort of neonates, without any known pathology, 2 infants exhibited RI's of < 0.55. It was noted that the infants had been fed immediately prior to the DUS examination. Increased CBFV's and lower RI's have been previously reported in infants during breast feeding (140). Using Near-Infrared Spectroscopy (NIRS), brain activations have been observed in neonates during pharyngeal infusions and during dummy (pacifier) sucking (257, 258).

4.4 METHOD

The method of obtaining the Doppler waveforms was as described in Chapter 2. A complete diagnostic cranial ultrasound was conducted with a Philips iU22 Ultrasound with a C8-5 curved ultrasound transducer suitable for neonatal cephalic use. All subjects presented with normal cranial anatomy with no abnormal pathology. Doppler waveforms were obtained using angle-correction by interrogation of the vertical portion of the Anterior Cerebral Artery (ACA) by two observers (this Principal Researcher and Head Sonographer of the Medical Imaging Department at the Mercy Hospital for Women) until three consistent RI's were observed; this resulted in 3-10 waveforms recorded for each participant. Where infants were unsettled or crying, they were settled with either feeding or sucking on a finger (with or without glycerol).

The CBFV's of PSV, EDV and TAV and the cerebrovascular indices RI and PI were recorded. The gestation, gender, post-natal age, mode of delivery, Apgar scores and birthweight were recorded as well as the post-prandial time, heart rate and behavioural state (calm, crying, sucking/feeding and post-event (immediately following crying or sucking).

Data were analyzed with Matlab 2017a (The MathWorks, Inc., Natick, Massachusetts, U.S.A). The Spearman's linear correlation coefficient and p-value is presented for comparison of similarity between continuous variables of different populations.

Ethics approval was obtained from the Mercy Health Human Ethics Committee prior to commencement and the study conforms to the provisions of the Declaration of Helsinki (as revised in Brazil 2013). Full informed, written consent was obtained from at least one parent before an infant was admitted into the study.

4.5 POPULATION

Forty-two singleton neonates were recruited from the Maternity Ward of the Mercy Hospital for Women in Heidelberg and studied whilst on the ward. The cohort had a mean post-natal age of 38 hours (range 1-98) and mean gestation of 39 weeks (range $37^{+0/7}$-$41^{+2/7}$) comprising: Early Term ($37^{+0/7}$-$38^{6/7}$wks, n=12), Full Term ($39^{+0/7}$-$40^{+6/7}$ wks, n=27), Late Term (≥41 wks, n=4) (240). Participants in the study had received standard antenatal surveillance as per hospital protocol, no antenatal corticosteroids were given and all were born in good condition (mean Apgar score at 5 mins of 9.1 (range 7-10)). 21 of 43 (49%) were female, 22 were male (51%). There was a mean post-prandial time of 101 min (15-300).

The birthweight range was 2850-4760g. All birthweights, bar two, were ≥ 10^{th} percentile according to the "Australian national birthweight percentiles by sex and gestational age, 1998-2007"(241). The infants with the lowest birthweights were males of $40^{+4/7}$ wks (2850g) and $40^{+0/7}$ wks (3000g) gestation who were 240g and 90g less than the 10^{th} percentile (3090g). The measured parameters for these infants were within the 95% confidence level of the group mean for all parameters and so were retained in the study.

4.6 RESULTS

There were no correlations detected with the CBFV's or cerebrovasular resistance indices of RI and PI with gestation, birthweight, mode of delivery, gender or Apgar scores. There was also no correlation with any of the CBFV's or cerebrovascular resistance indices and post-prandial time.

There were 65 recorded scans for the 43 infants. It was observed that some infants became unsettled and that their behavioural state, and the methods used for settling the infants, influenced their heart rate, the DUS waveform and the RI, causing transient changes during measurements. These changes were noted along with the accompanying behavioural state change to understand better the physiological mechanisms behind the changes. After a change to the CBFV's and subsequently the RI, it was noted that these parameters would then return to normal over a short period of time (5-10 mins). The behavioural state was recorded to account for any changes that these events may have on the CBFV's and cerebrovascular resistance.

The changes were classified by four identified behavioural states:

- normal i.e. infant was calm and not recently fed (post-prandial time > 30 mins)
- crying: infant was unsettled and crying, or immediately post-crying
- sucking: infant was being settled using either sucking (dummy or gloved finger) with or without the addition of glycerol.
- Post-event: a period of minutes after an infant had been crying or sucking

Results for the Cerebral Blood Flow Velocities (CBFV's) in cm/s, Resistive Index (RI) and Pulsatility Index (PI) are presented in Table 4-1 for this second cohort of healthy infants (referred to as Control Group 2, CG2) and for the cohort examined in the first study presented in Chapter 3. (referred to as the first control group or CG1). CG2 had CBFV's statistically similar to CG1. Although the CBFV's were statistically similar between the groups, the RI ($p < 0.05$) and PI ($p < 0.01$) were statistically higher in CG2.

In CG2, the CBFV's increased and RI and PI decreased with advancing post-natal age; low positive correlations were observed between PNA and the PSV, EDV and TAV (all $r=0.4$, $p<0.01$), and these translated to low negative correlations with PNA and the RI ($r=-0.4$, $p<0.05$) and PI ($r=-0.4$, $p<0.05$). The CBFV's and RI are displayed by post-natal age in Figure 4-1.

Table 4-1 The CBFV's, RI and PI for healthy neonates < 100 hours of age.

The Cerebral Blood Flow Velocities (CBFV's): Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV) and Time-averaged Velocity (TAV), the Resistive Index (RI) and Pulsatility Index (PI) for the previous study of 38 neonates, and the current cohort of 43 neonates and including 65 scans accounting for behavioural state and post-natal age.

STUDY POPULATION		RI	PI	PSV (cm/s)	EDV (cm/s)	TAV (cm/s)
Previous Study (n = 38)		0.67 ± 0.06	1.11 ± 0.18	36.3 ± 6.6	12.4 ± 3.9	22.0 ± 4.0
Current Cohort All scans (n=65)		0.71 ± 0.07*	1.29 ± 0.25**	37.9 ± 7.6	11.4 ± 4.6	21.5 ± 5.8
Behavioral State (n=65)	Normal (n=34)	0.74 ± 0.06	1.39 ± 0.22	36.9 ± 6.2	9.7 ± 2.9	20.2 ± 4.3
	Crying (n=13)	0.68 ± 0.09**	1.22 ± 0.32*	37.7 ± 6.5	12.3 ± 4.8**	22.3 ± 5.1***
	Sucking (n=8)	0.64 ± 0.06***	1.07 ± 0.19***	43.7 ± 10.5**	16.5 ± 5.9**	27.9 ± 7.5**
	Post-Event (n=10)	0.70 ± 0.06	1.26 ± 0.22	40.4 ± 7.6	12.6 ± 4.8*	23.1 ± 6.1
Post-Natal Age (Normal, n=34)	0-12 HRS (n=6)	0.80 ± 0.05	1.56 ± 0.19	33.3 ± 4.6	6.7 ± 2.0	16.7 ± 2.9
	12-24 HRS (n=10)	0.75 ± 0.05	1.38 ± 0.20	34.7 ± 6.1	8.7 ± 2.0	19.0 ± 3.5
	Day 2+ (n=18)	0.72 ± 0.05	1.33 ± 0.21	39.4 ± 5.8	11.2 ± 2.7	21.7 ± 4.1

Note: Significance levels for comparison are * - < 0.05, ** - <0.01 and *** - <0.001. Current cohort (CG2) compared against previous cohort (CG1). Behavioural states compared against "Normal" behavioural state.

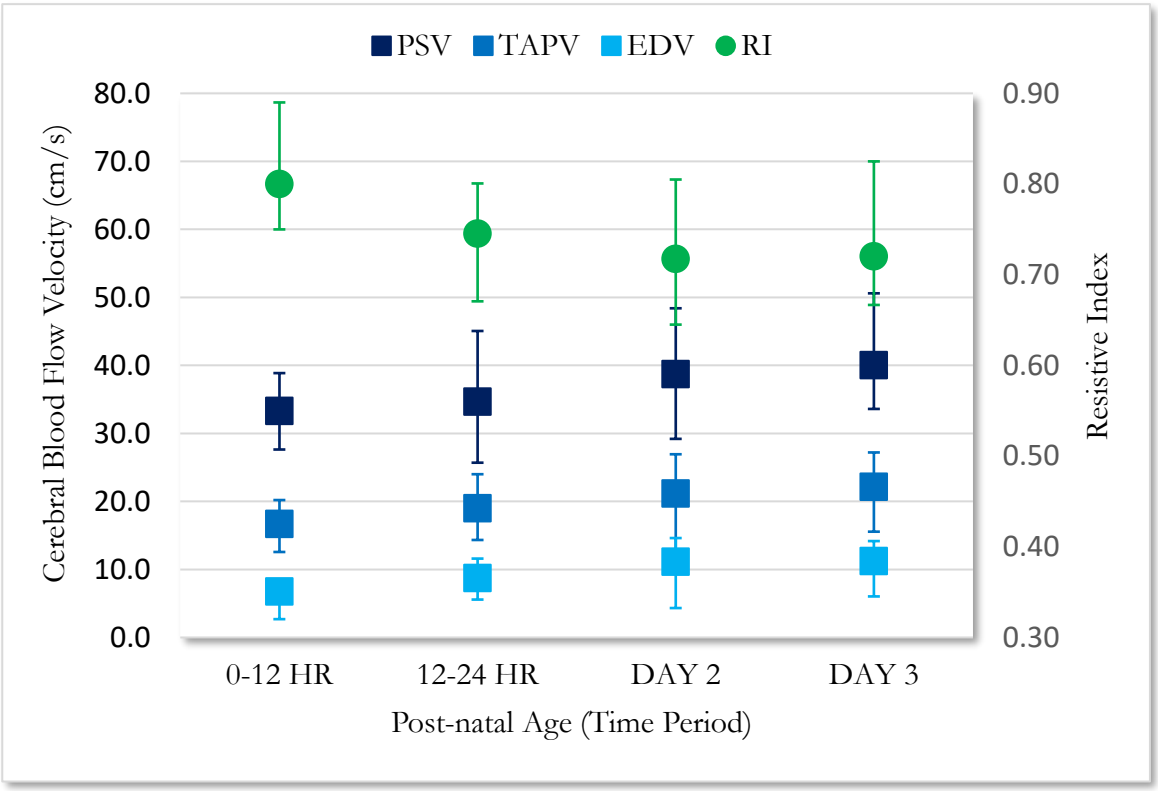


Figure 4-1 Cerebral Blood Flow Velocities (CBFV's) and RI by post-natal age (PNA).

The RI was significantly lower when the infant was or had just been crying ($p < 0.01$) or sucking ($p < 0.001$). A weak positive correlation was detected between heart rate (HR) and the EDV ($r=0.24$, $p < 0.05$), and and the RI ($r=-0.23$, $p <0.05$). There was one infant who exhibited a lower than the accepted normal limit of 0.55, the RI as measured in this infant was 0.54 whilst sucking a gloved finger with glycerol. The RI was strongly negatively correlated to the EDV ($r=-0.86$, p , 0.001), moderately negatively correlated to the TAV ($r=-0.67$, $p < 0.001$), and had a low negative correlation to PSV ($r=-0.45$, $p< 0.001$).

Interestingly, there was no correlation with post-prandial time as might have been expected from the first study. Two infants were measured before and after feeding specifically to explore the effect of feeding on the RI. The RI in one infant was decreased, but the RI in the second infant increased. These disparate results may explain the lack of correlation between post-prandial time and the RI in this second study. The RI was affected by the behavioural state, whether the infant had been crying or settled with sucking, and this most likely obfuscated any correlation with post-prandial time.

4.7 DISCUSSION

The CBFV's obtained in this study were in agreement to previously published studies and the first phase of this research study. The Resistive Index (RI) and Pulsatility Index (PI) were significantly lower than in the previous study; this is likely to be due to a greater proportion of infants who were examined before 12 hours of age (n=6 vs n=2 in the previous study). Access to infants of a lower post-natal age was a consequence of scanning infants in-situ in the maternity ward. Infants were moved to the Neonatal Intensive Care Unit on a different floor in the first phase of this research study, and this discouraged participant enrolment in the study on the first day of life.

The protocol of this second phase of the research study also included the placing of a headband (to secure NIRS sensors) on the head (as described in the second part of this study in Chapter 8) for a concurrent study and this resulted in a greater proportion of unsettled infants requiring settling with sucking and glycerol, which could have emphasized this effect. There was one infant with an RI of < 0.55, the RI in this infant was 0.54 whilst they were sucking a finger with glycerol placed on it. The use of sucking and glycerol to settle infants in the clinical setting including during cranial ultrasounds is common practice and it should be noted that this may alter cerebral haemodynamics and could result in a false positive result for a pathological condition.

There was a stronger correlation between the RI and EDV than any of the other CBFV's. This is in agreement with the first phase of this study, CG1, where the RI was correlated only to the EDV, and there was no correlation with the PSV or TAV. This indicates the variation in the RI is influenced more by the EDV than the PSV or the TAV. The benefit in the clinical use of the RI rather than the EDV is its angular independence, although with the improvement in Doppler measurement with modern Ultrasound machines, this benefit may be reduced. The EDV provides additional information which may be obscured by the calculation of the RI when the PSV changes concurrently.

An increased heart rate was associated with a decreased EDV, whilst a heart rate decrease was often associated with an increase in the EDV. Thus the EDV was also weakly negatively correlated to the heart rate and also corresponded to a relative change in the RI. It is possible that this was detected as a weak correlation as the RI only changes when there is a change to the EDV, as was empirically observed during transitions between behavioural states.

An increase in heart rate (HR) is associated with increased exertion and energy consumption. The inverse correlation of the HR to the EDV could be due to several mechanisms: a reduction in the resistance of the cerebral arteries induced by an increased energy demand, an increase in perfusion to

the brain as a consequence of an increase in cardiac output (CO) stimulated by feeding, or a causal consequence of the compliance of the blood vessels with a change of HR.

Sucking and pharyngeal infusions are associated with brain activations that have been detected by Near-Infrared Spectroscopy (NIRS) (257, 258) which could result in an increase in the metabolism in the brain. It is possible that a physiological mechanism may reduce the resistance of the cerebral arteries in order to increase blood flow to the brain to match demand, increased by stimulation, resulting in an increase in the EDV and thus a lowered RI.

During feeding, the blood flow to the stomach is increased by vasodilation of the superior mesenteric artery. Concurrently there is an increase in the peripheral resistance. The decrease in compartment volume and increase in blood flow to the main compartment may result in the knock-on effect of increased blood flow to the brain manifesting as an increase in EDV and thus a decrease in RI.

Feeding may also increase Cardiac Output (CO). An increase in CO has been observed in adults post-prandially, although for infants the reported results are conflicting. Nevertheless, the initial increase in HR associated with feeding would suggest that there is an increase in CO. Other behavioural events also cause the heart rate to increase, and EDV is increased resulting in a lower RI. Subsequent to the event, the HR tends to slow, to its initial speed, the EDV appears to increase and tends towards the initial EDV, and the RI therefore appears to return to normal. The time period for which this occurs appears to be variable, depending on the event and the subsequent behavioral state of the infant. The return to initial values has been observed over a 5 minute period, but may be slower in some case, as lower RI's were observed in infants who had been fed in a period of 15 mins prior to a CUS examination.

The regression to initial values could be captured where this occurred over a period of minutes due to the repeated Doppler measurements over the examination period. The examination was not extended to ensure the capture of the return to initial values, and so this was not observed in all cases. Future work may include extended study periods and longitudinal scans to be able to describe this phenomenon more completely.

4.8 CONCLUSION

The possible reasons for observing an RI < 0.55 in healthy neonates, with no known pathology, was explored in this study. This study suggests that there may be complex interaction of heart rate, cardiac output and changes to cerebrovascular resistance that impacts upon the measured cerebral blood flow velocities and therefore influences the cerebrovascular resistance indices, the RI and the PI. This

complex interaction could result in the RI or PI appearing to be lower that the accepted normal value of 0.55. This phenomenon should be considered when Doppler studies are conducted on infants who become unsettled and may be re-settled using the common clinical practices of sucking and glycerol. Where an RI < 0.55 is observed without any additional indicators of an hypoxic episode, it is suggested that a follow-up study is conducted to confirm the results.

CHAPTER 5. CBFV'S AND RESISTIVE INDEX (RI) AS MARKERS OF CEREBRAL PERFUSION IN HIE

5.1 INTRODUCTION

A Resistive Index (RI) < 0.55, as measured by Doppler Ultrasound (DUS), is a clinically accepted indicator of lowered cerebrovascular resistance (237). The initial question which launched this research project was whether the RI was a reliable indicator of an hypoxic-ischemic episode, particularly in cooled infants. The objective of this phase of the project therefore was to investigate the value of the measurement of the CBFV's and the RI in infants with HIE. This was achieved by monitoring the DUS parameters throughout the cooling and rewarming, and immediately following the rewarming. The previous research studies presented in Chapter 3. and Chapter 4. , conducted with healthy neonates (no known pathology), provide a baseline against which the CBFV's and indices of cerebrovascular resistance can be compared. The CBFV's and the determination of the RI, and their prognostic power in HIE infants were evaluated in the research study presented in this chapter.

5.2 HYPOTHESIS 1

5.2.1 Hypothesis 1

The Resistive Index (RI) is a reliable marker of altered cerebral perfusion marker and indicates the severity of Hypoxic-Ischaemic Encephalopathy (HIE) during TH.

5.2.2 Alternative Hypothesis 1

Derangements in the cerebral blood flow velocities (CBFV's), rather than the RI, more reliably predict the poor outcome in HIE.

5.3 METHOD

Infants diagnosed with HIE by the attending clinician, admitted to the Neonatal Intensive Care Unit (NICU) at the Mercy Hospital for Women (MHW), and prescribed Therapeutic Hypothermia (TH) were eligible to be enrolled in the study as per the hospital's "*Procedure for Cooling neonates*":

- Gestation over 35 weeks with birth weight > 1.8 kgs and under 6 hours of age AND

- Clinical evidence of encephalopathy – infants should have positive clinical indicators in at least three of the six categories under the 'moderate or severe encephalopathy' (as defined by Sarnat and Sarnat (66)(Table 1-1) AND
- At least one of the following:
    - Apgar score of 5 or less at 10 minutes, OR
    - Mechanical ventilation or need for continued resuscitation at 10 mins, OR
    - pH < 7.0 from cord blood, or arterial blood within 1 hour of birth; or base deficit > 12 within 1 hour of birth, OR
    - Amplitude integrated EEG (Brainz™ monitor) showing moderate or severe depression or seizures.

The HIE infants were examined with DUS, for determination of CBFV's, RI and PI, once per day during the TH, every 2 hours during rewarming and immediately following rewarming; results were compared against the baseline CBFV's, RI and PI established in healthy neonates (no known pathology) in the first 84 hours of life. Where possible, the diameter of the artery at the time of the DUS measurement was recorded. If infants remained in the NICU, DUS measurements were continued where possible until discharge. All DUS examinations and measurements were made by this author for operator and procedural consistency. The method of obtaining the Doppler waveforms was previously described in Chapter 2.

Data were analysed with Matlab 2017a (The MathWorks, Inc., Natick, Massachusetts, U.S.A). The Mann-Whitney-Wilcoxon test was utilized for comparison of similarity between different populations. Spearman's linear correlation coefficient and p-value are presented for correlations between variables and parameters.

Ethics approval was obtained from the Mercy Health Human Ethics Committee prior to commencement, and the study conforms to the provisions of the Declaration of Helsinki (as revised in Brazil 2013). Full informed, written consent was obtained from at least one parent before an infant was admitted into the study.

5.4 RESULTS

5.4.1 Healthy (Control) Population

Eighty healthy, normal (no known pathology) neonates, from the 2 previous studies were included in the Control group. The Control cohort had a mean post-natal age (PNA) of 36 hours (range 1-98 hrs), and a mean gestation of was $39^{+2/7}$ weeks (range $36^{+4/7}$-$41^{+6/7}$) comprising: $36^{+4/7}$ (n=1), Early Term

($37^{+0/7}$-$38^{6/7}$wks, n=27), Full Term ($39^{+0/7}$-$40^{+6/7}$ wks, n=46), Late Term (≥41 wks, n=7) (240) . There were 37 male (46%) and 44 female (54%) infants. The mean Apgar score at 5 mins was 9.25 (range 7-10). Mean birthweight was 3424g (range 2420-4760g).

The mean CBFV's of PSV, EDV and TAV, and the RI and PI (±SD) are presented in Table 5-1 Mean CBFV's of PSV, EDV and TAV, and the RI and PI (±SD).. The EDV, RI and PI did not vary significantly between Day 2 (24-48 hours) and Day 3 onwards (48+ hours), though the PSV and TAV were significantly different ($p < 0.05$) between those two periods. For this reason, Day 2 and Day 3+ were kept as two separate time periods for analysis.

Table 5-1 Mean CBFV's of PSV, EDV and TAV, and the RI and PI (±SD).

The mean±SD for the cerebral blood flow velocities (CBFV's): Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV) and Time Averaged Velocity (TAV), and the Resistive Index (RI) and Pulsatility Index (PI) in the Anterior Cerebral Artery (ACA) in 80 healthy neonates (PNA < 100).

PNA	RI	PI	PSV	EDV	TAV
0-12 HRS (n=8)	0.78 ± 0.05	1.48 ± 0.25	30.8 ± 4.6	6.6 ± 1.5	16.6 ± 3.1
12-24 HRS (n=22)	0.72 ± 0.05	1.26 ± 0.21	33.3 ± 6.3	9.6 ± 2.6	19.2 ± 3.9
24-48 HRS (n=26)	0.65 ± 0.06	1.10 ± 0.19	36.9 ± 6.4	12.9 ± 3.4	22.0 ± 4.4
48+ HRS (n=22)	0.67 ± 0.06	1.14 ± 0.18	42.3 ± 7.4	14.5 ± 5.1	25.1 ± 5.7

5.4.2 HIE (Exposed) Population

All 18 HIE infants included in this study met the Mercy Hospital for Women's eligibility criteria for TH. The HIE cohort had a mean gestation of $38^{6/7}$ weeks (range $35^{3/7}$-$41^{3/7}$) comprising: $35^{3/7}$ (n=1), $35^{4/7}$ (n=1), $36^{5/7}$ (n=1), Early Term ($37^{+0/7}$-$38^{6/7}$wks, n=4), Full Term ($39^{+0/7}$-$40^{+6/7}$ wks, n=9), Late Term (≥41 wks, n=2) (240). 12 of the 18 (67%) were male, 6 were female (33%). Mean birthweight was 3255g (range 2440-4400g).

Three infants were classified as Stage 1 (Mild HIE), 8 infants were classified as Stage 2 (Moderate HIE) and 4 infants were classified initially as Stage 3 (Severe HIE); 2 infants had no initial staging by the Consultant Neonatologist. Initial (Cord or Admission) pH was available in 15 infants with a mean pH of 6.9 (range 6.6-7.3), mean initial lactate (Cord or Admission) was 16.25 (range 7.9-35), mean initial base deficit of 17.4 (range 8-30), mean Apgar score at 1 min was 1.9 (range 0-5), at 5 mins mean

3.7 (range 0-7) and at 10 mins 5.2 (range 2-9). Moderate correlations were detected between outcome (Normal or Poor) and Initial Lactate ($r=54$, $p < 0.05$), and outcome and Sarnat stage ($r=0.54$, $p < 0.05$).

Outcome categorization was based on the initial diagnosis of the severity of the HIE by the attending clinician, the formal ultrasound report conducted in the first 3 days of life by a Mercy Health Sonographer, the formal report from the MRI (Magnetic Resonance Imaging) conducted after the infant was rewarmed (between day 4 and 6 of life) and the 2 year developmental review conducted by a Mercy Health Paediatrician, where available. An overview of the short- and medium-term outcomes for the 18 infants can be found in Overview of the short- and medium-term outcomes for the HIE infants enrolled in the studyAPPENDIX C

The HIE infants were numbered from HIE1 to HIE18, categorized by outcome and identified by letter as follows:

N: Normal outcome, no indication of HIE injury in MRI examination (n=9)

M: Evidence of mild-moderate HIE injury, with presumed normal outcome (n=3)

P: Poor outcome, deceased or significant developmental delay at 2 years of age (n=6)

5.4.3 Resistive Index

The mean RI (±SD) for time periods 0-12 PNA (Hrs), 12-24 PNA (Hrs), 24-48 PNA (Hrs), 48 Hrs PNA to rewarm (commencement of rewarming), rewarm (rewarming, 72-84 cooled hours) and post-RW (after rewarming) is presented in Table 5-2. Rewarming was separated into a distinct time period because the difference between groups and populations was more pronounced during this period of transition; the RI in both the Normal (N) and Poor (P) outcomes groups was significantly different, but opposite in derangement to the healthy control (HC) population ($p < 0.001$). The RI in the N and P groups remained significantly different to the HC's until after rewarming ($p < 0.05$); significantly higher RI in the N group and significantly lower in the P group. Initially the N and M groups had lower RI's which increased to and throughout rewarming, with the opposite trend occurring in the P group. The N and M outcome groups were statistically similar to each other and both were significantly different to the P outcome group ($p < 0.001$).

Table 5-2 CBFV's, RI and PI for HIE infants by outcome compared to healthy infants for periods 0-12, 12-24 and 24-48 hours PNA, 48 hrs PNA to rewarming, rewarming and normotemp.

Measurements of cerebral blood flow velocities (CBFV's): Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV) and Time-Averaged Velocity (TAV), the Resistive Index (RI) and Pulsatility Index (PI), for the HIE population separated by Outcome (N=Normal, M=Mild-Moderate HIE injury, and P=Poor outcome) by selected periods. Significant differences to the control population are indicated by * - $p < 0.05$, ** - $p < 0.01$ and *** - $p < 0.001$.

		0-12 PNA (Hrs)	12-24 PNA (Hrs)	24-48 PNA (Hrs)	48 PNA (Hrs) to Rewarm	Rewarm	Post-RW
RI	**Healthy**	0.78 ± 0.05	0.72 ± 0.05	0.65 ± 0.06	0.67 ± 0.06		
	N (n=9)		0.65 ± 0.15	0.70 ± 0.05	0.72 ± 0.09	0.75 ± 0.10***	0.74 ± 0.10*
	M (n=3)	0.54 ± 0.15*		0.68 ± 0.11	0.57 ± 0.17	0.72 ± 0.17	0.70 ± 0.14
	P (n=6)	0.63 ± 0.13	0.75 ± 0.10	0.74 ± 0.14	0.62 ± 0.06	0.58 ± 0.08***	0.60 ± 0.09*
PI	**Healthy**	1.48 ± 0.25	1.26 ± 0.21	1.10 ± 0.19	1.14 ± 0.18		
	N (n=9)		1.21 ± 0.53	1.21 ± 0.19	1.32 ± 0.32	1.49 ± 0.40***	1.46 ± 0.43*
	M (n=3)	0.77 ± 0.25*		1.26 ± 0.4	0.96 ± 0.4	1.59 ± 0.84	1.44 ± 0.7
	P (n=6)	1.03 ± 0.32	1.45 ± 0.52	1.45 ± 0.57	0.99 ± 0.16	0.90 ± 0.24***	0.97 ± 0.27*
PSV	**Healthy**	30.8 ± 4.6	33.3 ± 6.3	36.9 ± 6.4	42.3 ± 7.4		
	N (n=9)		17.1 ± 9.5*	36.3 ± 8.5	34.4 ± 9.0*	41.3 ± 6.9	41.9 ± 7.9
	M (n=3)	19.4 ± 11.3		39.1 ± 10.9	61.7 ± 24.8*	50.9 ± 18.1	57.4 ± 13.0*
	P (n=6)	30.7 ± 14.1	36.3 ± 11.9	44.4 ± 9.2*	55.0 ± 16.8*	58.2 ± 13.7***	55.3 ± 16.1*
EDV	**Healthy**	6.6 ± 1.5	9.6 ± 2.6	12.9 ± 3.4	14.5 ± 5.1		
	N (n=9)		5.0 ± 1.85**	11.2 ± 4.1	10.0 ± 5.0**	10.8 ± 5.6***	10.9 ± 5.2*
	M (n=3)	8.2 ± 2.3		13.2 ± 7.7	28.4 ± 21.1	16.9 ± 14.8	18.6 ± 10.2
	P (n=6)	11.6 ± 8.6	8.5 ± 3.7	10.9 ± 5.5	22.3 ± 9.0*	24.7 ± 8.5***	22.9 ± 11.2*
TAV	**Healthy**	16.6 ± 3.1	19.2 ± 3.9	22.0 ± 4.4	25.1 ± 5.7		
	N (n=9)		9.3 ± 3.5**	21.8 ± 6.5	19.7 ± 6.9*	21.8 ± 5.2*	22.8 ± 6.7
	M (n=3)	12.4 ± 6.0		21.7 ± 9.5	39.8 ± 21.9	27.9 ± 16.4	31.4 ± 11.4
	P (n=6)	19.6 ± 11.5	19.6 ± 6.9	25.4 ± 10.2	35.7 ± 11.6*	39.0 ± 10.6***	36.3 ± 13.9*

Figure 5-1 presents the plots of the mean RI (range indicated by vertical bars) of the M & N (as the RI in these groups were statistically similar) and the P groups. The P group has a significantly lower RI and the N group has a significantly higher RI than the healthy controls during and following the rewarming, whereas the M group had a mean RI that was not different to the healthy controls. The M group was made up of 3 infants, two of whom had mean RI's during rewarming of 0.87 and 0.73 respectively (HIE14-M and HIE17-M). The overall mean RI for this group during rewarming was reduced by the mean RI of the third infant of the M group, HIE2-M, who had an RI of 0.54 during rewarming.

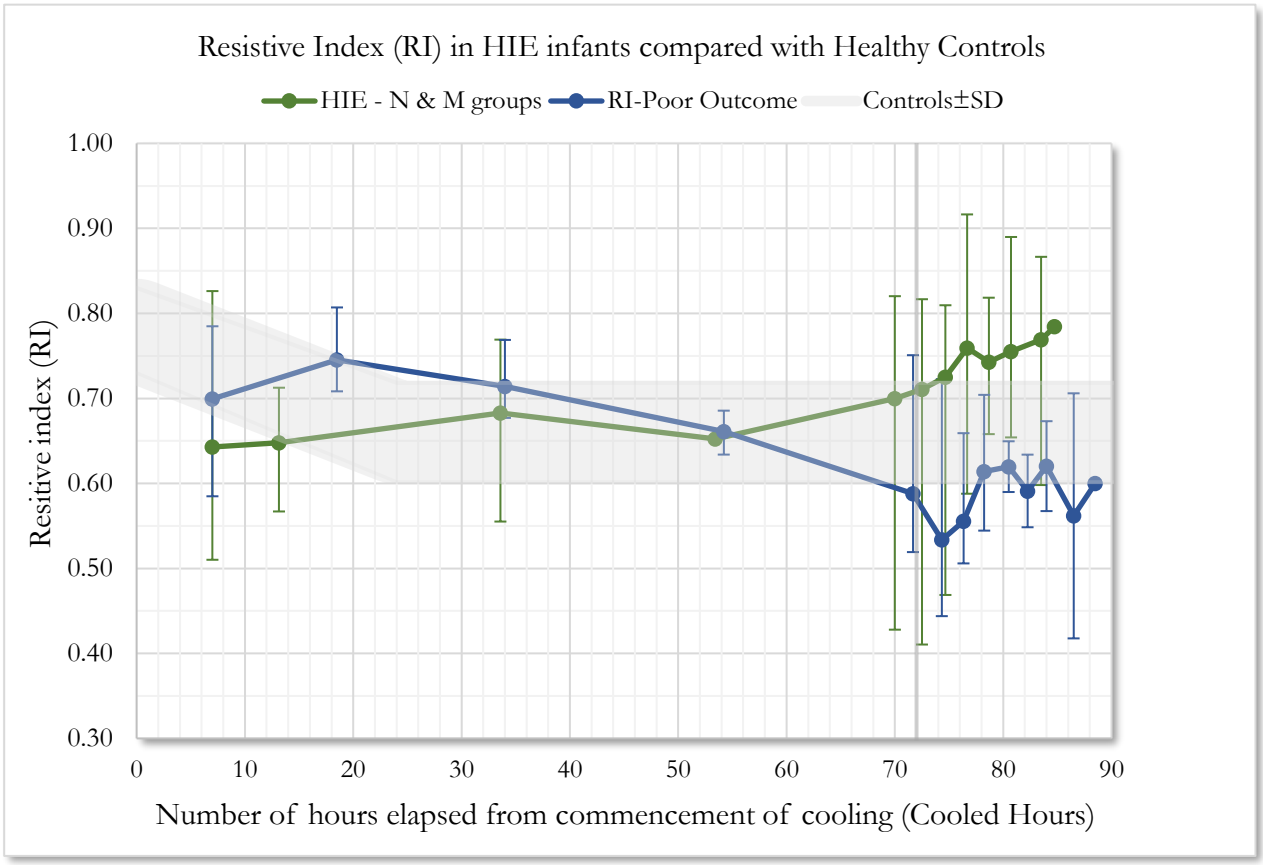


Figure 5-1 Resistive index (RI) in the HIE infants compared with Healthy Controls (mean±SD).

The HIE infants are separated into the Normal & Mild-Moderate HIE (green) & Poor Outcome groups (blue) combined. The RI in the Control population ± SD is indicated in grey.

The RI in the N group was weakly positively correlated with PNA (ρ=0.29, $p < 0.01$), cooled hours (number of hours elapsed from commencement of cooling) (ρ=0.36, $p < 0.001$) and temperature (ρ=0.26, $p < 0.05$). The RI in the Poor Outcome (P) group had a weak negative correlation with cooled Hrs (ρ=-0.30, $p < 0.05$) and PNA (ρ=-0.31, $p < 0.05$), but no correlation with temperature. In

addition, in the P group, there was a moderate negative correlation detected between the diameter of the artery measured in mm from the ultrasound image and the RI (ρ=-0.61, $p < 0.001$). No correlations were detected with the RI in the M group.

Table 5-3 Correlations between the Resistive Index (RI) and Post-Natal Age (PNA), cooled hours, temperature and the diameter of the artery.

Strength and significance of the correlations between the Resistive Index (RI) and Post-Natal Age (PNA), cooled hours (number of hours elapsed from commencement of cooling), temperature (rectal), and the diameter of the artery as measured from the Ultrasound image by outcome (Normal, Moderate or Poor outcome). Significance of correlation is indicated by * - $p < 0.05$, ** - $p < 0.01$ and *** - $p < 0.001$.

Correlation between given parameter and RI (ρ)	PNA	Cooled Hours	Temperature	Diameter of artery (mm)
Normal outcome group	0.29 **	0.36 ***	0.26 *	
Moderate outcome group				
Poor outcome group	-0.31 *	-0.30 *		-0.61 ***

The DUS measurements were abandoned prior to rewarming in 2 infants with severe neonatal seizures, both subsequently died. These 2 infants exhibited normal RI's of 0.89 and 0.78 at their first scans between 0-12 hours and both had RI's > 0.55 until the examinations were abandoned.

An indicator of a hypoxic episode is an RI < 0.55, as previously defined. Seven of the 18 infants (39%) presented with an RI < 0.55; of those 7, there were 3 infants with a known poor outcome so the RI had a sensitivity of 43%. There were 11 infants (61%) who did not have RI's < 0.55, 8 of whom had normal outcomes, so the specificity was 73%. Only 1 of the 3 infants with poor outcome presented with an RI < 0.55 in the first 24 hours, though all had RI measurements < 0.55 during rewarming, and one had an RI < 0.55 only during the rewarming period. By contrast, 3 of the 4 infants who had normal outcomes and RI's < 0.55 did so in the first 24 hours, 2 had RI's < 0.55 during rewarming, and 1 had an RI < 0.55 only during rewarmig.

The degree of derangement of the RI in the HIE infants relative to the healthy control (HC) population is presented in APPENDIX E. RI's more than SDs different to the HC's or < 2SD are highlighted. There were 6 different patterns observed in these infants:

- HIE4-P initially had lower RI (RI < 0.55); it then normalises, and has periods of elevated RI during rewarming

- HIE5-P has a normal RI which increases before dropping markedly to greater than 2SDs lower than the mean normal RI on Day 2 (D2). It remained low and dropped below 0.55 during rewarming, remaining significantly lower than the HC's until Day 6 (D6) when it recovers, and is 1SD higher than the mean RI of the normal population
- HIE8-P had an initially high RI which normalised prior to the rewarming; the infant subsequently clinically deteriorated during rewarming and was unable to tolerate further examinations.
- HIE9-P had a first initial RI that was normal before the RI became elevated (>3SDs). This infant also clinically deteriorated during rewarming and was unable to tolerate further examinations.
- HIE13-P had an initially normal RI that subsequently fell on D2, normalised on D3 before falling again during rewarming, and normalising afterwards.
- HIE15-P was initially more than 3SDs lower that the mean RI in the normal population and <0.55; the RI remained low until the last examination on D6.

5.4.4 Pulsatility Index

The PI for each of the groups at each time period is presented in Table 5-2. The PI results present with similar patterns to the RI: which is evident in the plot of the PI in the N&M group versus the P group (with an indication of the PI in the HC's) as displayed below in Figure 5-2.

The PI was initially significantly lower in the N&M group than in the healthy controls (HC's), in the P group it was initially similar to the HC's. Throughout the cooling, all groups had PI's statistically similar to the HC's although the mean PI of the N&M group was lower than the mean PI of the P group until 48 hours PNA. Prior to rewarming, the mean PI of the N&M group increases, and the mean PI of the P group decreases until the mean PI of the N&M group becomes higher than that of the P group. At the commencement of the rewarming the mean PI of the N group continues to increase and is significantly higher than the HC's. In contrast to the N group, the PI in the P group reduces at the commencement of rewarming, becoming (and remaining) significantly lower than the HC's throughout and immediately following rewarming. The M group is similar to the HC's throughout and immediately following rewarming, though this is the combination of one infant with a low PI (HIE2-M, mean PI during rewarming = 0.87±0.22), one infant with a high PI (HIE14-M, mean PI during rewarming=2.59±0.55), and HIE17-M with a moderate PI (mean PI during rewarming=1.36±0.24).

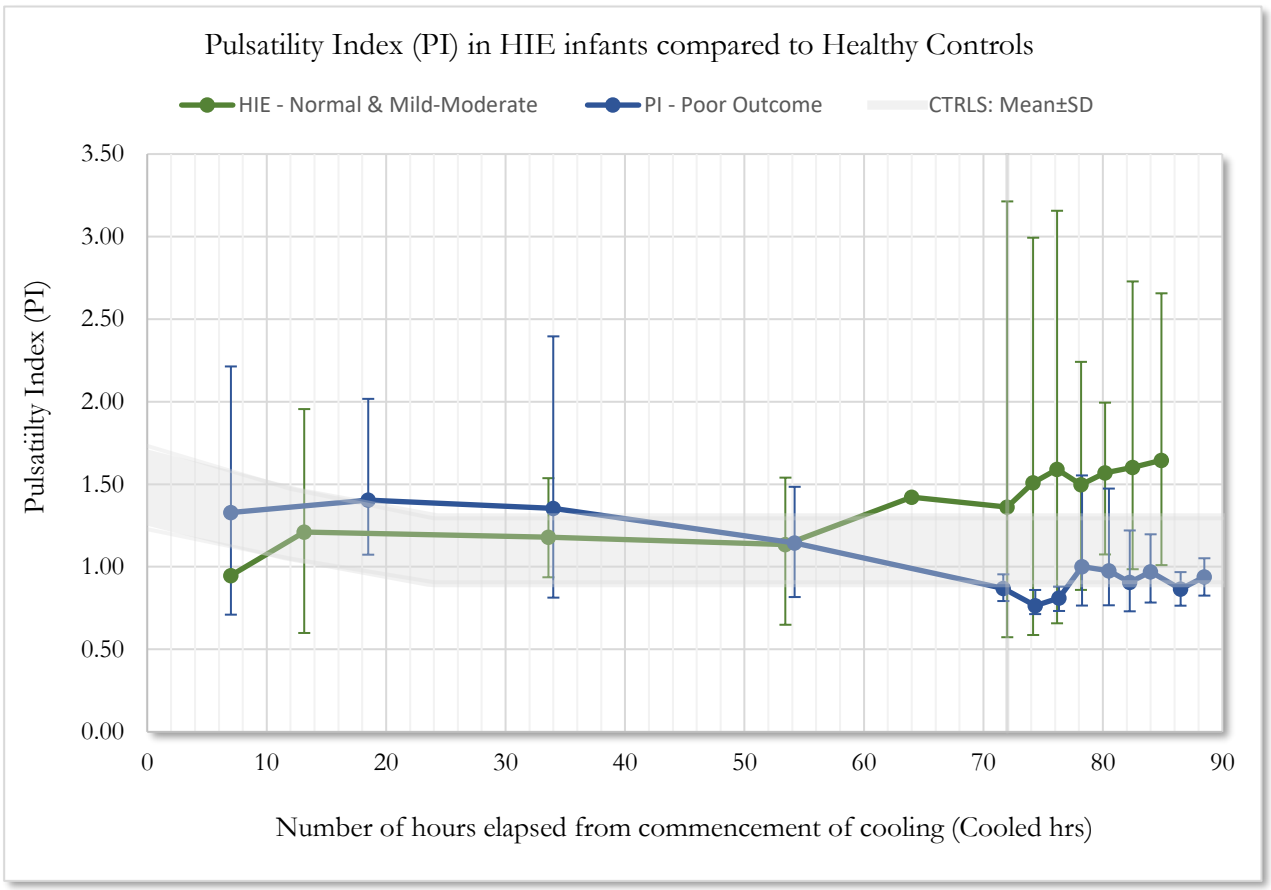


Figure 5-2 Pulsatility index (PI) in the HIE infants compared with Healthy Controls (mean±SD).

The HIE infants are separated into the Normal & Mild-Moderate HIE (green) & Poor Outcome groups (blue) combined. The RI in the Control population ± SD is indicated in grey

5.4.5 Cerebral Blood Flow Velocities (CBFV's)

Peak Systolic Velocity (PSV)

The Peak Systolic Velocity (PSV) in the M and P groups was statistically similar to each other, but significantly different to the N group. The M and P groups were therefore combined as an M&P group and plotted against the N group and the mean±SD of the HC's in Figure 5-3 Peak Systolic Velocity (PSV) in the HIE infants compared with Healthy Control (mean±SD).As can be seen from this graph, the mean PSV is higher throughout in the M&P group than in the N group.

The mean PSV for each of the groups is presented in Table 5-2. The PSV was significantly lower in the N group compared to the M and P groups, and significantly lower than the HC's in the time periods 12-24 PNA (hours) and 48 PNA (hours) to rewarm. The PSV in the N group was similar to the HC's during and immediately following the rewarming. In contrast the mean PSV in the P group was similar to the HC's initially, but became (and remained) significantly higher after 48 hours PNA.

The mean PSV in the M group was similar to the HC's initially but increased and was significantly higher than the HC's in the period immediately prior and following re-warming.

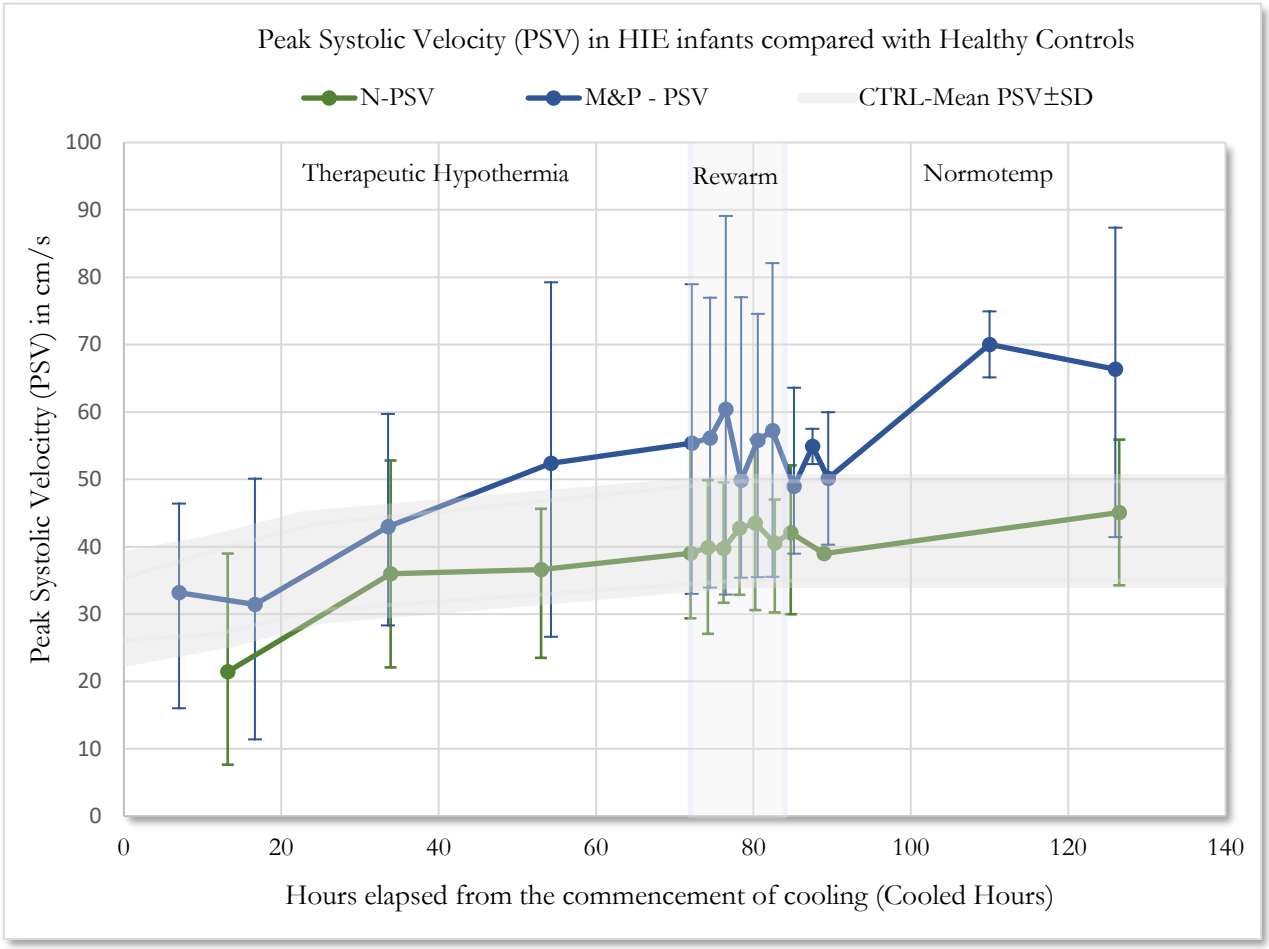


Figure 5-3 Peak Systolic Velocity (PSV) in the HIE infants compared with Healthy Control (mean±SD).

The HIE infants are separated into the Normal (green) and Mild-Moderate HIE & Poor Outcome groups (blue) combined. The RI in the Control population ± SD is indicated in grey.

End Diastolic Velocity (EDV)

As with the PSV, the EDV in the M and P groups was statistically similar to each other, but significantly different to the N group. The M and P groups were therefore combined and plotted against the N group in Figure 5-4.

As can be seen from the graph, the mean EDV is higher in the M&P group, than in the N group. The mean EDV for each of the groups is presented in Table 5-2. The EDV was significantly lower in the N group to the M and P groups and significantly lower than the HC's in the periods of 12-24 PNA (hours) and after 48 hours PNA. The EDV in the M group was statistically similar to the HC's

throughout. In contrast, in the first 24 hours the EDV in the P group was similar to the HC's and statistically higher to the N and M groups , and similar to both the HC's and the N and M groups in the period of 24-48 hours. The EDV in the P group increased to a significantly higher velocity from 48 hours PNA onwards.

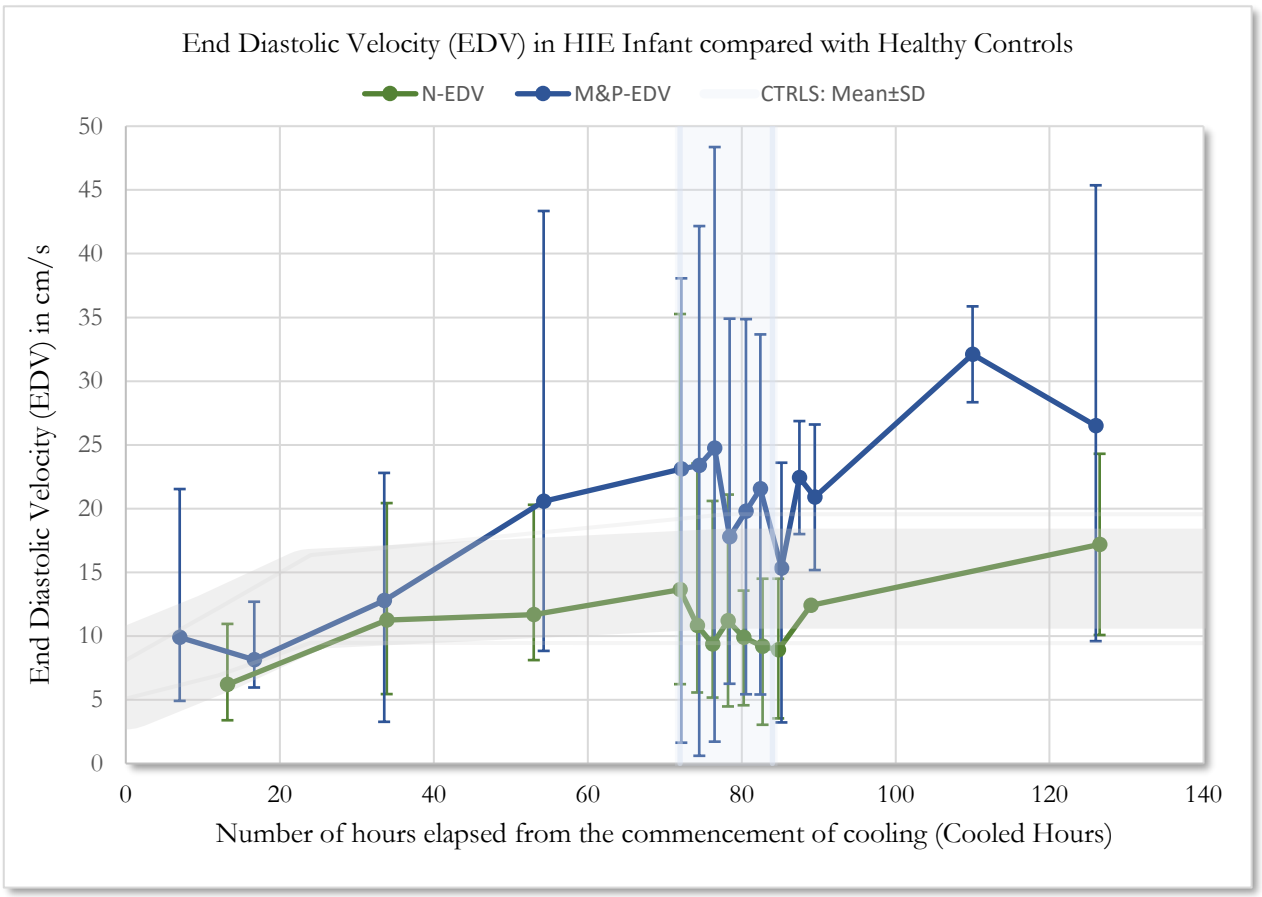


Figure 5-4 End Diastolic Velocity (EDV) in the HIE infants compared with Healthy Controls (mean±SD).

The HIE infants are separated into the Normal (green) and Mild-Moderate HIE & Poor Outcome groups (blue) combined. The RI in the Control population ± SD is indicated in grey.

Time-Averaged Velocity (TAV)

The TAV has a similar pattern to the EDV, as can be seen in Figure 5-5 Time-Averaged Velocity (TAV) in the HIE infants compared with Healthy Controls (mean±SD).The mean TAV is lower in the N group than the M&P group, and significantly lower than the Control population in the periods of 12-24 PNA (hours) and after 48 hours PNA to the end of rewarming. The mean TAV in the M group was statistically similar to the HC's throughout. In contrast, the TAV in the P group was higher than the in the N and M groups and statistically similar to the HC's in the first 48 hours PNA. The

TAV then increases to significantly higher TAV's than the Control population and remains so until the end and immediately following rewarming.

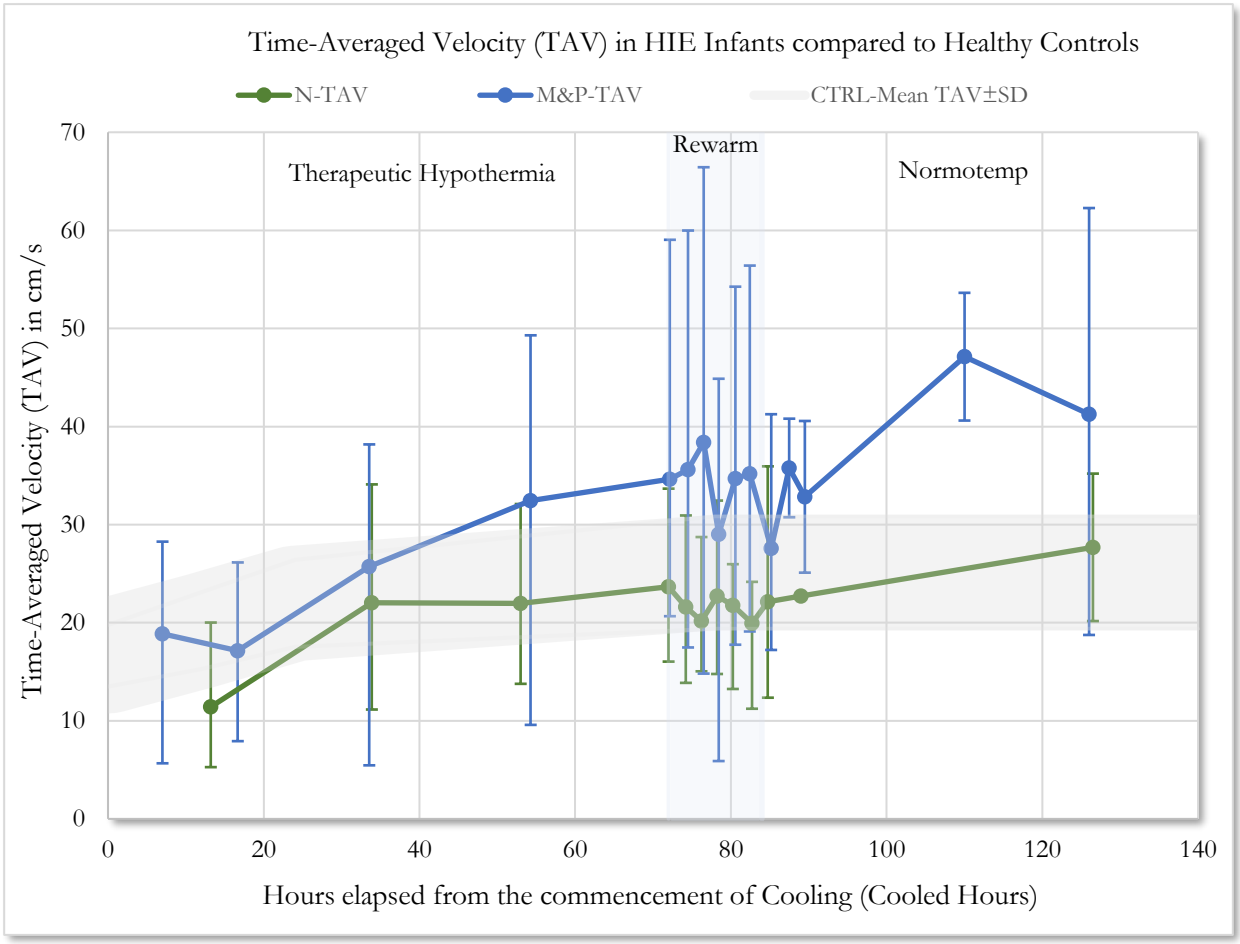


Figure 5-5 Time-Averaged Velocity (TAV) in the HIE infants compared with Healthy Controls (mean±SD).

The HIE infants are separated into the Normal (green) and Mild-Moderate HIE & Poor Outcome groups (blue) combined. The RI in the Control population ± SD is indicated in grey.

Derangements in the CBFV's and RI

There were derangements in the RI and CBFV's in the HIE infants compared to the HC's; these are displayed in APPENDIX E. There were several patterns of CBFV's which resulted in a variety of derangements to the RI; an overview of patterns of CBFV's and RI are displayed in APPENDIX F.

5.5 DISCUSSION

Statistical analysis of the results detected correlations between medium-term outcome and the initial lactate measure (from the cord blood or the first initial blood gas analysis on admission), and the Sarnat score (66). This is in agreement with previous published studies that indicate a higher lactate

may be predictive of moderate-severe HIE post-partum (259, 260). It also may be expected that the outcome is correlated to the Sarnat score, as this is a measure of the severity of the HIE, and the severity of the HIE is likely to be related to outcome.

In this study, if the cut-off for lactate were 13.90 mmol/L there would be 100% specificity, and 55% sensitivity for a poor outcome at this level. As a predictor of moderate-severe HIE, including the development of moderate HIE as a negative outcome, the sensitivity is improved to 75%. In contrast, the sensitivity for an Apgar score at 1 min ($Apgar_1$) <2 was 80% with specificity 37%). Lactate ≥ 13.90mmol/L and $Apgar_1$ < 2 therefore has a sensitivity of 80% and sensitivity of 100%. This was the most predictive combination of biomarkers and was in agreement withWhite et al (260) who also found theApgar score at 1 minute combined with the lactate was most predictive for the progression of moderate to severe HIE. The high sensitivity and specificity for the initial lactate and $Apgar_1$ together could make the combination a useful dichotomous indicator for candidates for hypothermia, especially within the 6 hour post-natal pre-treatment window. Beyond that window the initial lactate and $Apgar_1$ do not offer an ongoing, continous measure by which treatment may be guided..

The currently accepted dichotomous, and repeatable, indicator of a hypoxic event, and subsequent recovery, in neonates is the RI. Previous studies report that an RI < 0.55 is an indication that a sentinel hypoxic event has occurred, and has been previously been reported in infants with HIE (44, 75, 239, 244, 261-270). In the results of this study as presented here, the mean RI in the P group was similar to the Healthy Controls (HC's) until the commencement of rewarming. The mean RI was < 0.55 in the M group in the first 12 hours but returned to an RI similar to the HC's in the subsequent 24-hour period. The RI in the N group was similar to the HC's initially, but increased to significantly higher RI's during and immediately following rewarming. The differences of RI to the HC's were most evident in both the P and N groups only after rewarming had commenced. Moreover, a higher percentage of infants in the N group had an RI of < 0.55 in the first 72 hours than in the P group: 3 of 4 infants in the N group showed RI's < 0.55 in the first 72 hours (75%)compared to only 1 of 6 of the P group infants (17%).The P group infants were more likely to present with an RI < 0.55 after rewarming had commenced.

Although some of the previous studies have reported that low RI's < 0.55 on the first day of life are associated with moderate-severe HIE and poor outcome (46, 75, 239, 244, 261, 262, 266, 271, 272), there are several that have reported a delay before the RI falls (44, 263, 264, 273-276); in this study, a delayed drop in RI was observed in some cases. Some studies suggest that a delayed drop in the RI may be more predictive of a poor outcome (44, 264, 273, 275, 276). Moreover, there are also studies that have reported higher RI's in the first 24 hours in infants who later develop moderate-severe HIE

and ultimately have a poor outcome (46, 244, 264-267, 270, 277); some of these studies describe a subsequent fall in the RI over the following days (264, 267, 268). Still more studies have suggested that it is the infants with poor outcomes who have the widest fluctuations in RI (265, 278). There is an overview of previously published studies of the variation in the DUS parameters in infants with HIE and their reported results in APPENDIX D; Table 5-4 is a summary of those studies.

In the 6 infants with poor outcome in this study there were 2 that initially had lower RI's (HIE4-P and HIE15-P), 3 (HIE5-P, HIE9-P and HIE13-P) with RI's in the normal range (within 2 SDs of the mean RI of the HC's), and 1 (HIE8-P) with an RI > 2SD's of the HC's. Of the 2 with initially lower RI's, HIE4-P was normalised by D2, and was higher than the range of HC's during rewarming, HIE15-P remained low throughout and through to D6. The infants with the initially normal RI's also had disparate behaviours: 2 (HIE5-P and HIE9-P) became abnormally high on D2 (>2SDs), with the RI of HIE5-P dropping markedly at the commencement of rewarming, staying low until D6. Measurements of HIE9-P had to be abandoned due to a clinical deterioration after the increase in the RI. The RI in HIE13-P remained within 1 SD of the HC's, even though the RI was < 0.55 at times, until rewarming when it became severely deranged (<2SDs and <0.55). The RI in this infant returned to <1SD of the HC's immediately following rewarming. The one infant with the high RI initially (HIE8-P) had the RI remain high until normalising on D3, but then subsequently deteriorated and again measurements had to be abandoned. These 6 infants exhibited 6 different patterns, that could indicate, as in the previously mentioned published studies, that a low RI, a high RI, a falling RI or a fluctuating RI could all indicate a poor outcome.

Similarly, there was an absence of any pattern in the infants with a normal outcome. The RI was initially high and remained high (>2SDs higher than the HC's) in three infants. Four initially had RI's within 2 SDs of the HC's, 3 of which had RI's increasing to >2SDs of the HCs (2 prior to rewarming and 1 at the commencement of rewarming) and one in which the RI dropped to below < 0.55 and less than 3SDs of the HC's. In the two that had initially low RI's (<2SDs below the HC's), the RI steadily increased during treatment; though in one the RI had not returned to within 1SD of the HC's until D6.

Furthermore, of the 3 infants with evidence of mild-moderate HIE and normal outcome, there were 2 with initially high RI's: 1 had the RI increase to >0.9 during rewarming, and 1 which normalised (became within 1 SD of the HC's) prior to rewarming before increasing again to an RI >3SDs higher than the HC's. The third mild-moderate HIE infant had very low RI initially (<4SDs of the HC's, RI <0.44), that remained low until normalising during rewarming.

This variation in the RI suggests that there are not only individual differences between the infants, the nature of their hypoxic injury, and their response to the hypoxic event, but also other factors influencing the RI. Hypothermia could be one of these confounding factors. Many of the published studies were undertaken prior to the advent of hypothermia treatment, and so the majority have studied infants who are not cooled. In studies where infants are cooled, the RI has been shown to be less predictive (42, 279).

The RI is considered to be reflective of the cerebrovascular resistance (169, 238), and the inverse correlation with the diameter of the ACA found in this study supports this assumption. As hypothermia causes vasoconstriction (280, 281), it is possible that the RI measured in cooled infants is higher than it would be if the infants were not cooled, further confounding the meaning of the RI. Interestingly, in studies investigating the value of the RI in infants undergoing TH, there is no agreement regarding the value of the RI before, during or after cooling. Gerner et al reported that an RI < 0.6 prior to cooling was associated with poor outcome, with the post-cooling being not significant (271). Skranes et al suggested that the RI during cooling was not predictive, but an RI < 0.55 after rewarming was significant. Elstad et al found that although the predictive power of the RI during cooling was reduced when compared to studies at normothermia, it was most predictive at 48h PNA, whilst the infants were cooled (275).

It might not just be the vasoconstrictive effect on the cerebrovasculature that affects the predictive power of the RI during cooling and after cooling. The neuroprotective effect of the hypothermia may reduce the negative predictive value, by improving the prognosis of those infants who would otherwise have a poor outcome. There are also other factors that may affect the resistance of the cerebrovasculature and thus the predictive value of the RI, including the increased use of inotropes during cooling that may cause vasoconstriction thus increasing the RI (275). It is also possible that the use of inotropes is greater in infants with the worst clinical condition, who could initially have been more likely to have lower RI's.

Table 5-4 Overview of the Cerebral Blood Flow Velocities (CBFV's) and Resistive Index (RI), and their corresponding relationships as reported in previously published studies of infants with HIE.

	Low RI	High RI	Not Specified
Low or Reversed EDV	<6h: Eken, 1995 (2/32)	<12h: Simon, 2011 : Kirimi, 2002 (10/23) 12h: Barseem, 2015 <24h: Liu, 2007(1) D1-3: Yukinori, 1983 (1/5) N/S: Daneman, 2006	
Low TAV		4-31h: Levene, 1989 (4/34)	
Low PSV		12h: Barseem, 2015 <12h: Kirimi, 2002 (10/23)	
Low CBFV's (All or not specified)	<24h: Liu 2007(1)	<24h: Liu 2007(1), Liu 2007(2) Bennhagen (1998) <72h: Guan, 2017 (43/44)	
High EDV	<6h: Eken, 1995 (2/32), 6h+: Eken, 1995 (9/32) <24h: Liao, 1997 (PPV 91%) : Stark, 1994 (PPV 75%) >12h: Simon, 2011 D1-3: Kudreviciene, 2014 : Yukinori, 1983 (4/5) D1-D7 Bada,1979 D2-4: Van Bel, 1987 N/S* : Daneman, 2006		>24h: Low, 1994 >24h: Low, 1994 (19/26)
High TAV	<24h: Liao, 1997 (PPV 91%) 4-133h: Levene, 1989 (17/34) D2-4: Van Bel, 1987 D2-D5: Gray, 1993 (93%)		D1-D3: Fukuda, 2005
High PSV			>24h: Low, 1994 (19/26)
High CBFV's (All or not specified)	>12h: Ilves, 2004 <24h: Liu, 2007 (1) 30-85h: Bennhagen, 1998 (7/18) <72h: Guan, 2017 (1/44)		
Not Specified	Pre-cooling: Gerner, 2016 <24h: Jongling, 2002 (PPV 90%) 24-72: Elstad, 2011 (PPV 60%) 48h: Elstad, 2011 (PPV 64%) 48-72h: Mires, 1994 (MCA only) <62h: Archer, 1986 (PPV 86%) <72h: Jongling, 2002 (PPV 71%) Jongling, 2002 (PPV 78% - intrapartum hypoxia only) Pikala, 2018 (ACA-PPV 88%, MCA-PPV 50%) Post-Cooling: Skranes, 2014 (PPV 100%)		

N/S: Not specified. The number of affected infants out of the total number of infants in the indicated study or the Positive Predicitive Value (PPV) is given in the brackets.

Perhaps the RI should be not be used in isolation; the CBFV's from which the RI is calculated, the PSV and the EDV, or the time-averaged velocity provide us with additional information which may assist in differentiating between cases. Previously published studies indicate there are three patterns of alteration to the cerebrovasculature and CBFV's that are associated with HIE and poor outcome; a low RI (<0.6) most commonly associated elevated CBFV's (44, 46, 264, 266, 270, 273) (particularly EDV (75, 239, 244, 261-263, 268, 276)), a low RI (<0.6) paired less commonly with reduced CBFV's (239, 266), or an increased RI (RI > 0.75) with low CBFV's (46, 244, 264, 267, 268, 270, 277, 282). Increased CBFV's have been reported only with low RI's, which implies vasodilation, most likely as the response to a hypoxic event (75). Prolonged low RI's and increased CBFV's could therefore suggest vasoparalysis and hyper-perfusion, the hyper-perfusion known as "luxury perfusion", linked to moderate-severe HIE (266) and associated with poor outcomes. (44, 46). Decreased CBFV's would suggest hypoperfusion; low CBFV's have previously been reported in infants both with mild HIE (46, 270, 277, 282) and severe HIE with poor outcome (266).

In this study, the 4 infants (HIE3-N, HIE12-N, HIE16-N and HIE18-N) in the N group who had RI's initially within 2SDs range of the normal population, also had CBFV's within 2 SDs of the HC's. Three of these infants had an increase in the RI but the CBFV's remained within the normal range, except for one PSV measurement in HIE16-N between 74-76 cooled hours. The RI in HIE18-N dropped at the commencement of rewarming due to an increase in EDV. The 2 infants with normal outcomes and low RI's (HIE6-N and HIE7-N) both had PSV's (and therefore also TAV's) more than 2SDs lower than the normal population. The RI had normalised by the next examination and then increased in both these infants; 1 with normal or lower CBFV's, 1 with normal or higher CBFV's, and one with increased CBFV's. Two of the infants with increased RI's in the normal outcome group (HIE1-N and HIE11-N) had normal CBFV's throughout, the other with a high RI (HIE10-N) exhibited lower CBFV's initially that normalised in subsequent examinations. Interestingly, all of the normal outcome infants had normal or high RI's prior to rewarming, including the 2 infants with initially lower RI's, and all were more than 2SDs higher than the normal population during rewarming.

Only 1 of 3 infants in the M group (HIE2-M) had a low RI initially and this remained low throughout; initially presenting with low PSV/TAV, and then subsequently all CBFV's became high. The other two M group infants (HIE14-M and HIE17-M) had high RI's initially, but the CBFV's were within the normal range. Both had their RI normalise prior to rewarming, only to increase to greater than 2 SDs again during rewarming; this was due to an increase in the TAV of 1 of these infants (HIE14-M), but in the other (HIE17-M) the RI increase was due to an EDV drop and the EDV was less than 2 cm/s for the first half of rewarming. The very low EDV resulted in a very high RI (>0.9) in the first

half of rewarming and remained greater than 3SDs higher than the normal population for the latter half. Two of the M group infants had higher RI's during rewarming, the other one had a lower RI.

Likewise, it is difficult to ascertain any patterns in the infants in the P group. Infants HIE4-P and HIE15-P had low RI's initially, but the former had decreased PSV/TAV and the CBFV's in the latter were all increased. The RI and PSV normalised in HIE4-P but the EDV/TAV became elevated, prior to the RI increasing with normal CBFV's (<1SD of the Healthy Controls) during rewarming. HIE15-P had persistently higher CBFV's; the RI increased to within 1SD of the HC's prior to rewarming, but dropped again during rewarming.

Initially, HIE9-P and HIE13-P both had normal RI's and normal CBFV's. The RI remained normal in infant HIE13-P with initially increased PSV, then dropped during rewarming when the EDV increased. HIE9-P had a sharp drop in EDV with a corresponding jump in RI before clinically deteriorating. HIE5-P's RI was initially normal, though with decreased PSV/TAV. The RI then increased with a decrease in EDV but dropped to more than 2SDs below the RI in the normal population during rewarming, with an increase in all CBFV's. HIE8-P had a high RI with a high PSV during initially but the RI and CBFV's all normalised prior to the infant deteriorating clinically.

A lower than normal RI was observed more often when all CBFV's were increased, the EDV/TAV increased, or a decrease in the PSV only. Contrary to the studies of Eken et al (239) and Liu et al (266), a low EDV was not observed with a low RI at any time during this study. This could possibly be due either to the low precedence of this combination, or because these studies examined infants before 6h and 24h respectively or because the infants in this study were examined later (5 of 18 did not have their examination until after D2 due to delays in enrolment). Similarly, a low RI was not seen with all CBFV's in the normal range.

A high RI was the most frequent observation in HIE infants, especially in the infants with a subsequent normal outcome. Previous studies (46, 244, 264, 266-268, 270, 277, 282) reported that high RI's were most commonly seen with low CBFV's, whereas in this study a higher than normal RI was most often seen with normal CBFV's. In agreement with previous reports, high RI's were also seen with low EDV or low PSV, but they were also seen (albeit less often) with increased TAV/PSV. A normal RI could be observed with normal CBFV's, increased CBFV's, or a lower than normal PSV.

Daneman et al proposed that the degree of the decrease of the RI and its duration is indicative of the severity of the HIE (265). Certainly in this study, of the 6 infants with poor outcome, half had RI's lower than 0.55; two of which still had low RI's on D5/D6. Of the infants with normal outcome, one mild-moderate HIE infant had a low RI which normalised during rewarming, and 2 infants with

normal outcome who had low RI's initially did have the RI increased prior to the rewarming. 1 infant with normal outcome also had the RI drop during rewarming. Thus, these results from this study do not fully support Daneman's postulate.

Studies conducted by Levene, Ilves and Liu (44, 46, 266) have also suggested that an EDV <2SDs or CBFV's >3SD, signalling hypoperfusion or hyper-perfusion respectively, are most indicative of a poor outcome. In support of this, 5 of the 6 infants with a poor outcome in this study had either low or high CBFV's at some stage during the study (2 with EDV's less than 2SDs and 3 with CBFV's greater than 3SDs of the normal population). Although this means that extremely high or low CBFV's would have a high sensitivity, the specificity is not as favourable. All of the mild-moderate HIE infants (3 of 3, 1 low and 2 high CBFV's), and 5 of the normal outcome infants (5 of 9, 5 low CBFV's) had either extremely high or low CBFV's.

In general, infants with a normal outcome displayed lower CBFV's initially than those infants with poor medium-term outcome, and this was significantly lower than the normal population in the period of 12-24 hours. The mean PSV in the poor outcome group increased and only became significantly higher than the mean of the normal population in the period of 24-48 hours. After 48 hours PNA all infants with a poor outcome had PSV's higher than the mean PSV in the control population, and during rewarming, the EDV and TAV were also significantly higher than the normal population in the poor outcome group. This could suggest that the CBFV's, and particularly the PSV, may be more indicative of altered cerebral haemodynamics associated with HIE.

This extreme derangement of the CBFV's is the feature from the Doppler parameters that best distinguishes poor outcome infants from the normal outcome infants in the period immediately prior to rewarming. It is in this period prior to the rewarming that the CBFV, particularly the PSV, is more likely to be elevated, or the EDV more likely to be decreased in the infants with a poor outcome. Of the infants with a poor outcome, all had increased CBFV's (PSV only n=1, PSV/TAV n=1, EDV/TAV, n=1, all CBFV's n=1), or decreased EDV (n=2) which yields a sensitivity of 100%. In the infants with normal outcome only 1 of 9 had increased CBFV's in the period prior to rewarming, though 2 of the 3 mild-moderate cases with normal outcome also had increased CBFV's (TAV only n=1, all CBFV's n=1). The specificity of CBFV's being <2SDs or >2SDs of the normal population is 75% (9 of 12).

The RI in this same period did not distinguish between normal and poor outcome in this period; the RI of the P group was not significantly different to the HC's. All infants with poor outcome examined immediately prior to rewarming (5 of the 6 infants) had RI's within 1 SD of the normal population.

In the early study of Bada et al in 1979, the mean RI in the asphyxiated infants was lower than their normal counterparts; but there was overlap between the lower measurements in the normal infants and the higher RI measurements in the asphyxiated infants (75). Overlap of the RI and CBFV's between the HIE and normal population, and within the HIE population, has been reported in many of the subsequent studies in this field (44, 46, 244, 262-267, 270, 274, 277, 282, 283). In our study and in those considered during the analysis there is overlap in the RI between the normal outcome groups and the poor outcome groups. Thus, it is very unlikely that a single measurement of the RI would be adequate to identify those infants likely to have a poor outcome.

The extreme variability and lack of difference of the RI between poor and normal outcome HIE infants, and HC's, makes it an unsuitable as a parameter upon which to base or guide treatment protocols. The CBFV's contribute more information and may improve the predictive power of the RI. Nonetheless, there was still variability and a lack of clearly discernible patterns related to mediumterm so suggests that the CBFV's are also unreliable as parameters on which to base treatment. The use of the CBFV's also makes angle correction necessary (74) and would require an agreed and dependable set of normal mean CBFV's, with their corresponding SDs, against which operators could compare measurements. Nonetheless, CBFV's may be an area worthy of further study.

The high variability and fluctuations we have observed in this study have been reported previously, with suggestions that the infants exhibiting the greatest variability and widest fluctuations are at highest risk of poor outcomes (265, 278). As can be seen in APPENDIX E and APPENDIX F showing the derangements of the RI and CBFV's in all of the HIE infants, there were large fluctuations in the RI and CBFV's in all the HIE infants, regardless of outcome; the RI and CBFV's in some cases increased and in other cases decreased. The fact that this is not a clearly discernible or distinguishing feature between the normal and poor outcome infants makes this also an unsuitable criterion on which to base or guide treatment. Moreover, Daneman et al (265) have described brief transient variations in RI and EDV over short periods of only a few minutes. Variations such as this were also observed during this study when measuring the CBFV's in healthy infants. Transient variations further complicate the process of using CBFV's and the RI as determinant parameters; measurements attempted during transient periods could cause large variations between consecutive measurements. The possibility of large variations renders the Doppler parameters unsuitable for the purpose of treatment guidance where definite identification of the clinical and cerebral status is required.

Fluctuations in the CBFV's and subsequently the RI were evident throughout cooling, and particularly also during the rewarming process. There are several physiological parameters that have been found to influence the cerebrovascular resistance and the CBFV's, namely mean arterial blood pressure

(MABP) (169, 268, 284), intra-cranial pressure (ICP) (169, 284, 285), and the partial pressures of carbon dioxide ($PaCO_2$) and oxygen ($PaCO_2$) (268). Myocardial function (265, 267) and cardiac output (CO) (267), blood viscosity (263) and hypovolaemia (265) could also have an impact on the cerebral haemodynamics. All of these parameters could be altered by a hypoxic event and subsequent HIE, including cerebral oedema as a result of hyper-perfusion and by the cooling and the rewarming processes, by prescription medications for clinical regulation, by increasing the fraction of inspired oxygen (FiO_2), or by mechanical ventilation. Additionally, there may also be variation due to neonatal seizures, common in HIE infants.

Perlman et al monitored 12 preterm neonates during seizures and in all seizures observed in all 12 infants there was an increase in MABP and ICP which was accompanied by an increase in CBFV's and a decrease in RI, without any changes to $PaCO_2$ or PaO_2 (284). The RI returned to initial values in around 5 minutes post-seizure. Similarly, while analysing seizure events Greisen et al (169) demonstrated a direct correlation between systemic haemodynamic events and cerebral haemodynamic changes. They found that seizures caused an increase in the HR and MABP, the TAV increased accordingly to the MABP and the RI lowered, which establishes a causal link between the MABP, cerebral blood flow velocity and RI. These changes were seen even in subtle seizures (284); in cooled, sedated infants such as those in this study, seizures that may not be obvious without reference to aEEG.

Thus an increase in MABP may cause an increase in ICP, which could consequently increase the CBFV's, and in particular this increase was observed in the EDV (284). An increase in EDV would lower the RI (169, 263, 284, 285). Also, the increase in ICP may not be related directly to an initial increase in MABP. The hyper-perfusion common in infants with HIE can cause cerebral oedema that would increase the ICP independently to the MABP and might result in an increase in the RI (263, 286, 287). The competition of the two mechanisms may be the reason that Behrens et al found that the association between RI and ICP was not reliable when conducting a study to investigate the feasibility of using the RI as a surrogate measure of ICP (288).

An increased MABP is also likely to result in a decrease in the compliance of the cardiovascular and cerebral arteries: that would primarily affect the PSV, and would thus be reflected in the RI (263). The correlation between the MABP and the PSV observed by Simon et al would support this causal link in HIE, although there were also correlations detected with the CBFV's, EDV and PSV to the both the $PaCO_2$ and PaO_2 (inverse) in their study that would have also resulted in some variation in the CBFV's not related directly to MABP (268).

The impact that these have on the cerebrovasculature and the CBFV's is likely to depend on whether the infants have intact cerebral autoregulation, an ability to maintain cerebral blood flow (CBF) with varying MABP. Cerebral autoregulation is disturbed in HIE (43, 75, 169, 178, 244, 284, 286) and explains both the changes in CBFV's and RI with changes in the MABP and the exaggerated fluctuations observed in the CBFV and RI measurements observed in this and other studies (265). A measure of cerebral autoregulation would probably be a better reflection the severity of the HIE, and restoration of cerebral autoregulation could be an improved indicator of recovery. The difficulty lies in the assessment of cerebral autoregulation, as it firstly requires measurement of the CBF, then a variation of MABP must occur to assess whether or not CBF remains stable.

Cerebral blood flow can be estimated from the CBFV's if the diameter of the vessel is constant (169, 178, 289). The correlation detected between the diameter and the RI of the ACA, and the variation of the RI suggests that there are dynamic changes occurring to the diameter of the vessel. These dynamic changes would most likely make any estimation of the CBF inaccurate. Moreover, hypo- or hypertension, or vasoconstriction could cause overestimation of up to 100% of the CBF (289). Not only does hypothermia cause changes to MABP and the cerebrovasculature, which disturbs the relationship between the CBFV's and CBF, but infants undergoing TH are also sedated and often given anticonvulsant drugs, further impairing normal thermoregulatory vasoconstriction (280). All of this indicates that assessments of cerebral autoregulation (CA) using CBFV's or RI would be problematic.

Even if CBF could be measured reliably, then a change to the MABP would be necessary to make an assessment of whether there is a corresponding change in CBF: that would indicate such lapsing in CA. The common use of inotropes to maintain blood pressure would most likely make this difficult as there would be little change in the MABP during clinical care, as was seen in the study by van Bel et al (263). If it were possible to use the spontaneous fluctuations in MABP, there is the further difficulty of reliably and concurrently obtaining the DUS measurements before and after the change in the MABP, given the fast, transient nature of those changes. DUS is a one-off measurement, so co-ordination with changes to the MABP would most likely be challenging. This one-off nature of the measurements of the DUS parameters also offers a further explanation for the variation of the CBFV's and RI seen in the various studies, presented in Table 5-3, as the examinations were made at different times depending on the design of the studies. The HIE evolves over a period of days from the initial hypoxic insult; the duration and intensity of this insult will impact on the progression, severity, and duration of the HIE, but the epidemiology and timing of the hypoxic event is often unclear. As the development of vasoparalysis associated with HIE reportedly takes at least 6 hours, infants with

changes in cerebral perfusion markers measured by Doppler Ultrasound prior to 6 hours of age would suggest that they have had an earlier insult. As an example, an infant exhibiting higher CBFV's and a lower RI by 3 hours of age was found to have had an early hypoxic event caused by an abruption of the placenta around 6 hours before birth (44).

After the initial hypoxic insult, the individual's physiological response evolves into HIE which has a progression causing changes to the cardiovascular and cerebrovascular parameters in subsequent hours and days. Several of the studies mentioned describe a variety of changes to both the CBFV's and the RI occurring in the first hours, first day and up to several days after the hypoxic event (42, 239, 263, 267, 268, 278, 290). Nonetheless, there are distinctions between these studies, and also differences between individuals and groups within studies. Indeed, there were variations in the derangement and the timings of the derangements between infants and groups in this study.

The cerebral perfusion, and therefore the DUS parameters as measures of cerebral perfusion, will depend on the timing of the measurement and the recovery of the patient. If a measurement of the DUS parameters is made near to the time of a seizure, or other clinical event such as the dispensing of a prescribed medication, then the parameters may not reflect that the alterations are due to the HIE at all, rather a reflection of the changes to the physiological parameters caused by the seizure or clinical event. The time-dependent progression of HIE, and the impact of clinical interventions, makes the timing of the examination important, and the comparison between infants, and the correlation of measures to outcome, extremely difficult.

The location of the measurement, the cerebral artery or area of insonation, may also yield different results. Given the variation in CBFV's, reference values must match the artery being interrogated. In addition to normal differences in CBFV's between arteries, differences have also been reported in HIE infants in the RI (44, 274, 290) and its predictive power (273, 287) calculated from CBFV's in different arteries. Thus, CBFV's and RI measurements made in only one artery may not be representative of the global cerebral perfusion. In a controlled study of non-human primates, the RI in the anterior artery was significantly lower in the neonates who died or developed CP compared with the Controls, whereas the RI in thalamus was significantly higher in the poor outcome group, suggesting that there was local hypoperfusion only in the thalamus (291). Interestingly, the RI was also significantly higher in the thalamus in the cooled versus the non-cooled asphyxiated infants, an indication of the vasoconstriction caused by hypothermia, but the RI in the ACA was not higher in this hypothermic group. This signifies that CBF and therefore cerebral perfusion might not be homogenous: a further complication.

5.6 HYPOTHESIS 1 OUTCOME

5.6.1 Hypothesis 1 Outcome

In this study, the Resistive Index (RI) was found not to be a dependable marker of altered cerebral perfusion and did not reliably indicate the severity of Hypoxic-Ischaemic Encephalopathy (HIE) during TH.

5.6.2 Alternative Hypothesis 1 Outcome

Severe derangements in the cerebral blood flow velocities (CBFV's) were more indicative than the RI's though there was no reliable pattern of derangement in the HIE infants with poor outcome. As the CBFV's were more indicative than the RI, this may be an area for further research. A disadvantage of the CBFV's is that a baseline of all CBFV's would be required in a healthy control population to compare against should the CBFV's be used as a clinical indicator to guide treatment. Furthermore, CBFV's also suffer from being a one-off measurements, that are not able to be continuously monitored.

5.6.3 Hypothesis 1 Conclusion

Neither the CBFV's nor the RI are suitable indicators of hyper-perfusion, and therefore severity in HIE infants. Due to the lack of reliability and discontinuous nature of the DUS parameters, neither the CBFV's nor the RI are suitable for use as a clinical indicator for guiding TH treatment.

5.7 CONCLUSION

The CBFV's and the RI, an accepted indication of cerebrovascular resistance and of a prior hypoxic event, were thoroughly investigated in this study. This was not only to evaluate their reliability to predict poor outcome in HIE, but primarily to determine whether any DUS parameters are reliable enough to guide treatment in the future. If a parameter is to be used to guide treatment, it must be specific and sensitive, and reliable with a clear and precise meaning.

The results of this study did not find a clear pattern of alteration to the RI, and in fact demonstrated that the RI may be normal whilst CBFV's are not and vice versa. The CBFV's were the most predictive of the DUS parameters, with a derangement of <2SDs or >2SDs having a sensitivity of 100%. Severe derangement of the CBFV's was also observed in normal and mild-moderate infants, which resulted in the specificity of severe derangement being only 75%. Thus, neither the CBFV's nor the RI was able to distinguish reliably between normal and poor outcomes in HIE infants.

This lack of reliability is most likely due to the often uncertain timing and severity of the initial hypoxic episode, the timing of the measurement in relation to the progression of the HIE (and therefore the alterations to the cerebral perfusion), and individual differences in response to the hypoxic event. $PaCO_2$, PaO_2, MABP and ICP have all been found to be correlated to the RI and CBFV's in some studies, although also not correlated in others. These contrasting findings are most likely due to the clinical condition of the infants and whether or not they have intact CA, the assessment of which cannot be made accurately when there are concurrent dynamic changes to the cerebral vasculature. Furthermore, the changes to the RI and CBFV's are probably also obscured by not only the TH treatment, but most likely also the sedatives and inotropes given to the infants whilst they are cooled.

Given the wide range of influencing factors and thus the uncertainty in the DUS parameters in infants with HIE, these parameters cannot be considered reliable markers of cerebral perfusion and are not suitable to guide treatment. Thus the hypothesis that the RI would be a reliable indicator of the severity of HIE has been rejected by theseresults. Furthermore, the conclusion from this study must be that there are no DUS parameters that, inidividually, are clear and precise enough, with sufficient predictive power, to be suitable for use as a clinical indicators to guide treatment for HIE infants.

CHAPTER 6. FREQUENCY DOMAIN NEAR-INFRARED SPECTROMETRY (FD-NIRS) AS AN ALTERNATIVE BIOMARKER OF CEREBRAL PERFUSION

6.1 INTRODUCTION

The Resistive Index (RI), the currently accepted indicator of a hypoxic-ischemic insult, and the CBFV's were investigated as markers of cerebral perfusion in HIE infants in Chapter 5. Although there were derangements in the CBFV's and RI, as derived by Doppler Ultrasound (DUS), the derangements not reliable cerebral perfusion markers; thus a better, more reliable marker of cerebral perfusion was sought.

The most important requirement for a cerebral perfusion marker in HIE infants is that it is able to distinguish between normal cerebral perfusion (that which is matching metabolic demand) and abnormal cerebral perfusion – hyper-perfusion resulting from a hypoxic-ischemic (HI) event. As HIE infants are cooled, which may alter cerebral perfusion and oxygen saturation, this cerebral perfusion marker needs to be able to distinguish between alterations resulting from Therapeutic Hypothermia (TH) and those due to HIE. The Doppler parameters are also just a "*snapshot*" view of the cerebral perfusion; it would be better if this cerebral perfusion marker could monitor the cerebral perfusion continuously and track trends. In addition, a method of assessing cerebral perfusion non-invasively would promote its use and acceptance.

Accordingly, Near-Infrared Spectroscopy (NIRS) was explored as an alternative to Doppler Ultrasound on account of its potential to indicate the adequacy of the cerebral perfusion non-invasively and continuously.

6.2 NEAR-INFRARED SPECTROMETRY AS A MEASURE OF HYPER-PERFUSION

Hypoxic Ischemic Encephalopathy (HIE) results from a deprivation of oxygen to the brain of the infant during birth. The lack of oxygen forces the cells to revert to producing energy anaerobically. The initial physiological response to the shortage of oxygen is known as the "*brain-sparing*" effect; reducing the resistance of the cerebral arteries, and increasing peripheral vascular resistance, preferentially redirecting blood flow to the brain to restore oxygen (O_2) delivery to the brain tissue for normal aerobic energy production.

After this period of brain-sparing, the resistance of the cerebral arteries may not recover immediately, resulting in prolonged vasoparalysis and subsequently hyper-perfusion: perfusion exceeding metabolic demand. Hyper-perfusion accelerates restoration of the O_2 supply, causing rapid production of injury provoking oxygen free radicals, increasing cerebral blood volume (CBV) and intracranial pressure (ICP) which may cause additional injury to the brain tissue. Therapeutic Hypothermia (TH) ireduces brain injury associated with HIE by slowing the metabolism, thereby moderating the production of oxygen free radicals, and mitigating increased cerebral blood flow and hyper-perfusion.

Asphyxiated infants who later develop Hypoxic-Ischaemic (HI) brain injury have been reported to have hypo-perfusion on D1 and hyper-perfusion on D2 and D3, despite TH (21). It is possible therefore that the severity of the HIE will be mirrored by the extent and duration of the hyper-perfusion; and the infants with prolonged hyper-perfusion continuing beyond the 72h window of TH will be at the highest risk of poor outcome, death or disability.

The first hypothesis of this study was that the RI would be a reliable indicator of hyper-perfusion given the clinical acceptance. Although there were alterations to the CBFV's and RI, as determined by DUS, indicating alterations to the cerebral perfusion in infants with HIE, they were not found in the earlier part of this study to be trustworthy indicators of hyper-perfusion; they were also not correlated with the severity of the HIE or outcome. Thus additional forms of monitoring were investigated to determine whether they may be able to offer a quantitative, reliable indication of the changes to cerebral perfusion related to HIE.

Near-Infrared Spectrometry (NIRS) is a non-invasive technique for determining the concentrations of HbO and HbR, or simply the proportion of HbO to HbT, present in the body, and brain, tissues by calculating the amount of near-infrared light absorbed by that tissue (292). Blood present in the tissue structures is a combination of oxygen-rich arterial blood delivered via the arteries, and oxygen-depleted venous blood prior to its return to the heart and lungs for reconditioning. The tissues metabolise the HbO in the blood to HbR, so there is expected to be a lower saturation, or lower proportion of HbO to HbT in the cerebral tissues than is found in arterial blood. Due to low absorption of haemoglobin in the near-infrared range above 650nm, near-infrared light can penetrate several centimetres into the body, even through the skull or scalp. Thus when near-infrared light is emitted into a body, a measurement of the light reflected allows the calculation of the concentrations of those substances that absorb the light in the underlying tissues.. Cerebral oxygenation, the oxygen saturation of the brain tissue, and thus the adequacy of the cerebral perfusion can therefore be assessedby interrogation of the brain tissue with near-infrared light, through the scalp and skull.

Near-Infrared Spectroscopy (NIRS) devices are able to measure and monitor the cerebral HbO and HbR concentrations in neonates utilizing light in the near-infrared spectrum, as the light can penetrate into the grey-matter due to the neonate's delicate skin and skull structure. The NIRS measurement is achieved by placing a sensor consisting of near-infrared light emitters and corresponding light detectors, on the scalp. The detectors measure the amount of light not absorbed but reflected; this relates directly to the concentrations of substances present in the brain tissue. This relationship for a sample containing HbO, HbR and H_2O, such as brain tissue, can be described by the Beer-Lambert Law (Equation 1-1), which relates how much light is absorbed to the concentrations of substances present:

$$\mu_a = K_{HBO}[HbO] + K_{HBR}[HbR] + K_{H_2O}[H_2O] \quad (293)$$

Equation 6-1 Beer-Lambert Law for HbO, HbR and H_2O

The absorption co-efficient μ_a, measured in cm^{-1}, at a given wavelength is the linear addition of the light absorbed by each individual component; and each component absorbs light depending on its concentration and its absorbance at that wavelength which is given by *K*, the extinction coefficient. The extinction coefficients indicate the rate of light-absorbance of the chromophores, the light-absorbing parts of the consitutent molecules of the substance. The extinction coeffients for HbO and HbR have been previously derived (294, 295).

For each wavelength an equation can be formed relating the amount of light absorbed to the concentrations of the consitutent components; if the number of constituent components equals the number of wavelengths, and the extinction coefficients are known, then the set of simultaneous equations can be solved. Wavelengths are chosen to optimise measurements for the desired components to be measured, whilst minimising absorption by components present in the sample which will not be measured.Thus, with the use of 2 suitable wavelengths this principle can be used to focus on the measurement of Oxy- and Deoxy-Haemoglobin (HbO and HbR) in the the neonatal brain. The neonatal brain has a significant concentration of water in tissue which is known to be between 70-80% in neonatal brain tissue. Thus even with optimal choice of wavelenths, water needed to be accounted for. With only 2 wavelengths used, the concentration of only 2 components, HbO and HbR, can be measured. An additional wavelength could be included and a 3rd equation added. An alternative method, and the one used in this study, was instead to assume a water concentration of 75% in calculations in line with similar studies (296), to account for the interaction of the water with light.

All clinically-available NIRS devices provide a measure of cerebral oxygenation; the oxygen saturation of the interrogated tissue known as the regional cerebral oxygen saturation. Although current papers use various abbreviations to represent cerebral oxygen saturation, it will be referred to herein as $rScO_2$. Similar to the arterial oxygen saturation (SaO_2), the cerebral oxygen saturation $rScO_2$ is the ratio of HbO to total haemoglobin (HbT=HbO+HbR); though the $rScO_2$ refers to the oxygen saturation of the tissue rather than the blood.

Hyper-perfusion related to HIE is cerebral blood flow (CBF) above and beyond the metabolic need for O_2, so it is expected that the $rScO_2$ will be higher than in healthy neonates. Previous studies have used NIRS to monitor $rScO_2$ in infants with HIE undergoing TH and have not only observed higher $rScO_2$ measures in HIE infants as compared to their normal age-matched counterparts (150), but $rScO_2$ was also found to be significantly higher on the first day of life in those infants with HIE who developed brain injury compared with those who did not (297).

These studies were encouraging for the possible use of $rScO_2$ determined by NIRS as a cerebral perfusion marker of HIE and initially appears to meet the initial requirement for a monitor of cerebral perfusion. NIRS is also non-invasive; the sensors are placed flat against the surface of the body and the light is emitted percutaneously into the tissue. Morover the placement of the sensors does not require specialist skills or training and so could be placed on the scalp by clinical staff if the technology were adopted in the future to monitor HIE infants. NIRS also has the advantage that it is safe for long-term continuous measurement as it emits low power and does not involve ionising radiation. This summarises the reasoning behind the following investigation of using NIRS to monitor the cerebral perfusion of infants with HIE, and to evaluate the efficacy of using NIRS parameters as cerebral perfusion markers of HIE that could be used to guide TH or other treatments in the future.

6.3 SELECTION OF NEAR-INFRARED SPECTROSCOPY (NIRS) MONITOR

There are many types of NIRS devices available that differ in their type, the number of sensors with varying numbers of sources and detectors and all with proprietary algorithms that are used to calculate the HbO and HbR. All of these factors affect the accuracy of the measured HbO and HbR and influence the $rScO_2$ determination. The first step in this investigation was therefore to review the available NIRS devices and to select the most suitable NIRS monitor for the purpose of obtaining cerebral perfusion markers for infants with HIE.

The main consideration in choosing a NIRS device was its ability to non-invasively monitor cerebral oxygenation as an indicator of cerebral perfusion, in order to define or determine which alterations in

cerebral perfusion are due only to the HIE. The problem is that during TH, the metabolism of oxygen is slowed and so regardless of the cerebral blood flow there will be less HbO metabolised to HbR, and thus the ratio of HbR to HbT (HbO+HbR) will be higher than normal, with a consequent increase in $rScO_2$. This hypothermia-related elevation in $rScO_2$ has been reported in studies of infants cooled for cardiopulmonary bypass surgery (296). In the Dehaes et al study mentioned earlier, not only was a higher $rScO_2$ observed in HIE infants as compared to age-matched controls, but the mean $rScO_2$ was higher in the HIE infants during TH versus post-TH (150). The higher $rScO_2$ could therefore have been due either to the hypothermia or hyper-perfusion caused by HIE.

NIRS monitors calculate the $rScO_2$ from measured HbO and HbR. Given the influence of hypothermia on the $rScO_2$, this author proposes the second hypothesis of this study; that HbO will better detect hyper-perfusion in infants with HIE whilst they are cooled than $rScO_2$. The basis for this hypothesis is this: with hypothermia alone, CBF is reduced and thus HbO delivery is reduced, but the metabolism is slowed so there is also less HbO metabolised, therefore $rScO_2$ is elevated. However, in a state of hyper-perfusion associated with HIE, CBF is increased and so delivery of HbO is higher than normal, and thus HbO tissue concentation will be higher than normal, even during TH. It is also possible that during this period of hyper-perfusion, the increased CBV resulting from increased CBF, will be reflected also in a higher than normal concentration of HbT. To test these assumptions, the NIRS monitor must provide accurate, absolute measurements of HbO and HbR, so that they are inter-comparable between infants.

There are three main approaches for the measurement of HbO and HbR in NIRS monitors; continuous-wave, frequency-domain and time-domain NIRS measurements. All these approaches attempt to calculate the HbO and HbR concentrations, they differ in how theythey incorporate light scattering into their calculations. The general Beer-Lambert Law assumes that the difference in the light intensity, between the emitted and detected light, is entirely due to the absorption of the light by each chromophore present in the sample. In fact in reality the majority of the light is scattered rather than absorbed. The modified Beer-Lambert Laws attempt to take into accountscattering by including a Differential Path Length (DPL) factor, a measure of how far the photons have travelled before they appear at the detector.

6.3.1 Continuous-Wave NIRS (CW-NIRS)

The majority of the clinically available NIRS devices are *Continuous-Wave* NIRS (CW-NIRS). CW-NIRS emits continuous-wave light from the source which is detected by one or more detectors. All the prevailing commercial clinical NIRS monitors available in Australia when this project commenced

in 2013 were continuous-wave. Various monitors were available with permanent or disposable sensors and could be purchased either outright or supplied as part of a consumables package, the advantage of which is that the NIRS monitor could be obtained at a very low cost, with a small outlay for each research participant. The CW monitors are readily obtained and are easily usable by clinical staff. Measurements are easy and quick to obtain. The information is displayed in a convenient graphical interface display and is available for download. Owing to its ease of use, reliability and stability, CW-NIRS is used most often in functional NIRS where localised brain activations can be detected with complex emitter-detector configurations.

Spatial resolution is achieved in CW-NIRS by placing detectors at varying distances from the emitters; in this way light detected from deeper structures such as the brain tissue is separated from reflections detected from superficial structures such as the skin and scalp. The differential path length (DPL) included in the calculations is estimated. The disadvantage therefore of CW-NIRS is that, because it estimates DPL, it does not provide absolute measurements of HbO and HbR. For this reason HbO and HbR are often not available as individual parameters on a CW-NIRS monitor. Even when HbO and HbR are available, as they are not absolute but relative measurements, they are not directly comparable between infants and consequently neither is the $rScO_2$ that is calculated from the HbO and HbR.

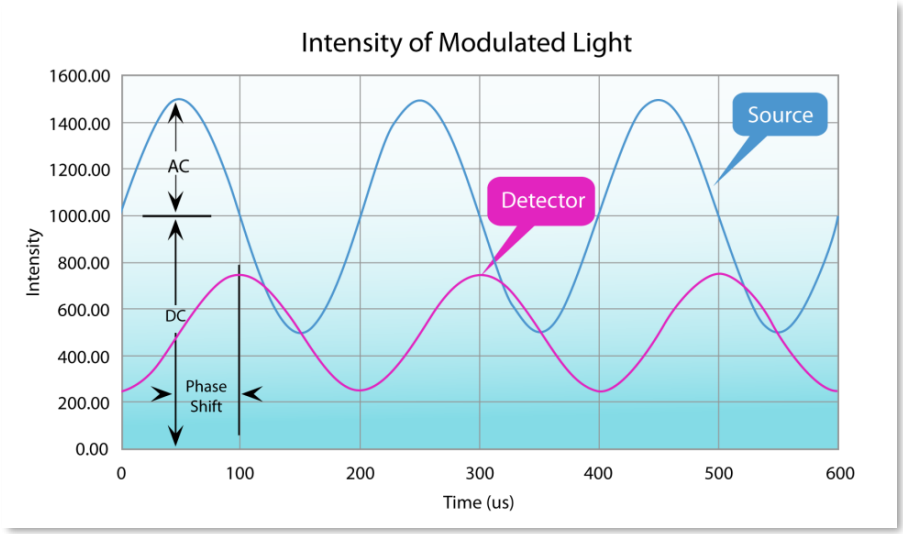
Due to this limitation, the inability to obtain absolute concentration measurements of HbO and HbR, the CW-NIRS monitors are unsuitable for the purpose of testing the hypothesis that the HbO would be more indicative than $rScO_2$ of the status of the cerebral perfusion in infants with HIE.

6.3.2 Time-Domain NIRS (TD-NIRS)

Time-Domain NIRS (TD-NIRS) or *Time-of-Flight (TOF)* NIRS does allow the absolute measurement of Haemoglobin because it can directly calculate the differential path length (DPL). TD-NIRS emits a very rapid pulse of light (around 1 pico-second) and measures the time-of-flight from emitter to detector: in doing so the TD-NIRS monitor can calculate the DPL. Although this can be a highly accurate method, due to the nature of the TD-NIRS it can be difficult to obtain reliable results, and, at the time of the selection of a NIRS monitor for this study, there were no TD-NIRS monitors commercially available.

6.3.3 Frequency-Domain NIRS (FD-NIRS)

Frequency-Domain NIRS (FD-NIRS) is so-named because if frequency modulates the emitted light, it also uses spatial resolution. As the light is modulated it is made up of three components, the baseline or DC, the modulated or AC component, and a phase as shown in Figure 6-1.



Reproduced from the OxiplexTS™ Operation Manual (293)

Figure 6-1 Diagram of the constituent components of modulated light: AC, DC and Phase.

The detected light will therefore have a change of intensity in the AC and DC component, and also a phase shift. With the additional information of the AC intensity and phase shift the scattering coefficient, μ_s, can be determined, and the scattering of photons are then accounted for. μ_s quantifies the amount of light light is scattered rather than absorbed by the tissues, and is measured in *per cm* (cm^{-1}).

The light source intensity as a function of time is described by:

$$I_0 = I_{DC_0} + I_{AC_0} \sin(2\pi f t - \Phi_0) \quad (293)$$

where: I_0 = source intensity, I_{DC_0} = DC or baseline component of light source intensity, I_{AC_0} = AC or alternating component, f = modulation frequency, Φ_0 = phase of the light source

Equation 6-2 The light source intensity as a function of time.

The light source intensity is modelled as a photon density wave travelling through the highly scattering tissue with scattering given by μ_s; the modulated light travels away from the source as a photon density wave at a constant speed and with decreasing intensity; the phase shift is a measure of thepropagation speed of that wave. Using this model, the scattering coefficient μ_s can be determined and used to calculate directly the absolute concentrations of HbO and HbR.

6.4 SELECTION OF NIRS DEVICE

The four NIRS monitors that were listed on the Australian Register of Therapeutic Goods (ARTG) for the purpose of cerebral oximetry (measurement of oxygen saturation in the brain tissue) were:

- INVOS™ – Manufactured by Covidien IIIc (Sponsor: Medtronic Australasia Pty. Ltd.)
- ForeSight™ – Manufactured by Cas Med Systems (Sponsor: Edwards Lifesciences Pty. Ltd)
- Equanox™ – Manufactured by Nonin Medical Inc (Sponsor: Device Technologies Australia Pty Ltd)
- NIRO-200NX™ – Manufactured by Hamamatsu (Sponsor: SDR Scientific Pty ltd.

The first three of these devices offer only the $rScO_2$, i.e. an index or percentage of cerebral oxygenation only, and so after the initial review, were regarded unsuitable and rejected.

The Hamamatsu NIRO 200-NX (Hamamatsu Photonics K.K, Hamamatsu City, Japan) is the only NIRS device approved for use by the TGA that offers Haemoglobin concentration measurements: HbO, HbR and HbT as well as a normalised Total Haemoglobin Index (nTHI) and a Total Oxygenation Index (TOI), the equivalent of $rScO_2$. The NIRO-200NX™ is a CW-NIRS monitor which uses spatial resolution to distinguish between the superficial signal and the light reflected from the brain tissue, but in calculations it uses an estimate of the DPL. The nTHI is a parameter designed to be a quantitative indication of the HbT, though it does not relate directly to concentrations and is presented in arbitrary units. The NIRO-200NX™ is therefore the most suitable commerical monitor for monitoring changes or trends in the concentrations of haemoglobin, but still does not provide an absolute measurement of haemoglobin.

There are no Frequency-Domain NIRS or Time-Domain NIRS devices commercially available in Australia and on the Australian Register of Therapeutic Goods (ARTG). The lack of commercially available FD- and TD-NIRS devices reflects the difficulty of reliably obtaining Haemoglobin measurements with these devices, as opposed to the ease of use of the Continuous-wave NIRS devices. The ease of use and ability to monitor trends in cerebral oxygenation makes CW-NIRS monitors most suitable for clinical, continuous long-term monitoring. Nevertheless, for the purposes of this study it was important to obtain a monitor that could offer absolute measurements of the Haemoglobin concentrations, and this outweighed any difficulty in obtaining measurements.

The Biomedical Optics group at the Athinoula A. Martinos centre (Massachusetts General Hospital, Charlestown, USA), associated with both Harvard Medical School and the Massachussets Institute of Technology in Boston, were involved in the development of the OxiplexTS FD-NIRS device now

manufactured by ISS Inc (Champaign, US). Using the Frequency-Domain (FD) NIRS method, the OxiplexTS is the only available commercial device that provides absolute values of HbO, HbR and HbT.

6.4.1 The OxiplexTS™ FD-NIRS Monitor

Overview

The OxiplexTS had European certification and was available for purchase for clinical research. The Athinoula A. Martinos Centre in conjunction with the Boston Children's Hospital had used a prototype Oxiplex device in several studies of neonates, including HIE affected infants, to measure haemoglobin (94, 150, 296). The ISS Inc. OxiplexTS was therefore chosen as the preferred device because due to the frequency multiplexing of the light source it was the only device offering absolute measurements of haemoglobin. Furthermore, the OxiplexTS™ has previously been used for research with neonates:

- **Cerussi et al:** Non-invasive monitoring of red blood cell transfusion in very low birth weight infants using diffuse optical spectroscopy. (298)

- **Roche-Labarbe:** Near-infrared spectroscopy assessment of cerebral oxygen metabolism in the developing premature brain. (299)

- **Dehaes et al:** Cerebral oxygen metabolism in neonatal hypoxic ischemic encephalopathy during and after therapeutic hypothermia. (150)

- **Dehaes et al**: Quantitative effect of the neonatal fontanel on synthetic near infrared spectroscopy measurements. (300)

The cofficients of scattering, U_S and absorbtion, U_A, measured by an OxiplexTS™ correlated with the thickness of the skin, bone and cerebrospinal fluid in neonates. (301), verifying the FD-NIRS method.

The previous use of the OxiplexTS™ for studies in neonates, in neonates with HIE and the validation of the absorption and scattering coefficients indicated that the OxiplexTS™ would allow accurate measurement of HbO, HbR and $rScO_2$ for this study.

Procurement

The OxiplexTS was available for purchase only from ISS Medical in Illinois, U.S.A. As it was an overseas purchase, procurement challenges caused a delay of almost 6 months prior to ordering. Once ordered, the device was manufactured and arrived in Australia 2 months later.

The OxiplexTS was not listed on the ARTG and required a Clinical Trial Notification (CTN) for use in the trial; Human Research Ethics Committee (HREC) approval for the use of the OxiplexTS™ was obtained from The Mercy Hospital for Women's HREC, before submission of the CTN to the Therapeutics Goods Administration (TGA). The initial research ethics included monitoring and recording all of the physiological parameters via the patient monitor connected as per standard hospital protocol, and measuremenet of DUS parameters; the inclusion of monitoring with FD-NIRS was proposed to the HREC as an amendment to the initial ethics approval. The ethics approval included FD-NIRS monitoring with the OxiplexTS™ from enrolment on the study (after consent from at least one parent), throughout the TH treatment and subsequent 12 hr rewarming. Approval was given in full for both the continuous monitoring and also for the use of a non-TGA approved device.

In general, Medical Devices for therapeutic use in Australia must be approved by the TGA. The OxiplexTS™ is not currently approved for use in Australia and is not on the Australian Register of Therapeutic Goods (ARTG). The approval gained from the Mercy HREC provided the required ethics approval enabling use of the device under the TGA's Clinical Trial Notification (CTN) scheme; this scheme allows devices not yet been approved by the TGA to be used as part of a clinical trial. The CTN subsequently applied for from the TGA was successfully obtained prior to the commencement of the FD-NIRS study.

Sensors

The OxiplexTS was purchased with the configuration of two flexible neonatal sensors for left and right, as shown in Figure 6-2. The flexible sensors accommodate curvature and can be positioned flat against the infant's head and mechanically held in place with a hat or head band that could avoid the use of adhesive; there were concerns that the fragile skin of the neonate could be damaged or torn when removing sensors if they are adhering to the skin. Each sensor consists of 8 laser diodes, which emit modulated near-infrared light at the 2 chosen frequencies 690nm and 830nm. These wavelengths have been chosen as they are able to pentrate the skull, and with optimal differentiation between absorption of HbO and HbR at these wavelengths; there is minimal absorption of HbO, with greater absorption of HbR at 690nm, and more absorption by HbO than HbR at 830nm.The emitters are spaced 1.5, 2.0, 2.5 and 3.0 cm from the detecting photodiode, a high-sensitivity photomultiplier tube specifically selected for a superior response in the near-infrared spectral range. The sensors are fully enclosed and waterproof, and connected to the OxiplexTS device by optic fibres. There is no electrical connection from the sensors to the device, and it does not pose an electrical hazard while connected to the infant.

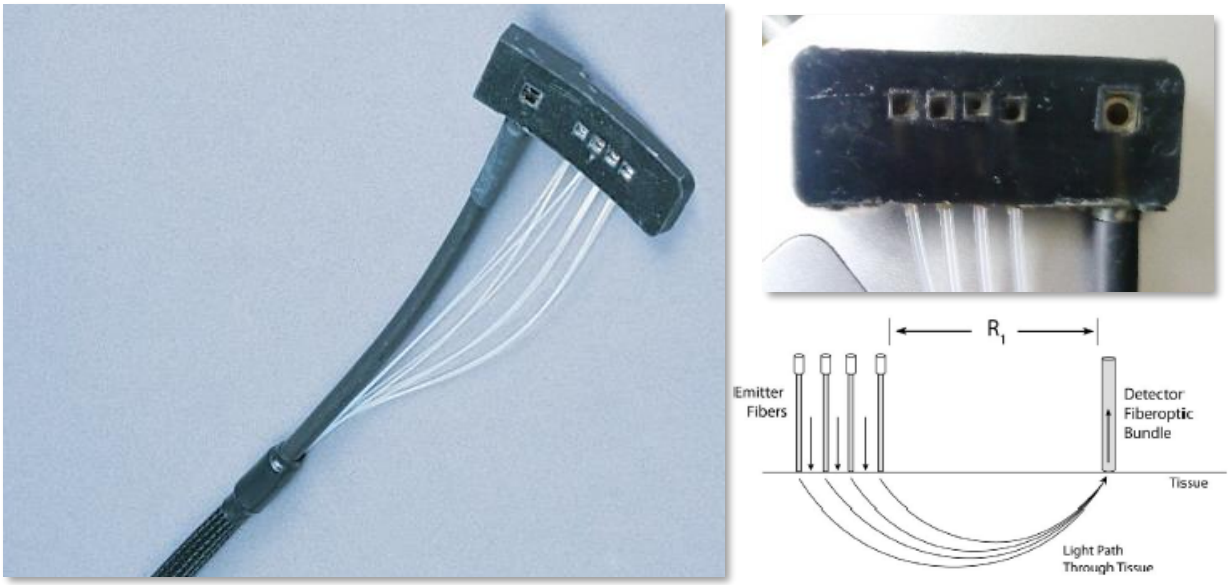


Figure 6-2 OxiplexTS Side Entry Flexible Infant Head Sensor and Configuration

[*From OxiplexTS™ Operation Manual* (293)]

The optical fibres connect the laser diodes within the OxiplexTS™ to the sensors; there are eight fibres for each sensor corresponding to each laser diode. The front panel of the OxiplexTS™ is shown in Figure 6-3. The optical fibre connection and the orientation of the fibre affect the integrity of the light intensity. When attaching the individual fibres, it was important to ensure firm connections for

maximal output, and to restrict movement or yield during the measurement period. Any movement of the fibre during the course of measurement could alter the light intensity output from the source, and thus could affect the calibration. The amplitude of the output from the laser diode at the sensor could be checked at any time in the OxiplexTS software by verifying the strength of the raw analogue signal from the detector.

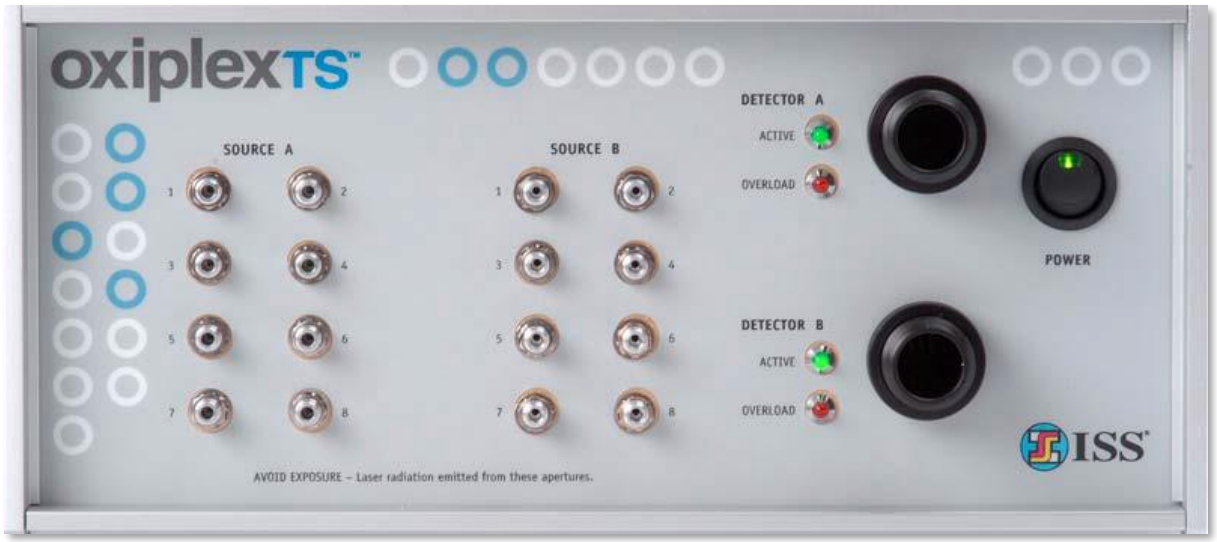


Figure 6-3 Front panel of the OxiplexTS™ (FD-NIRS) device.

The signal intensity is established prior to calibration. Two optical phantoms are supplied by ISS for the purposes of calibrating and checking the sensors. The optical phantoms have known scattering and absorption characteristics and so, by placing the sensors on the phantoms, the correlation between the light intensity and the optical characteristics of the phantom can be calculated and the calibration factors for each diode can be determined. One phantom is used to calibrate, the other for verification of the calibration. In practice, the calibration factor provided is relative to the first LED (thus the first calibration factor is always 1). Thus, the raw data can be converted to optical intensity or vice versa using the calibration factors. Recording over a short measurement period on the phantom before and after an experimental measurement allows for the assessment of drift or change in the optical intensity during the measurement period.

Data Acquisition and Processing

The software provided with the OxiplexTS™, trade-marked as OxiTS™, provides real-time displays of the data in both numeric and graphical form. The raw data are stored in OxiplexTS™ proprietary

format as the raw analogue signal intensity; the HbO and HbR concentrations are calculated and displayed graphically in real-time.

The OxiTS™ software is designed to provide a user-friendly graphical interface for the user to acquire, analyse and extract the measurement data. The format enables the user to view or replay the recording, and displays the graphs of HbO, HbR, HbT and $rScO_2$ measurements. Figure 6-4 is an image of the typical graphs of HbO, HbR, HbT and $rScO_2$ as viewed in the OxiTS™ graphical user interface. The data from the graphs can be saved in csv format and can also be converted to an ASCII text file which includes the calibration factors, and the raw data of the light intensity measured at the detector from each of the 8 sources.

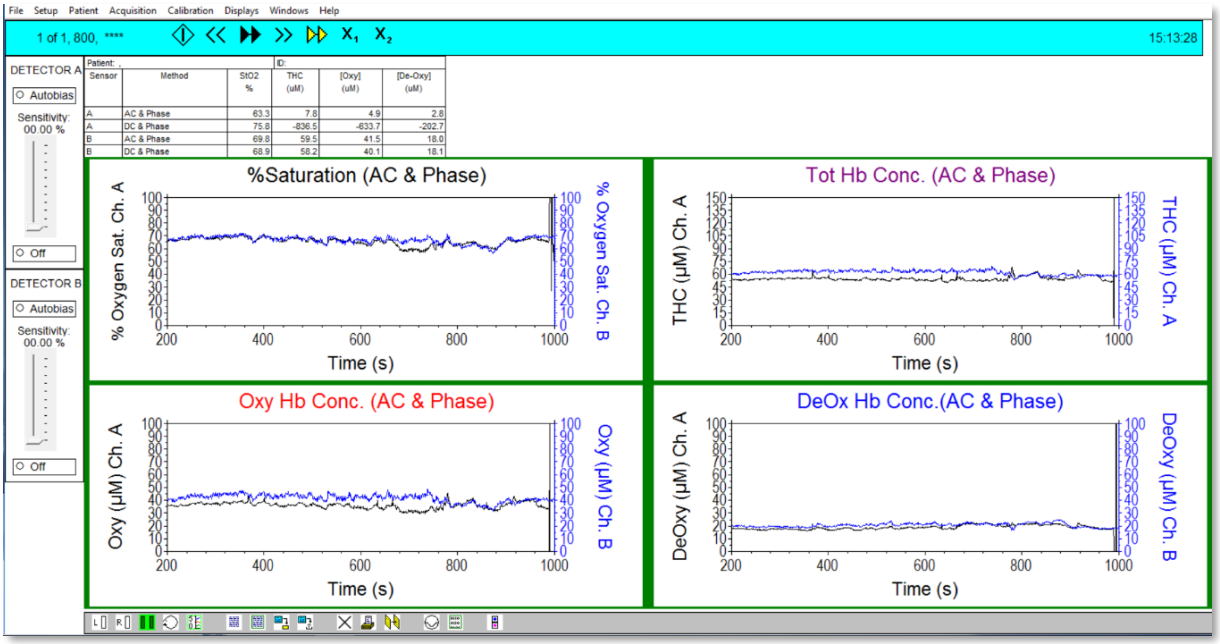


Figure 6-4 Illustration of the graphs of $rScO_2$, HbO, HbR and HbT as viewed in the OxiTS™ software.

The HbO and HbR concentrations are calculated from the raw light intensity by modelling the emitted light as a photon wave and making the assumption that $\mu_s >> \mu_a$. The propagation of the light through the tissue can then described by the following set of equations (293):

$$\ln(R^2 \cdot DC) = (R \cdot S_{DC}) + K_{DC}$$

$$\ln(R^2 \cdot AC) = (R \cdot S_{AC}) + K_{AC}$$

$$\Phi = (R \cdot S_{\Phi}) + K_{\Phi}$$

Where R=source-detector separator distances.

Equation 6-3 Set of equations describing the propagation of light through tissue.

These equations form straight lines of the form $y=mx+b$ where S_{DC}, S_{AC} and S_{Φ} are the slopes of those lines when the equations are plotted against the distances given by R. S_{DC}, S_{AC} and S_{Φ} are functions of U_S and U_A, and can be calculated directly from any 2 of the slopes. In practice, the DC component of the signal is most susceptible to background light, so S_{AC} and S_{Φ} were used preferentially in the calculation of U_S and U_A in this study. These lines indicating the DC and AC light intensity and phase shift for each of the 4 source-detector differences can be plotted and viewed in the OxiTS™ software. An example plot is shown in Figure 6-5 .

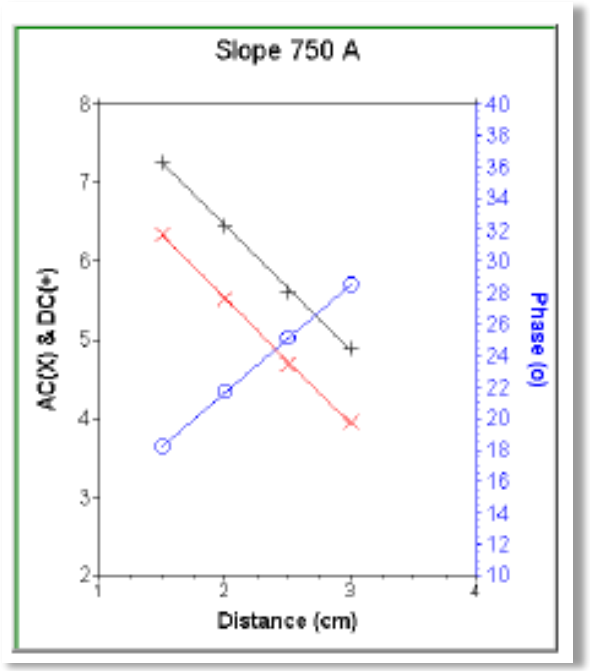


Figure 6-5 Plot of the AC and DC intensity and Φ, the phase shift over 4 source-detector differences.

Theoretically, only two distances would be required to obtain the slope, but with four distances a line of "best-fit" can be used thus giving a better accuracy and signal-to-noise ratio. An assessment of the linearity of the four points provides an indication of the calibration and therefore reliability of the measurement at any time. The plot for each wavelength was regularly reviewed prior to and during recordings to check the calibration and reliability.

Extinction Coefficients

Conversion of the data from a calibrated light intensity to a concentration of HbO, HbR and subsequently HbT necessarily relies on several assumptions. Although the contribution to the NIRS signal from water in the near-infrared range is minimal due to the limited absorption of near-infrared light by water, the absorption is non-zero and thus an assumption needs to be made regarding the

expected water in the tissue, and this needs to be accounted for in the calculations. The reported percentage of water in the adult brain is 60-70%; the water content of the neonatal brain is reportedly at a higher percentage, 70-80%. In order to be able to compare the results of this research project with similar projects, the water content has been assumed as 75%; this is the water content value recommended by ISS and is known to have been used in previous published studies of neonates (296).

The ability to measure HbO and HbR using near-infrared light is based on the principle that the chromophores, the parts of the molecules which absorb light, absorb differently at different wavelengths. The amount that a substance or molecule absorbs at a given wavelength can be measured and described by the extinction coefficient, *K*. The extinction coefficients have been previously determined experimentally by measuring the amount of light absorbed by samples of all HbO or all HbR (294, 295). The extinction coefficients used were those published by Wray et al in 1988 (294); these were recommended by ISS Inc., and are default in the OxiTS™ software.

It should be noted that the choice of the percentage of water content and the extinction coefficients can alter the HbO and HbR calculations. Thus, if NIRS measurements are to be compared, the same water content percentage and extinction coefficient values should be used.

Technical Challenges

There were several technical issues and challenges to be overcome during the study. Although the OxiTS™ software provides a facility to log the data, logging is not automatically initiated and must be manually enabled and activated. While becoming familiar with the correct sequencing for starting a recording, some data were lost. Also, the data were not saved to file in real-time; the data were stored in RAM until the recording is stopped, only then is the data saved to the file. If the measurement recording is stopped in an unexpected way or the software is closed before the log is ended and data saved manually, then the data for the session were lost. For longer recording sessions, if the RAM becomes full, the software "crashes" resulting in the loss of data for the session.

The data acquisition rate determined the amount of data that was stored in the RAM per second, and thus how long a recording could continue before the RAM was full and the software would crash. Initially the data acquisition rate was set at 5Hz, but at this rate the recording session was limited to approximately 2 hours. By reducing the sampling rate to 1 Hz, the recording session could last up to 10 hours before the RAM would become inundated. This still necessitated the check and re-starting of the log at regular intervals throughout the 84-100 hour period.

Clinical care of the infant was paramount, and thus the infants would be managed clinically corresponding to standard protocols. In the course of standard care, infants were moved regularly and

the head would be repositioned. In addition, clinical care and treatment would sometimes require an examination light; the introduction of bright lights and movement of the sensors, so they were no longer covered by the hat, could expose the sensors saturating the signal. The OxiTS™ software would automatically detect an over-exposure and deactivate the sensors to protect the sensitive photodiode detectors. Deactivation of the sensors resulted in data loss, until the sensors were reactivated. Data could also become unreliable due to the movement of the sensors and this data were discarded.

Safety Considerations

The American National Standards Institute (ANSI) has determined that the maximum permissible skin exposure limit (MPE) for light in the Near-Infrared spectrum to be below 200mW/cm^2 (302). The optical power output of the laser diode sources of the ISS Oxiplex is 800μW at the fibre optic tip. This focused signal is dispersed over an area of approximately 1cm^2 which results in an exposure of less than 1mW/cm^2, which is 200 times less than the ANSI MPE limit. Similarly, the Australian Standard AS/NZS IEC 60825.14:2011 – Safety of Laser products (see relevant excerpt regarding MPE for skin in APPENDIX G) also recommends the MPE to be less than 200mW/cm^2 in the Near-Infrared Spectrum for longer periods of exposure (the MPE for wavelengths greater than 700nm is actually greater than 200mW/cm^2 but this has been taken as the lower limit). The OxiplexTS emission is therefore more than 200 times less than the Australian, American and International standards and is therefore safe for long-term clinical use with neonates.

Limitations of the OxiplexTS™

The OxiplexTS™ has only 2 wavelengths and so only 2 chromophores can be measured. The prototype OxiplexTS™ developed at the Athinoula A. Martinos centre has 8 wavelengths. This allows for the measurement of additional chromophores, such as water that might affect the accuracy of the measurement. A greater number of wavelengths also allows the measurement of additional components such as cytochrome oxidase (OxCCO, associated with cerebral metabolism). The prototype Oxiplex monitor had 8 wavelengths, as it was initially thought that the HbO and HbR calculations would be more accurate, but ISS found experimentally that with only 2 wavelengths, the accuracy was not reduced. Moreover, the reduced complexity increased the reliability of the measurements. However, the use of only 2 wavelengths restricts the measurement to only HbO and HbR, and does not allow the measurement of OxCCO. Thus cerebral metabolism, via the measurement of OxCCO is not possible with the OxiplexTS.

CHAPTER 7. FREQUENCY-DOMAIN NEAR-INFRARED SPECTROSCOPY (FD-NIRS) PARAMETERS IN HEALTHY FULL-TERM NEONATES

7.1 INTRODUCTION

Near-infrared Spectrometry (NIRS) is increasingly being used for the monitoring of cerebral oxygenation of neonates in clinical care; most commercially available monitors are Continuous-Wave NIRS monitors. These are easy-to-use and the cerebral oxygen saturation ($rScO_2$) is a parameter which can be easily interpreted, much like SpO_2, peripheral oxygen saturation, which is already well understood by clinical staff. An FD-NIRS monitor was chosen for this study because it promised to provide absolute measurements of Oxy- and Deoxy-Haemoglobin (HbO and HbR respectively) as well as $rScO_2$, the ratio of HbO to total Haemoglobin, HbT where HbT=HbO+HbR.

In previously published studies the $rScO_2$ in normal-term neonates varies significantly as they were obtained with various different NIRS devices. There are limited numbers of studies using the OxiplexTS™, the FD-NIRS monitor chosen for this study, that provide a baseline of $rScO_2$ for healthy full-term neonates with no known pathology. There is only one study known by this author that reports HbO, HbR and HbT meaured by an OxiplexTS™ in healthy full-term neonates (303).

A baseline of NIRS parameters, HbO, HbR, HbT and $rScO_2$ therefore had to be established in a healthy full-term neonate population, prior to evaluation of the potential of the FD-NIRS parameters as cerebral perfusion markers of HIE.

7.2 OBJECTIVE

The objective of the study was to determine the baseline FD-NIRS parameters measured within the first four days in healthy neonates. This baseline will then be utilised to compare the FD-NIRS measurements from infants with Hypoxic-Ischemic Enceephalopathy (HIE) undergoing Therapeutic Hypothermia (TH).

7.3 METHOD

The infants studied were the same 43 neonates from the previous Doppler Ultrasound Study who were all recruited from the maternity ward of the Mercy Hospital for Women in Heidelberg. In 3 cases

the NIRS recording was lost and so there were recordings of 5-10 minutes for 40 infants available to the study.

The cohort of 40 had a mean post-natal age of 37 hours (range 1-98) and mean gestation of $39^{3/7}$ weeks (range 36-$41^{2/7}$) comprising: Early Term ($37^{0/7}$-$38^{6/7}$ wks, n=12), Full Term ($39^{+0/7}$-$40^{+6/7}$ wks, n=24), Late Term (≥41 wks, n=4) (240). Participants in the study had received standard antenatal surveillance as per hospital protocol; no antenatal corticosteroids were given, and all were born in good condition (mean Apgar score at 5 mins of 9.1 (range 7-10)). The cohort included 20 female (50%) and 20 male (50%) participants. 17 were born by Caesarian section (40%), the rest were by vaginal delivery (60%).

The mean birthweight was 3409g and birthweight range was 2580-4760g. All birthweights, except two, were ≥ 10^{th} percentile according to the "Australian national birthweight percentiles by sex and gestational age, 1998-2007" (241). The infants who were less than the 10^{th} percentile were males of 2850g and 3000g who were $40^{+4/7}$ and 40 weeks gestation respectively. The reported 10^{th} percentile in the Australian population was 3090g for male infants of 40 weeks gestation. All measured parameters for these infants were within 2 Standard Deviations (SDs) of the group mean and were retained in the study.

Parents were approached in the maternity ward, and consent was obtained from one parent prior to the study. Infants were studied in the nursery of the maternity ward, and when possible, the lights were turned down and blinds closed to avoid inadvertent saturation of the sensors.

The study was conducted with the dual Channel Oxiplex TS; 06208 model (ISS Inc., Champaign, IL, USA) with two flexible neonatal sensors for the left and right-hand sides. Each sensor, housed in a flexible black rubber probe, consists of one detector and eight sources, four each of wavelengths 690 nm and 830 nm. Sources are modulated at 110MHz and multiplexed (rapidly cycled with only one enabled at any time) so that separation of signals can be achieved with software. The sources are at four distances (two wavelengths at each distance) of 1, 1.5, 2 and 2.5 cm. These distances allow the photons to interrogate the cerebral cortex whilst allowing the sensor to be small enough for a newborn infant's head, without excessive curvature (94).

The sensors were initially placed on either side of the forehead under an elastic headband which held the sensors firmly onto the head, as shown in Figure 7-1. The forehead site was chosen as this was mostly free of hair, the sensors could be aligned with the ears/sides of the forehead for consistent placement and was most convenient for the infant when supine: all infants were supine during measurements, though the head could be turned for improved access. To reduce the possible error

that may be introduced by the flexibility of the sensors on the slightly curved surface of the neonatal forehead, the calibration was performed on a similarly curved surface of an optical phantom. To reduce the likelihood of interference from the alternate set of sensors on the other side of the head, the detector was always distal to the middle of the forehead. The infants would often become unsettled by the placement of the headband but would settle soon afterwards.

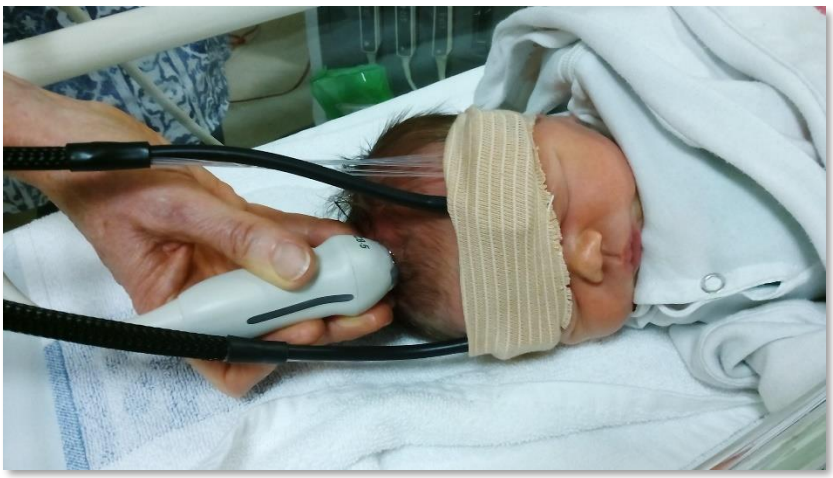


Figure 7-1 Placement of the OxiplexTS™ sensors on the infants.

The OxiplexTS sensor were placed on either side of the forehead below the hairline.
The placement of the sensors allowed for Ultrasound to be undertaken simultaneously.

The FD-NIRS signal was visualised using OxiTS™, the proprietary software of the OxiplexTS™. The sensors would remain on the neonate's head for a period of 15 minutes. As described in Chapter 6. , the OxiplexTS uses light multiplexing and spatial resolution to determine the absorption and scattering coefficients (μ_a and μ_s respectively) and calculate concentrations of HbO and HbR. The extinction coefficients published by Wray et al were used in these calculations (294). Sampling of the NIRS was at 1 Hz.

HbO and HbR were calculated using the AC and Phase components of the signal as this method was less prone to movement artefacts. Movement artefacts were then identified using a standard deviation threshold of 20% in HOMER2 (304), and confirmed visually. An example of motion artefact that would be identifed and removed is demonstrated in Figure 7-2.

The NIRS parameters of HbO, HbR, HbT and $rScO_2$ were recorded on the left (L) and the right-hand (R) side of the forehead, where:

$$rScO_2 = \frac{HbO}{HbO+HbR} = \frac{HbO}{HbT}$$

Equation 7-1 Calculation of Regional Cerebral Oxygen Saturation, $rScO_2$

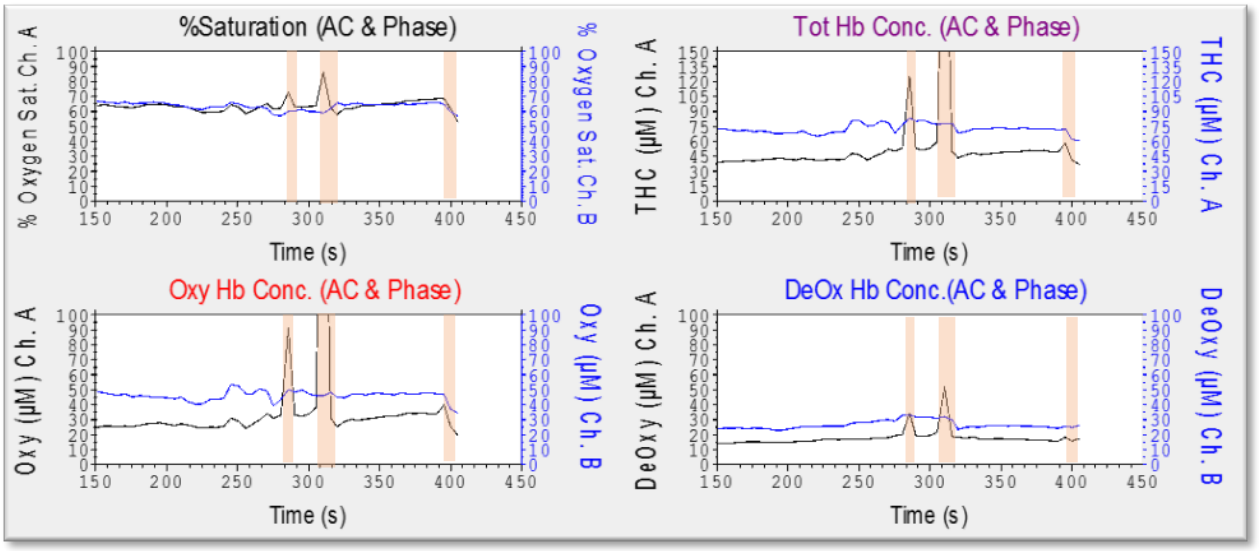


Figure 7-2 Example of motion artefact identified and removed using HOMER2.

Motion artefact that was identified and removed using a standard deviation threshold of 20% in HOMER2 is highlighted in pink.

The NIRS parameters are hyphenated with L, R to indicate the measurements at the left and right sensors respectively e.g HbO-L and HbO-R, for HbO concentration on the left and right-hand sides.

Statistical analysis was undertaken with Matlab 2018a (The MathWorks, Inc., Natick, Massachusetts, U.S.A). The Anderson-Darling test was used to determine whether the data had a normal (Gaussian) distribution, and as some of the data series were found to be non-Gaussian, the following non-parametric statistical tests were used to analyze the data: the Wilcoxon signed-rank sum to compare paired data between the left and right sides, Spearman's linear correlation for correlations between data and continuous variables, and Point Bi-serial correlation for dichotomous variables (305). Differences were considered statistically significant when $p < 0.05$. Significance levels of $p< 0.05$, $p < 0.01$ and $p < 0.001$ are indicated where relevant.

7.4 RESULTS

The mean, standard deviation (SD), minimum and maximum of each of the NIRS parameters (HbO, HbR, HbT and $rScO_2$) for the 40 healthy neonates (no known pathology) are displayed in Table 7-1.

Boxplots of the HbO, HbR, HbT and rScO2 are displayed in Figure 7-3: pairwise measurements of HbO, HbT and rScO2 at the left and right sensors were statistically similar for all parameters, whereas the HbR measurements between left and right were statistically different ($p<0.05$).

Table 7-1 NIRS parameters in the normal term neonates as measured by the OxiplexTS™

The mean, standard deviation (SD), minimum (Min) and maximum (Max), and confidence level (CL) of the NIRS parameters Oxy-Haemoglobin (HbO), Deoxy-Haemoglobin (HbR) and total haemoglobin (HbT) measured in μM and the regional cerebral oxygen saturation ($rScO_2$) as a percentage as determined in normal full-term neonates.

NIRS Parameter	Measure	Mean	SD	Min	Max
HbO (μM)	L	42.7	16.4	13.1	91.7
	R	46.6	17.7	17.5	83.9
HbR (μM)	L	17.2	4.2	8.5	25.4
	R	19.0	4.6	12.2	30.4
HbT (μM)	L	59.9	19.9	21.6	113.9
	R	65.6	21.5	29.8	109.6
$rScO_2$ (%)	L	70.3*	4.9	59.4	80.5
	R	69.8*	5.3	58.0	78.6

* Indicates a significant difference in the measurements between the Left and Right sensor with $p < 0.05$.

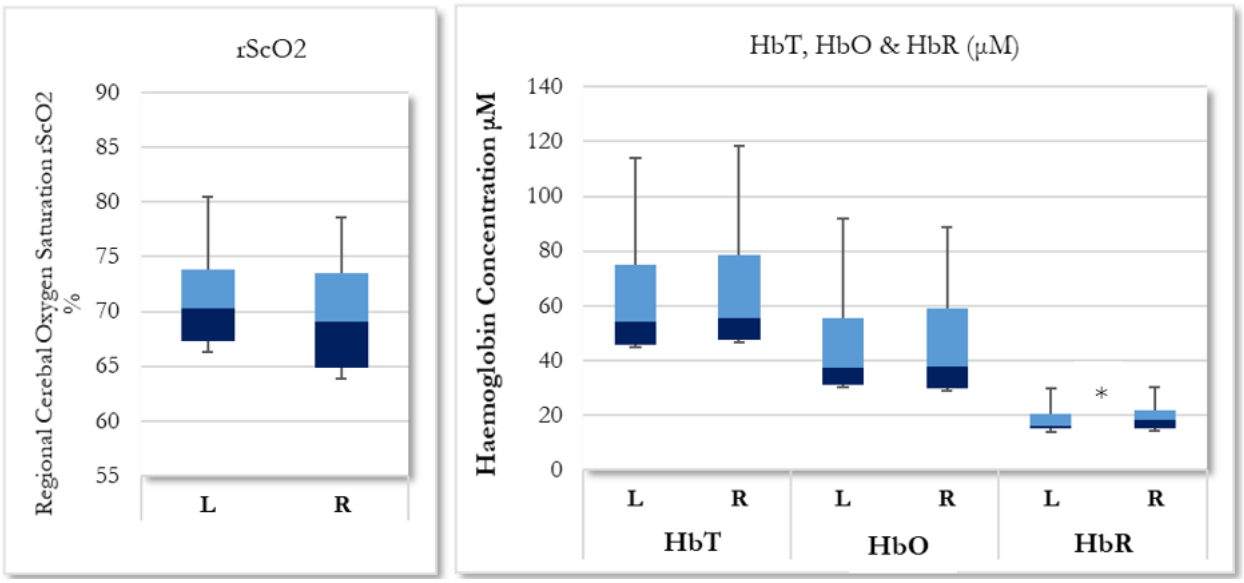


Figure 7-3 Boxplots of HbO, HbR and HbT in the Normal Full-Term Neonates

Boxplots of the NIRS parameters of Oxy-Haemoglobin (HbO), Deoxy-Haemoglobin (HbR) and Total Haemoglobin (HbT) as concentration in μM for the Left (L) and Right (R) sensors.

* Indicates a significant difference between the left and right sensors with $p < 0.05$.

Correlations

Correlations with sex, gestation, mode of delivery (MoD), Apgar Score at 5 min, birthweight, behavioural state and post-prandial time were all explored for all FD-NIRS parameters. There were weakly-positive correlations with birthweight and HbO-L (ρ =0.34, $p<0.05$), HbO-R (ρ=0.42, $p<0.01$), HbR-R (ρ=0.37, $p<0.05$), HbT-L (ρ =0.35, $p<0.05$), HbT-R (ρ =0.44, $p<0.01$) and $rScO_2$-R(ρ=0.34, $p<0.05$). A low-value correlation detected between $rScO_2$-L and MoD (ρ =0.35, $p<0.05$) indicated that a Caesarian Delivery (CD) is weakly associated with lower $rScO_2$-L. A weak correlation was also detected between the post-prandial time and HbR-R only ($p<0.05$, ρ =0.34).

7.5 DISCUSSION

It was necessary to conduct a study to establish a baseline of NIRS parameters because there is no absolute reference (306) and few studies reporting the $rScO_2$ in normal healthy infants in the first 5 days. Moreover, of the 16 published studies of NIRS parameters in healthy term neonates, only 3 of these used frequency-domain NIRS, and all used the OxiplexTS™ (94, 150, 303). Bokiniec et al (303) reported all NIRS parameters (HbO, HbR, HbT and $rScO_2$), whereas Franceschini et al (94) reported only $rScO_2$ and HbT, including also a calculation of CBV. Dehaes et al provided a baseline of $rScO_2$ for comparison with infants with HIE, also with CBV and additionally indices of CBF and cerebral oxygen metabolism ($CMRO_{2i}$). An overview of all the NIRS studies of neonates that were found, with their reported results, is given in APPENDIX H.

The majority of the NIRS studies in term neonates are conducted with CW-NIRS monitors. Each of these NIRS monitors has their own proprietary algorithms and sensors that vary with the light -source type and intensity, the number and period of the wavelengths used, and varying optode source-detector distances. Studies comparing different CW-NIRS devices have found that the device and algorithm, the sensors, the number of wavelengths and the interoptode distance can all affect measurements (91, 102-106).

Importantly, although absolute values of NIRS parameters differ by a reported 10% between NIRS devices (106), the trends were still comparable (91, 103-105). These studies involved CW-NIRS devices, so this is likely a consequence of the measurement method as discussed in Chapter 7. Highlighting this factor was the study of the precision of measurement by Sorensen et al (307) in which the re-siting of a probe resulted in a 5.2% variation on the same infant, similar to the 6.9% between infant variation. Therefore the strength of the CW-NIRS devices is in trend monitoring.

One of the reasons for using the FD-NIRS device was to avoid this variation by directly calculating the scattering coefficient, allowing absolute measurements of haemoglobin. Those studies that demonstrate the differences between devices indicate also that the NIRS parameters are only comparable between studies that have been conducted with the same individual NIRS device. This is therefore a challenge when there are only 3 studies against which to compare, and necessitated the establishment of a baseline of FD-NIRS parameters in healthy full-term neonates using an OxiplexTS™. Moreover, it was hoped that by using the exact same device and sensors, any variation resulting from device and sensor specifics could be avoided.

Many of the studies of healthy neonates focus on the transitional period following birth (110, 308-316). Although these studies have been performed with various devices, together they confirm that the $rScO_2$ increases during the first few minutes after birth and then plateaus out and remains constant after 7-8 mins.Van Bel et al (317) reported that HbT (HbO+HbR), as an indicator of CBV, also remained stable in the first day in their healthy new-born group. These studies indicate that after the initial, rapidly changing, cerebral haemodynamics associated with birth, the NIRS parameters stabilise and remain relatively constant for a period of time thereafter. Thus it is assumed here that once the cerebral haemodynamics have stabilised after the birth transition that they will at least be stable for the period of interest in this study, the first 4 days. This was supported by the results, as there was no correlation between the post-natal age and any of the NIRS parameters.

In this study, the average regional oxygen saturation $rScO_2$ was found to be 70±5% (mean±SD), with a range of 58-80%, for both frontal left and right sensor placements. This was in close agreement with Dehaes et al (150); their mean $rScO_2$ in 22 healthy controls (HC's) centred on 70%, with a range approximately from 65% to 75% (without outliers). There was also good agreement with the previously-mentioned study by Fransceschini et al (94), in which the mean $rScO_2$ was 66% in the first week of life measured in the frontal region using an OxiplexTS™ FD-NIRS monitor. Interestingly, although the $rScO_2$ did not change significantly and HbT increased by 0.5μM over the period of a year, the $rScO_2$ and HbT decreased in the first 6 weeks. It is feasible therefore that the $rScO_2$ may have been higher in the first 5 days, as they only included 8 infants of PNA 0-2.5 months. It is also possible that the $rScO_2$ was higher in this research study, compared to the study by Franceschini et al, because there was a larger number of infants measured at an earlier post-natal age. Similarly, the HbT of 62.8±18 μM (mean of left and right measurements) in this study, was also similar to the measured HbT of 53 μM in that same study by Franceschini et al (94). Francesshini et al also found that the HbT decreased in the first 6 weeks, so the slightly higher $rScO_2$ and HbT measured in this current study, may be due to the greater number of infants examined at less than 4 days post-natal age.

Bokiniec et al reported all the NIRS parameters including HbO and HbR in their study (303). They also found, in accord with Franceschini et al, that HbT decreased in the first 4 weeks. This was the result of both HbO and HbR decreasing in this period; though as both HbO and HbR decreased there was no significant change in $rScO_2$. Bokiniec et al measured the NIRS parameters in 5 locations: 2 left frontal sensors, 2 temporal sensor and one occipital sensor. The placement of sensors in this study was fronto-temporal, somewhere between the Bokiniec et al frontal and temporal measurements. The Bokiniec et al results for all frontal and temporal measurements on D1 were transcribed from their graphs and are presented in Table 7-2.

Although the HbR measurements were in fairly close agreement between this study and the study of Bokiniec et al (303), there was a obvious differences in the HbO measurements. The HbO measurements in the Bokiniec et al study was approximately half of the HbO concentration measurements in this study, and because of the lower HbO measurement, the $rScO_2$ was also lower. Initially, this could have been thought to be due to the timing of the measurements. Bokiniec et al conducted their measurements immediately after birth so the infants may have still been in the birth transition phase; the $rScO_2$ has been shown to increase from 18±1% at 1.5 mins (309) to 55-85% at 15 mins (308-315). This does not seem to be the case though, as the $rScO_2$ was not significantly different, and the HbO decreased, in the Bokiniec et al study at the subsequent measurement at Day 28 of life. As the $rScO_2$ was less than 50% in the Bokiniec et al study, compared to this study, and their values are lower than all known studies of $rScO_2$ in healthy full-term neonates subsequent to the birth transition, it can only be assumed that there was a device or sensor configuration or other issue that resulted in their lower value.

Table 7-2 Table comparing the results of this study to the Bokiniec et al (2012) study of FD-NIRS parameters in healthy neonates.

	Bokiniec et al (303)		
NIRS parameter	**Frontal**	**Temporal**	**This Study**
HbO-L	21μM (7-35μM)	25μM (10-39μM)	42.7 μM (13.1-91.7 μM)
HbO-R	17μM (5-28μM)	24μM (13-35μM)	46.6 μM (17.5-83.9 μM)
HbR-L	23μM (16-31μM)	27μM (20-34 μM)	17.2 μM (8.5-25.4 μM)
HbR-R	23μM (16-28μM)	26μM (20-32μM)	19.0 μM (12.2-30.4 μM)
HbT-L	45μM (24-65μM)	50μM (30-70μM)	59.9 μM (21.6-113.9μM)
HbT-R	40μM (20-55μM)	48μM (30-65μM)	65.6 μM (29.8-109.6 μM)
$rScO_2$ -L	42% (28-55%)	44% (30-55%)	70.3% (59.4-80.5%)
$rScO_2$-R	40% (25-50%)	45% (32-56%)	69.8% (58.0-78.6%)

The Bokiniec study (318) is the only one against which we can directly compare, as it was the only one conducted with the OxiplexTS™ involving infants within the first 5 days that report all the NIRS parameters. Other studies of healthy term neonates using different devices, the $rScO_2$ falls within the reported range of 55-80%. The HbR in this study corresponded well to the Bokiniec et al study, and also agrees with Cooper et al (319) who derived an absolute HbR measure of 14.6 ± 4.0 μM in healthy neonates, using the ratio of the second differential of the HbR near infrared spectroscopy signal to that of the water. As noted previously the HbT agreed well with Franceschini et al (94). Therefore, as HbR, HbT and $rScO_2$ all correspond with previously published data, and we know they have relationship $rScO_2 = \frac{HbO}{HbT} = \frac{HbO}{HbO+HbR}$, then it is likely that the HbO measurements in this study are a fair reflection of the HbO concentration in the brain.

Wijbenga et al reported that the HbO, HbT and $rScO_2$ measurements between the left and right sensors were all statistically similar (101). Wijbenga et al also found no difference in $rScO_2$ between the left and right sides, suggesting that cerebral oxygenation across the brain is uniform. HbR was significantly different between the left and right sides; there is no study known by this author reporting differences in HbR between the left and right sides. HbR was directly related to cerebral metabolism, the amount of oxygen used by the cells, so perhaps the difference is due to some variation in the function, and therefore energy requirements, on the opposite sides. It is postulated that this could be due to increased cerebral activity due to development and/or myelination (94); although this

difference was not uniform with 24 of 40 infants having right HbR concentration greater than the left HbR concentration and vice versa in the remaining 16. Regardless of the reason for the difference, these results would suggest that CBF is uniformly maintaining HbO concentrations, and consequently $rScO_2$ even with what appears to be varying levels of cerebral activity and metabolism.

7.6 CONCLUSION

The baseline NIRS parameters of HbO, HbR, HbT and $rScO_2$ were established in a cohort of 42 healthy term infants. It was paramount to establish this baseline because differences between NIRS devices and sensors can cause significant variation in measurements, and there was only one study (Bokiniec et al) reporting all NIRS parmeters of HbO, HbR, HbT and $rScO_2$ as determined by using an OxiplexTS™ in healthy neonates in the first 4 days; they reported that a lower HbO, and therefore lower $rScO_2$ was expected. Thus, confirmation of the baseline NIRS parameters was important to ensure there were measurements against which we could compare for future research projects utlizing our device. The OxiplexTS™ device and sensors will be used for the next stage of the research to investigate the feasibility of using these NIRS parameters, and especially HbO, as cerebral perfusion markers in infants with HIE.

CHAPTER 8. INVESTIGATION OF FD-NIRS PARAMETERS AS MARKERS OF HYPER-PERFUSION IN INFANTS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY (HIE)

8.1 INTRODUCTION

The Resistive index (RI) as determined by Doppler Ultrasound is currently the accepted method to test whether the vascular tone of the cerebral arteries has been altered, thereby modifying cerebral blood flow, after a hypoxic event. The underlying basis is to test whether a hypoxic event has caused an increase in blood flow to the brain. As discussed previously the RI may indicate a lowered resistance of the cerebral arteries but does not necessarily indicate hyper-perfusion.. In addition, Doppler Ultrasound is a one-off measurement requiring by a skilled operator. Therefore, a method of continuously measuring hyper-perfusion of the brain was sought.

Frequency Domain Near-Infrared Spectroscopy (FD-NIRS) has the potential of providing quantitative measurements of Oxy- and Deoxy- Haemoglobin (HbO and HbR) concentrations as well as the cerebral oxygen saturation, $rScO_2$. FD-NIRS determines the concentrations of HbO and by the use of light-emitting diodes and detecting sensors placed on the head. The FD-NIRS emits frequency modulated light in the near-infrared range, detects the reflection of this light and from the amount of light absorbed calculates the concentrations of HbO and HbR in the interrogated cerebral tissue using modified Beer-Lambert laws as discussed in Chapter 6.

Hypothesis 2 of this study is that hyper-perfusion resulting from a hypoxic event in neonates with Hypoxic Ischemic Encephalopathy (HIE) would result in a higher concentration of HbO in the cerebral tissue, and this can be measured by an FD-NIRS monitor. The reason for undertaking the study with an FD-NIRS monitor rather than a conventional continuous wave monitor is that the conventional NIRS monitor provides only regional cerebral oxygen saturation ($rScO_2$) as a parameter.

Although Dehaes et al (150) reported higher Regional Cerebral Oxygen Saturation ($rScO_2$) in neonates with HIE undergoing hypothermia treatment versus normal non-cooled neonates, this study also observed higher $rScO_2$ measures in HIE neonates during cooling as opposed to after cooling. Hypothermia reduces metabolic demand, thus reducing the rate of metabolism of HbO in the brain tissue. With less HbO metabolised, there will be more HbO and less HbR than at normal temperature. As rScO2 is the ratio of HbO to total Haemoglobin (HbT=HbO+HbR), a higher $rScO_2$ may be caused by the hypothermia treatment itself. A subsequent study by Peng et al did observe $rScO_2$ in

cooled infants developing HIE injury higher than in those infants who did not develop HIE injury but their study did not include non-cooled counterparts (297). Thus, an increased rScO2 may indicate hyper-perfusion subsequent to a hypoxic event or decreased metabolic demand due to hypothermia treatment.

FD-NIRS was explored as an alternative that, by measuring HbO and HbR directly, may be able to provide a more precise indication that there is hyper-perfusion rather than just a lowered metabolism. The foundation of the hypothesis is that hyper-perfusion will result in delivery of HbO beyond metabolic demand, which is not directly related to the altered metabolism due to the hypothermia. Moreover, it is theorised that the severity and persistence of the hyper-perfusion, which is related to the severity of the HIE and long-term outcome, will be related to the concentration of the HbO in the brain tissue.

This stage of the study is intended to test this hypothesis. The study involved continuously monitoring cerebral oxygenation of HIE infants with an FD-NIRS monitor and comparing the measurements of HbO, HbR, HbT and rScO2 against the baseline FD-NIRS parameters from the control group of healthy neonates, with no known pathology, as described in Chapter 7.

8.2 HYPOTHESIS 2

8.2.1 Hypothesis 2

Oxy-Haemoglobin (HbO) as measured by Frequency-Domain NIRS (FD-NIRS) is a marker of cerebral hyper-perfusion in infants with Hypoxic-Ischemic Encephalopathy (HIE) during therapeutic hypothermia (TH).

8.2.2 Alternative Hypothesis 2

Oxy-Haemoglobin (HbO) in infants with Hypoxic-Ischemic Encephalopathy (HIE) undergoing therapeutic hypothermia (TH) is a consequence of the hypothermia and not correlated to hyper-perfusion related to the HIE.

8.3 METHOD AND POPULATION

Six infants diagnosed with Hypoxic Ischemic Encephalopathy (HIE) undergoing therapeutic hypothermia (TH) were continuously monitored with Frequency-Domain Near-Infrared Spectroscopy (FD-NIRS). These were the same last 6 of the 18 infants as described in Chapter 5. that compared the Resistive Index (RI) in the HIE neonates with the healthy age-matched controls.

The 6 infants had a mean gestation of 39 weeks (range $36^{+5/7}$-$41^{+3/7}$) with mean birthweight of 3016g (range 2440g-4230g) with cooling commencing at a mean of 4 hrs post-natal age (range 1-6 hours). There were 2 female and 4 male infants. The infants were recruited in the Neonatal Intensive Care Unit of the Mercy Hospital for Women in Heidelberg; five were born at other centres and transported to the Mercy Hospital, one was inborn.

The infants were de-identified and are numbered in the sequence in which they were enrolled on the research project, with HIE13 being the identification for the first infant monitored with FD-NIRS (the 13th in the project) through to HIE18, the 6th monitored with FD-NIRS (the 18th study participant). For continuity, the identifiers remain the same as Chapter 5 for these infants. A table of their short- and medium-term outcomes can be found in APPENDIX C. Outcomes are based on the initial diagnosis of the severity of the HIE by the attending clinician, the formal ultrasound report conducted in the first 3 days of life by a Mercy Health Sonographer, the formal report from the MRI (Magnetic Resonance Imaging) conducted after the infant was rewarmed (between day 4 and 6 of life) and the 2 year developmental review conducted by a Mercy Health Paediatrician, where available.

The previous Chapter described the FD-NIRS parameters in the 40 infants with no known pathology; these were the 2nd control group of the overall study and the same infants as described in Chapters 3 and 4. This was the normal control cohort of infants against which the exposed group, the infants diagnosed with HIE, were compared. The control group were recruited from the Maternity Ward of the Mercy Hospital for Women in Heidelberg and consent was obtained from at least one parent. The control group was 50% female and 50% male with a mean post-natal age of 37 hours (range 1-98), mean gestation of $39^{3/7}$ weeks (range 36-$41^{2/7}$), mean Apgar score at 5 mins of 9.1 (range 7-10) and mean birthweight of 3409g (range 2580-4760g). 17 were born by Caesarian section (40%), the remainder were by Vaginal delivery (60%).

The FD-NIRS recordings were made with the OxiplexTS™ (ISS, Illinois, United States) FD-NIRS monitor, as previously described. Two frontal sensors were placed on either side of the forehead within the hairline with maximum separation between each other. Although the OxiplexTS™ cycles are time-multiplexed to avoid interference between light sources (the 8 light sources across the 2

sensors are cycled so that no two sources are on at the same time), the separation was a secondary mechanism for avoiding interference and interrogating different areas of tissue.

The 40 infants enrolled in the control study had discrete recordings of FD-NIRS for a period of 15 minutes. The 6 HIE infants were monitored with the FD-NIRS monitor from recruitment to the research project through to normal temperature. The continuous recording was separated into 15-minute periods. All recordings were made between 0 and 96 hours post-natal age.

Movement artefacts were removed from each of the 15-minute recordings, by the method previously described in Chapter 7, and the final measurements were recorded as the mean HbO and HbR concentrations over the 15-minute period in both the control and HIE populations. The total Haemoglobin (HbT) was calculated as HbO+HbR=HbT. The regional cerebral oxygen saturation $rScO_2$ was calculated as the ratio of the concentration of HbO to HbT, presented as a percentage as given in Equation 7-1. Statistical analysis was undertaken with Matlab 2018a (The MathWorks, Inc., Natick, Massachusetts, U.S.A). The Anderson-Darling test was used to determine whether the data was of a normal, Gaussian distribution and some of the data series were found to be non-Gaussian. F-tests were used to compare the variance between the control population and the separated periods for the HIE infants; there were significant variances due to a varying numbers of data points. For this reason, non-parametric methods of comparison were used for comparing the control data of 40 infants and each time series for the HIE infants; the Mann-Whitney-Wilcoxon test for comparing data between 2 independent continuous variables, the Wilcoxon signed rank sum to test data in dependent pairs (305). Differences were considered statistically significant when $p < 0.05$. Significance levels of $p< 0.05$, $p < 0.01$ and $p < 0.001$ are indicated where relevant. Due to the non-normality of the data, correlations were evaluated using the Spearman Correlation or the Point-Biserial Correlation where one of the variables was dichotomous.

The data from the HIE infants were divided into time periods. The division of the time periods was designed to focus on the time periods of most interest. The ultimate aim of the Optimising Cooling for HIE (OCHIE) research project is to be able to determine whether an infant will have a poor prognosis prior to rewarming, and consider them for an altered treatment protocol. Thus, in this part of the study, there is a focus on the 3rd day of cooling, the period of 48-60 hours from the commencement of cooling and the rewarming period. Also taken into consideration was the available data. 2 out of the 4 infants were not recruited until the second day of cooling, and there was some data loss with movement of sensors during clinical and nursing interventions. As all infants had complete data for the time period 64-66 hours following the commencement of cooling, this has been chosen as a particular period of comparison between HIE infants whilst cooled. This period may also

be useful as it is 6-8 hours prior to the commencement of the rewarming and thus may be an appropriate time to review the cerebral oxygenation status of the infant with a view to prognosticate prior to rewarming.

8.4 RESULTS

The FD-NIRS parameters that were recorded were the Oxy-Haemoglobin (HbO), Deoxy-Haemoglobin (HbR), Total Haemoglobin as the addition of HbO and HbR (HbT), and Regional Cerebral Oxygen Saturation ($rScO_2$) calculated as described in the Method. The FD-NIRS parameters on the left and right are indicated by a suffixed -L or -R denoting the left or right-hand sensor (e.g. HbO-L). The similarity between the measurements at the left and right sensors was analysed using the Wilcoxon signed rank sum test, as data from Left and Right are paired at each time point.

The FD-NIRS parameters measured in the healthy control (HC) neonates were presented in Chapter 7. No correlation was found with any of the FD-NIRS parameters and the Post-Natal Age (PNA). Given there was no correlation with PNA, this allowed the use of the number of hours elapsed from the commencement of cooling as the time variable rather than the PNA. As each of the HIE infants was cooled at a different post-natal age, this was a way of being able to compare each of the infants directly at the same time period of treatment. This number of hours elapsed from the time of cooling is referred to as "Cooled Hrs" or C-Hrs.

HIE infants were not birthweight matched to the healthy controls, as the discovered correlations between FD-NIRS paramters and birthweight were weak, and birthweight matching would limit an already limited population.

Measurements of Haemoglobin concentrations (HbO, HbR and HbT) are in micromoles (μM) and $rScO_2$ as a percentage (%) unless otherwise stated.

8.4.1 Outcomes for infants monitored with FD-NIRS

The short- and medium-term outcomes for each of the 6 HIE infants can be found as the last 6 infants in the Outcome table in APPENDIX C. To summarise:

- 2 infants, HIE13 and HIE15, had initial Ultrasound examinations reporting generalised echogenicity and Resistive Indices (RI's) of < 0.55 and abnormal MRI's subsequent to therapeutic hypothermia (TH). HIE13 was reported to have significant developmental delays at 2 years of age. Infant HIE15 had treatment withdrawn following the TH and died on Day 5 of life.

- The radiologist's report for HIE14 and HIE17 indicated some echogenicity consistent with HIE but normal RI's > 0.55. The MRI reports for both HIE14 and HIE17 indicated probable HIE injury; in the basal ganglia for HIE14 and white matter for HIE17. HIE14 had no significant developmental delays at 2 years of age. The parents of HIE17 declined the 2-year appointment with a paediatrician; the reason for declining the appointment is not known.
- Infants HIE16 and HIE18 had normal Ultrasound examinations with RI's > 0.55 and no indication of echogenicity. HIE16 had a normal MRI subsequent to the TH. Neither infant attended their 2-year paediatric review appointment; once again the reason for declining the appointment is not known.

For the purposes of this study, poor outcome is defined as any significant development or delayor death at 2 years of age. By this definition, HIE13 and HIE15 have poor outcomes and so for classification purposes will be defined as P for Poor Outcome designated by an P in HIE13-P and HIE15-P for easier identification.

HIE14 and HIE17 had indications of mild-moderate HIE in ultrasound and MRI investigations and so are classified here as "Mild-Moderate HIE" and designated by an M as HIE14-M and HIE17-M.

HIE16 and HIE18 had no indications of HIE injury in either ultrasound or MRI and have been classified as "No known HIE injury" for the purposes of this study and designated with an N; HIE16-N and HIE18-N.

8.4.2 FD-NIRS parameters in the HIE infants

Overall comparison between HIE infants and healthy controls

When comparing the HIE infants against the Healthy Control (HC) population for each FD-NIRS parameter, and including all data, many of the FD-NIRS measurements in the HIE infants were significantly different to the HC's (39 out of 48 comparisons, 81%). This is summarised in Table 8-1 which presents the p-values for each FD-NIRS parameter comparison between the individual HIE infant and the HC population. All FD-NIRS parameters in HIE15-P were significantly different to the HC's. In contrast, the only infant with an FD-NIRS parameter statistically similar to the HC population at both the Left & Right Sensors was HIE16-N, for the HbO concentration only.

Table 8-1 P-values for comparison of FD-NIRS parameters between Healthy Controls and HIE infants.
Table of p-values for the comparison for each FD-NIRS parameter in the individual HIE infants to the Healthy Control (HC) population. Yellow highlights indicate significant difference to the HC population, whereas green indicates no significant difference as determined by Mann-Whitney-Wilcoxon test, with p-value shown.

	HbO-L	HbO-R	HbR-L	HbR-R	HbT-L	HbT-R	rScO2-L	rScO2-R
HIE13-P	$p < 0.001$	$p < 0.001$	0.33	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
HIE14-M	0.39	$p < 0.001$	0.07	$p < 0.001$	0.19	$p < 0.001$	0.20	$p < 0.001$
HIE15-P	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
HIE16-N	0.48	0.44	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
HIE17-M	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.26	$p < 0.001$	$p < 0.001$
HIE18-N	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.68	$p < 0.001$	$p < 0.001$

Correlations

The hours elapsed from the commencement of cooling (C-Hrs) was significantly correlated to at least one of the FD-NIRS parameters in all HIE infants. Table 8-2 indicates the ρ value for each of the significant ($p < 0.05$) correlations discovered. The strongest correlations of $\rho > 0.80$ were between the HbR and Cooled Hours in HIE13-P (Left and Right-hand sides) and HIE16-N (Left hand-side only). In HIE13-P, HbO-L, HbR-L, HbR-R and HbT-L were all negatively correlated to Cooled Hours, which corresponded to a positive correlation between Cooled Hours and $rScO_2$ on both the Left ($\rho = 0.74$) and Right-hand ($\rho = 0.68$) sides. Similarly, in HIE16-N, the $rScO_2$ was correlated to Cooled Hours on the Left-hand side ($\rho > 0.73$), the same side which had a strong negative correlation with HbR ($\rho = -0.88$).

There were significant correlations detected between the FD-NIRS parameters and the recorded temperature at the time of the measurement. These correlations are presented in Table 8-3. There were correlations detected between the temperature and all HIE infants; the strongest correlations of greater than $\rho > 0.80$ were negative correlations in HbR in infants HIE13-P (HbR-L and HbR-R) and HIE16-N (HbR-L only).

Table 8-2 Correlations between the Cooled hrs (C-Hrs) and FD-NIRS parameters in HIE infants.

The ρ value of each of the significant correlations (significance $p < 0.05$) between an FD-NIRS parameter in a HIE infant and the number of hours elapsed from commencement of the therapeutic hypothermia (cooling). High positive or negative correlations (±0.7-0.9) are indicated in yellow, moderate positive or negative correlations (±0.5-0.7) are indicated in orange. The remaining ρ-values are either low positive/negative (±0.3-0.5) or negligible correlations (±0.0-0.3) (256).

C- Hrs	HbO-L	HbO-R	HbR-L	HbR-R	HbT-L	HbT-R	$rScO_2$-L	$rScO_2$-R
HIE13-P	-0.59		-0.84	-0.81	-0.65	-0.34	0.74	0.68
HIE14-M			0.36				-0.67	
HIE15-P		-0.29		-0.32		-0.18		0.30
HIE16-N	-0.56	0.38	-0.88		-0.73	0.58	0.73	
HIE17-M	-0.73	-0.68	-0.59	-0.49	-0.71	-0.72		-0.23
HIE18-N	-0.36	0.21		0.42	-0.23	0.30		-0.26

Table 8-3 The ρ value of each of the significant correlations (significance $p < 0.05$) between an FD-NIRS parameter in a HIE infant and temperature.

The ρ value of each of the significant correlations (significance $p < 0.05$) between an FD-NIRS parameter in a HIE infant and the recorded temperature at the time of the measurement (Temp). High positive or negative correlations (±0.7-0.9) are indicated in yellow, moderate positive or negative correlations (±0.5-0.7) are indicated in orange. The remaining ρ-values are either low positive/negative (±0.3-0.5) or negligible correlations (±0.0-0.3) (256).

Temp	HbO-L	HbO-R	HbR-L	HbR-R	HbT-L	HbT-R	$rScO_2$-L	$rScO_2$-R
HIE13-S	0.30	0.46	-0.24	-0.246	0.20	0.38	0.44	0.34
HIE14-M	0.53		0.60	0.226	0.60		-0.39	
HIE15-S		-0.36	0.18	0.184			-0.22	-0.24
HIE16-N	-0.43	0.32	-0.75	0.187	-0.60	0.57	0.60	
HIE17-M	-0.58	-0.72	-0.33	-0.207	-0.54	-0.72	-0.15	-0.46
HIE18-N	-0.27		0.37	0.356			-0.40	-0.43

For each FD-NIRS parameter, each HIE infant was compared against the HC's for all data points (across all time periods), and in the periods of Therapeutic Hypothermia (TH), rewarming, and when at normal temperature (immediately following TH). The comparisons are presented as boxplots including indications of significant differences in APPENDIX J.

8.4.3 Oxy-Haemoglobin Concentration (HbO) in HIE Infants

The Oxy-Haemoglobin (HbO) concentration at the left and right sensors at the commencement of each 2 hour period is plotted on a graph for each of the 6 HIE infants in Figure 8-1. Plotted against the HIE measurements is the mean of the HC's (continuous line), and the dashed horizontal lines

When comparing the data for all time periods for the HIE infants to the HC's, the HbO Concentration at the left sensor was statistically and significantly:

- Higher in the HIE population as a whole ($p < 0.001$)
- Higher in participants HIE13-P, HIE15-P and HIE17-M ($p < 0.001$)
- Higher in HIE 18-N ($p < 0.05$)

when compared to the HC population.

At the right sensor, HbO Concentration was statistically and significantly:

- Higher in the HIE population as a whole ($p < 0.001$)
- Higher in participants HIE13-P, HIE15-P and HIE17-M ($p < 0.001$)
- Higher in HIE18-N ($p < 0.01$)
- Lower in HIE14-M ($p < 0.001$)

when compared to the HC population. The HbO concentration in HIE16-N was not different to the HC's when comparing data across all time periods.

During both the TH and rewarming periods, the HbO concentration in the HIE population as a whole was statistically higher than in the HC population. The HbO concentration was not statistically significantly higher when HIE infants were at normal temperature. During TH, all HIE infants exhibited statistically different HbO concentrations at both sensors, except HIE16-N which was not different at either sensor. HIE13-P, HIE15-P, and HIE17-M were significantly higher ($p < 0.001$), HIE18-N was statistically higher ($p < 0.01$), and HIE14-M was statistically lower ($p < 0.001$).

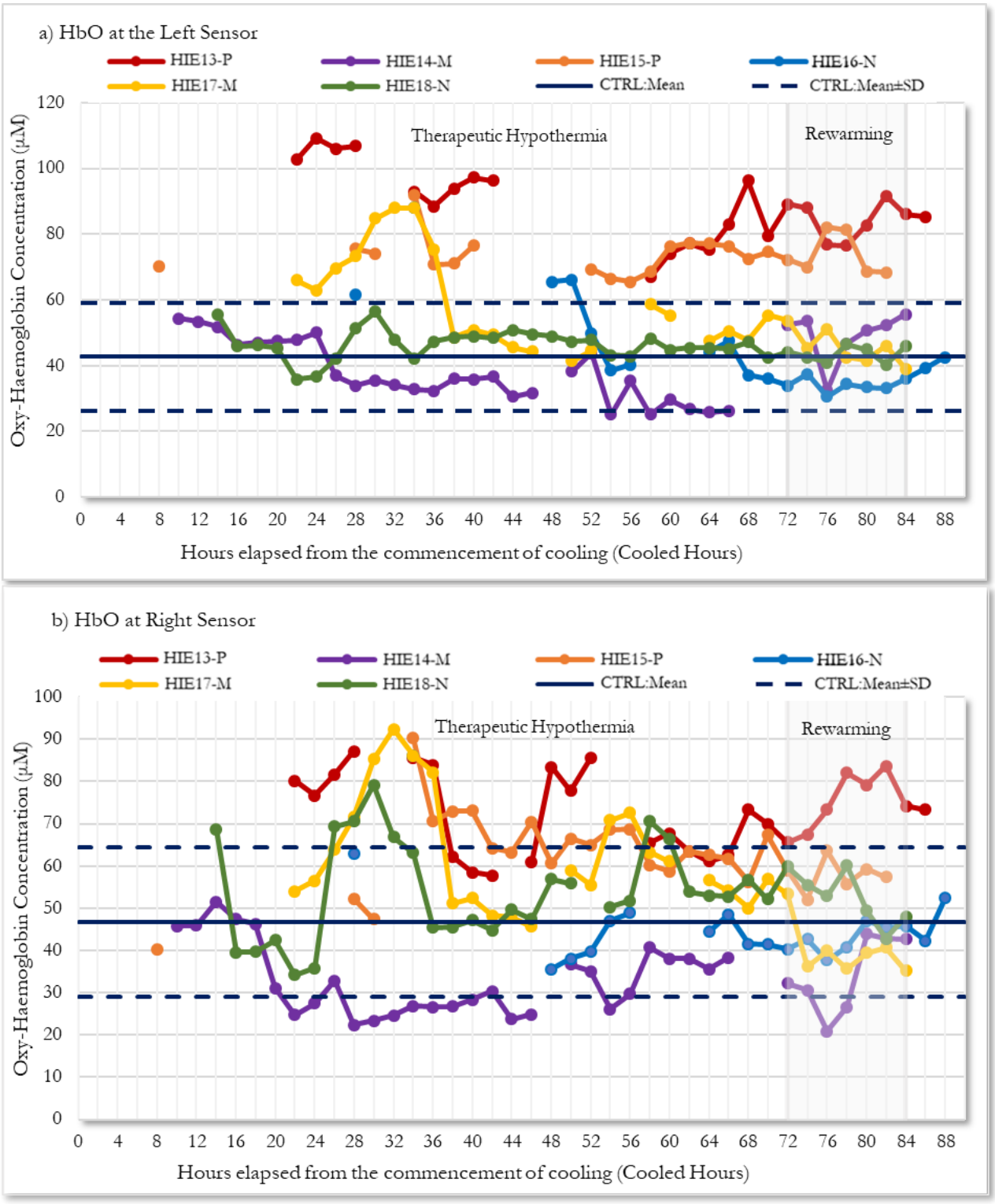


Figure 8-1 The Oxy-Haemoglobin (HbO) Concentration in HIE Infants at the Left (a) and Right (b) Sensor at commencement of each 2-hour interval.

The Oxy-Haemoglobin (HbO) Concentration at the Left (a) and Right (b) Sensor at each 2-hour interval for the HIE infants (where data was available). On the same graphs is plotted a line indicating the mean HbO concentration for the Healthy Controls and the dotted lines indicate ±1SD (standard deviation) from the mean of the Healthy Controls.

Note that all infants are undergoing therapeutic hypothermia treatment from 0-72 Cooled Hours, rewarming occurs between 72-84 hours and that infants are at normal temperature after the rewarming.

Whilst being rewarmed, HIE13-P and HIE-15-P were significantly higher than the HC's at both sensors ($p < 0.001$). HIE17-M was statistically different at the right sensor ($p < 0.001$) but not the left. HIE16-N was statistically different at the left sensor ($p < 0.001$) but not the right. HIE14-M was higher on the left ($p < 0.05$) but lower on the right ($p < 0.001$). HIE18-N was not statistically different to the HC's either sensor. The higher HbO concentrations on the left-hand side persisted in HIE13-P and HIE15-P through to returning to normal temperature after rewarming (normotemp) ($p < 0.001$). HIE13-P also had a higher HbO concentration on the right side ($p < 0.01$).

Analysis by Time Periods

Initially the HbO concentrations in the HIE infants were analysed by comparing each time period against the measurements for all HC infants. These comparisons, with indications for the significant differences between the HIE and HC infants are displayed in APPENDIX K. For each time period, the mean±SD (Standard Deviation) are shown and the colours and the number of asterisks indicating significant differences between the HIE infant and the control population to significance levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$. Yellow was used to indicate a significantly higher HbO concentration, whereas blue is used to indicate a significantly lower HbO concentration to the HC's. The colours have been used for easier identification of higher HbO, which was hypothesised by this author to indicate hyper-perfusion.

The difficulty in comparing different time periods to the HC's was in the varying number of data points for that time period, depending on the chosen length of the time period and the number of data points available. The varying number of data points used in the comparison resulted in inconsistencies in the significances of those comparisons. Furthermore, if the eventual outcome is to be able to give clinicians a parameter upon which they can prognosticate and base decisions regarding treatment, this needs to be a set value and not one contingent on having a healthy population, of an appropriate size in relation to the window of analysis, against which to compare.

Another comparison method was therefore devised. For each time period analysed, if there were four consecutive 15-min epochs in which the mean HbO remained 1SD above or below the mean of the HC population, then it was considered statistically abnormal. Being above 1SD for each 15-min epoch has a probability of <0.16, so the probability of 4 consecutive epochs above 1SD is $0.3^4=0.0007$ (if each measurement was independent from the others, which corresponds to a probability or p-value < 0.001. Although the measurements are not independent, the occurrence of four measurements >1SD is likely to be statistically significant.

To simplify the comparison, the highest of 1SD above the Left and Right mean in the HC's was taken as the Upper Limit, the lowest of the 1SD below the Left and Right-hand side measurements in the HC's was taken as the Lower Limit. One SD above the mean on the left-hand side in the HC's was 59μM, and on the right-hand side it was 64 μM, so the Upper Limit was taken as 64 μM. Similarly, 1SD below the mean HbO in the HC's was 26μM on the left-hand side and 29μM on the right-hand side, so 26μM was taken as the Lower Limit.

Table 8-4 displays the HbO concentration mean±1SD for each HIE infant for each analysed time periods. The period of 12h from 60 to 72 Cooled Hrs was a particular period of interest, as it is possible in the future that the 12 hours prior to rewarming will be important in influencing treatment, especially if the infants who might have a poor outcome could be identified. A period of normality is defined by neither sensor having 4 or more consecutive 15-min epochs outside derived limits (26 μM < HbO < 59 μM).

The same patterns in HbO could be discerned from both methods of comparison. HIE13-P has a very high initial HbO concentration at both sensors and remains high in both sensors apart from 4 time periods in which the HbO measurements on the left side are not greater than the Upper Limit. The HbO in HIE13-P remains high through to normotemp. HIE15-P has 4 or more consecutive HbO measurements greater than the Upper Limit in every time period on the left-hand side. On the right-hand side, the mean HbO in HIE15-P is always greater than Upper Limit for at least 4 consecutive 15-min epochs in all time periods except two, which were 0-24 and 62-64 Cooled Hours. HIE13-P and HIE15-P both had poor outcomes.

HIE16-N, HIE17-M and HIE18-N all had periods where the HbO was greater than the Upper Limit for 4 or more consecutive 15-min epochs in the first 60 hrs of cooling; after 60 Cooled Hours none had any periods with consecutively high HbO (4 measurements >Upper Limit) in the 12-hour period prior to rewarming. Indications from other examinations with US, MRI and clinical condition subsequent to rewarming suggest these three infants have had a normal outcome. The HbO concentration in HIE14-M was either lower or within 1SD of the mean HbO of the HC's on both sides throughout, including the 12-hour period prior to rewarming. HIE14-M had no severe developmental delays at 2 yrs.

Table 8-4 Oxy-Haemoglobin (HbO) in the HIE infants (Mean±1SD) by Periods.

Mean HbO for the Time Periods shown (mean±1SD) for each of the HIE infants. Yellow indicates that there were 4 consecutive 15-min epochs with mean HbO >64μM (Upper Limit, >1SD than mean of Healthy Controls) during that time period. Blue indicates that there were 4 consecutive 15-min epochs with mean HbO <26μM (Lower Limit, <1SD of than the mean HbO in Healthy Controls), and green otherwise; if there were not 4 or more consecutive 15-min epochs where the mean HbO in the HIE infant was outside of derived Upper & Lower Limits.

Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L		101 ± 3	48 ± 3	69 ± 1		64 ± 2	46 ± 5
	R		78 ± 3	38 ± 13	41 ± 2		55 ± 3	41 ± 8
24-48 Cooled Hrs	L		99 ± 8	34 ± 2	76 ± 7	65 ± 1	67 ± 16	48 ± 3
	R		73 ± 11	25 ± 2	67 ± 15	36 ± 0	69 ± 18	54 ± 10
48-54 Cooled Hrs				39 ± 4		54 ± 11	44 ± 2	48 ± 1
				32 ± 4		41 ± 4	59 ± 2	57 ± 1
54-60 Cooled Hrs	L		65 ± 2	29 ± 4	69 ± 3	39 ± 1	60 ± 3	44 ± 3
	R		63 ± 2	27 ± 2	68 ± 2	46 ± 2	71 ± 7	62 ± 8
60-62 Cooled Hrs	L		69 ± 3	28 ± 2	69 ± 0		57 ± 2	45 ± 1
	R		66 ± 3	39 ± 1	61 ± 1		61 ± 0	63 ± 6
62-64 Cooled Hrs	L		75 ± 3	28 ± 2	74 ± 2		46 ± 2	43 ± 2
	R		64 ± 1	37 ± 1	56 ± 5		60 ± 3	51 ± 2
64-66 Cooled Hrs	L		76 ± 2	27 ± 2	78 ± 1	46 ± 1	48 ± 1	47 ± 1
	R		61 ± 1	37 ± 2	63 ± 1	46 ± 2	55 ± 1	53 ± 1
66-68 Cooled Hrs	L		75 ± 5		77 ± 1	41 ± 1	48 ± 2	44 ± 2
	R		59 ± 3		62 ± 1	43 ± 1	51 ± 3	53 ± 2
68-70 Cooled Hrs	L		94 ± 2		77 ± 0	38 ± 1	53 ± 1	44 ± 2
	R		74 ± 6		63 ± 1	41 ± 1	56 ± 2	52 ± 3
70-72 Cooled Hrs	L				75 ± 2	35 ± 2	53 ± 2	43 ± 2
	R				60 ± 2	41 ± 2	53 ± 2	52 ± 1
Rewarm 72-78 Hrs	L		84 ± 5	45 ± 8	71 ± 2	34 ± 2	47 ± 3	43 ± 3
	R		67 ± 3	24 ± 4	59 ± 6	40 ± 2	39 ± 5	56 ± 7
Rewarm 78-84 Hrs	L		84 ± 5	53 ± 3	80 ± 4	35 ± 2	44 ± 3	44 ± 3
	R		78 ± 3	41 ± 5	60 ± 4	47 ± 2	38 ± 3	48 ± 5
Normo-temp	L	43 ± 16	85 ± 1	56 ± 1	69 ± 0	40 ± 3	39 ± 1	45 ± 2
	R	47 ± 18	72 ± 2	43 ± 1	59 ± 0	47 ± 5	36 ± 1	43 ± 3
Period of Normality? Yes (Y) or No (N)			**NO**	**YES**	**NO**	**YES**	**YES**	**YES**

8.4.4 De-Oxyhaemoglobin (HbR) Concentration in HIE Infants

The Deoxy-Haemoglobin (HbR) concentration at the commencement of each 2-hour period for each of the 6 HIE infants is displayed in graphs for the left and right-hand sensors in Figure 8-2; a) and b) respectively. Plotted against the HIE measurements is the mean of the control group (continuous line) and the mean plus one standard deviation (SD) and the mean minus one standard deviation which are both shown as dotted lines, above and below the mean for the controls accordingly.

Overall Analysis

To analyse the overall differences in HbR concentration measurements, all data points from the HIE infants were compared against the Healthy Controls (HC's), individually and as a group. The boxplots of individual HIE infant comparisons for all data points, during TH, rewarming and at normotemp (immediately following rewarming) are presented in APPENDIX J.

In general, HbR was significantly lower in both the left and right-hand sensors in the HIE population than in the HC's when pooling data from all time periods. The HbR was significantly lower in the HIE population in general at both sensors because it was significantly lower for all stages of the treatment; whilst cooled, rewarmed and also after being returned to normal temperature immediately following the TH. To summarise by HIE infant:-

At the left sensor, the HbR concentration was statistically and significantly:

- Lower in the HIE population altogether ($p < 0.01$)
- Lower in HIE16-N, HIE17-M and HIE18-N ($p < 0.001$)
- Lower in HIE14-M ($p < 0.05$)
- Higher in HIE15-P ($p < 0.05$)

in comparison with the HC's. The HbR concentration was not significantly different to the HC's on the left side in infants HIE13-P.

At the right sensor, the HbR concentration was statistically and significantly lower in the HIE population and in all individual HIE infants:

- Lower in the HIE population altogether ($p < 0.001$)
- Lower in HIE13-P, HIE15-P, HIE16-N, HIE17-M ($p < 0.001$)
- Lower in HIE14-M ($p < 0.01$)
- Lower in HIE18-N ($p < 0.05$)

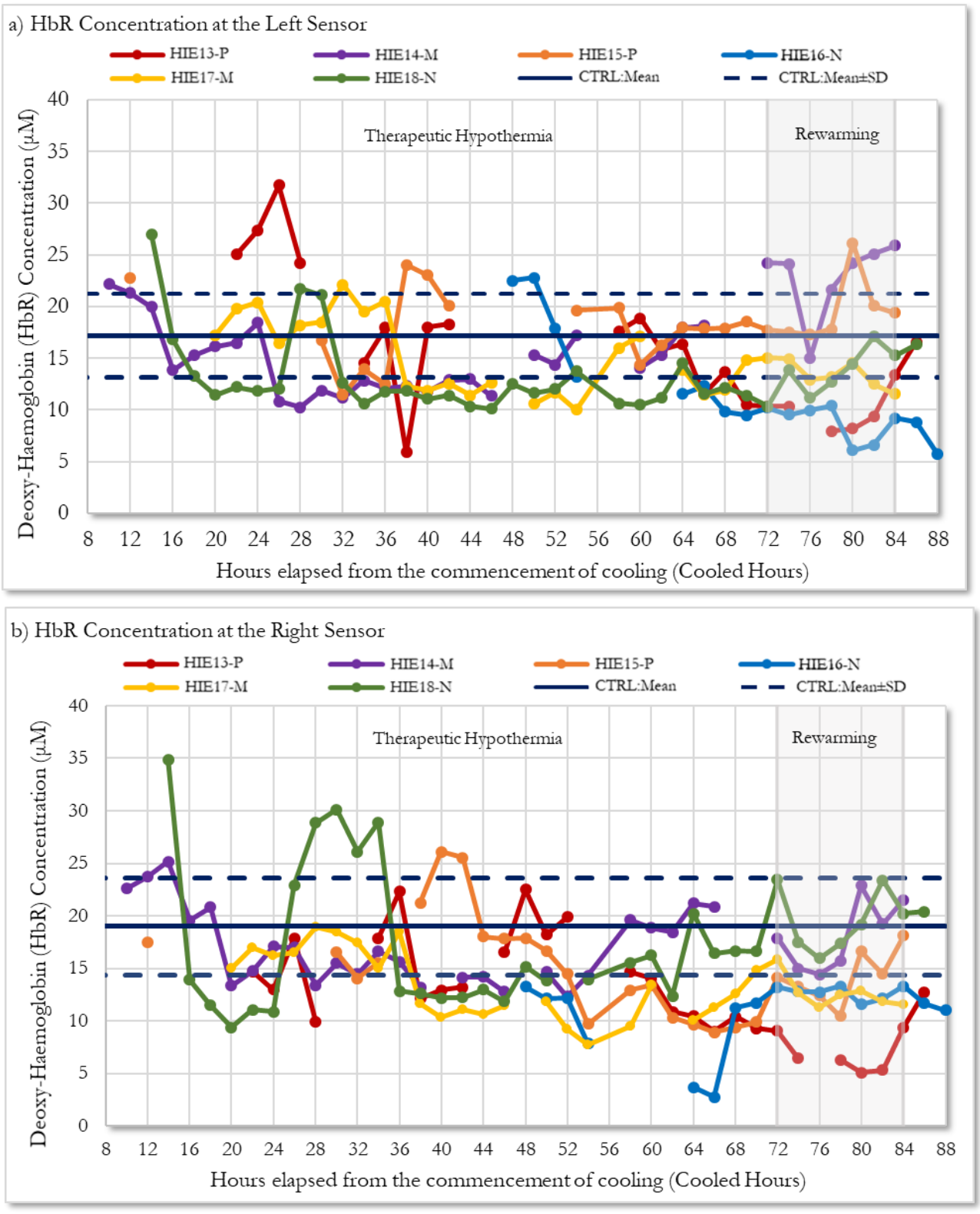


Figure 8-2 The Deoxy-Haemoglobin (HbR) Concentration in HIE Infants at the Left (a) and Right (b) Sensor at commencement of each 2-hour interval.

The Deoxy-Haemoglobin (HbR) Concentration at the Left (a) and Right (b) Sensor at each 2-hour interval for the HIE infants (where data was available). On the same graphs is plotted a line indicating the mean HbR concentration for the Healthy Controls and the dotted lines indicate ±1SD (standard deviation) from the mean of the Healthy Controls.

Note that all infants are undergoing therapeutic hypothermia treatment from 0-72 Cooled Hours, rewarming occurs between 72-84 hours and that infants are at normal temperature after the rewarming.

During TH, compared to the HC's all HIE infants except HIE15-P had significantly different HbR measurements on both sides, and HIE15-P had significantly lower HbR measurements on the right side:

- HIE13-P (higher on the left: $p < 0.05$, lower on the right: $p < 0.001$),
- HIE14-M (lower on the left and right: $p < 0.001$),
- HIE15-P (not significantly different on the left, lower on the right: $p < 0.001$),
- HIE16-N (lower on left: $p < 0.01$, lower on right: $p < 0.001$),
- HIE17-M (lower on the left: $p < 0.05$, lower on the right: $p < 0.001$),
- HIE18-N (lower on the left: $p < 0.001$, lower on the right: $p < 0.01$).

During rewarming, all HIE infants had HbR measurements significantly different to the HC's:

- HIE13-P, HIE 16-P, HIE-17-P and HIE18-P were all lower on the left side and HIE13-P, HIE14-M, HIE15-P, HIE16-N, HIE17-M were all lower on the right side (all $p < 0.001$).
- HIE14-P ($p < 0.001$) and HIE15-P ($p<0.01$) were higher on the right side,
- HIE18-N was not significantly different on the right side.

After rewarming, at normotemp, the HbR concentration remained significantly different to the HC's on the left-hand side for infants HIE14-M (higher, $p< 0.001$), HIE16-N (lower, $p < 0.001$) and HIE17-M (lower, $p < 0.01$). On the right-hand side HIE13-S ($p < 0.01$), HIE16-N ($p < 0.001$) and HIE17-M ($p < 0.01$) continued to have significantly lower HbR concentrations than the HC's.

Analysis by Time Period

The HbR was analysed divided into time periods, in the same way as the HbO in the previous section. HbR measurements in each time period for each HIE infant were directly compared with the HC's using the Mann-Whitney-Wilcoxon test. The table of the mean HbR for each infant by time period (mean±1SD) with the significance of each of comparison is displayed in APPENDIX K. The same issues with inconsistencies of significances due to varying numbers of time points were encountered.

The same alternative method of comparison as described for the HbO was therefore used. Four consecutive 15-min epochs of HbR measures greater than or less than 1SD of the HC's were taken as an indication that the HbR concentration was abnormally high or low, relative to the HC's. Thus, the higher mean+1SD of the left and right in the HC's, 23μM (right) was taken as the Upper Limit and the mean-1SD in the HC's, 13 μM (left) was taken as the Lower Limit. The mean HbR (±SD) for each HIE infant by time period is presented in Table 8-5.

Table 8-5 Deoxy-Haemoglobin (HbR) in the HIE infants (Mean±SD) by Periods.

Mean HbR for the Periods shown (mean±1SD) for each of the HIE infants. Yellow indicates that there were 4 consecutive 15-min epochs with mean HbR >23μM (Upper Limit, >1SD than mean of Healthy Controls) during that time period. Blue indicates that there were 4 consecutive 15-min epochs with mean HbR <13μM (Lower Limit, <1SD of than the mean HbR in Healthy Controls), and green otherwise; if there were not 4 or more consecutive 15-min epochs where the mean HbR in the HIE infant was outside of derived Upper & Lower Limits.

Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L		24 ± 2	17 ± 3	20 ± 4		19 ± 2	15 ± 4
	R		14 ± 1	20 ± 4	16 ± 2		16 ± 1	13 ± 6
24-48 Cooled Hrs	L		22 ± 6	12 ± 1	20 ± 5	22 ± 0	17 ± 4	12 ± 3
	R		15 ± 3	15 ± 2	22 ± 5	13 ± 1	15 ± 3	18 ± 7
48-54 Cooled Hrs	L			16 ± 2		19 ± 4	11 ± 1	12 ± 0
	R			14 ± 3		11 ± 2	9 ± 1	14 ± 1
54-60 Cooled Hrs	L		18 ± 0	15 ± 2	19 ± 1	12 ± 1	17 ± 1	12 ± 1
	R		15 ± 0	14 ± 2	12 ± 1	7 ± 1	10 ± 2	15 ± 2
60-62 Cooled Hrs	L		16 ± 2	15 ± 2	15 ± 0		18 ± 1	10 ± 0
	R		13 ± 1	19 ± 2	14 ± 0		13 ± 2	16 ± 2
62-64 Cooled Hrs	L		16 ± 0	16 ± 2	17 ± 1		13 ± 1	15 ± 2
	R		11 ± 0	19 ± 2	10 ± 0		12 ± 2	21 ± 4
64-66 Cooled Hrs	L		16 ± 1	16 ± 2	18 ± 1	12 ± 1	13 ± 1	13 ± 1
	R		11 ± 1	19 ± 2	9 ± 0	2 ± 1	12 ± 1	18 ± 2
66-68 Cooled Hrs	L		14 ± 2		18 ± 0	10 ± 0	12 ± 0	12 ± 0
	R		10 ± 1		9 ± 0	11 ± 0	13 ± 1	17 ± 0
68-70 Cooled Hrs	L		12 ± 1		18 ± 0	10 ± 1	15 ± 1	13 ± 2
	R		9 ± 1		10 ± 0	12 ± 1	14 ± 1	18 ± 3
70-72 Cooled Hrs	L				18 ± 1	10 ± 1	14 ± 1	12 ± 1
	R				10 ± 1	13 ± 1	15 ± 1	17 ± 1
Rewarm 72-78 Hrs	L		11 ± 1	20 ± 4	17 ± 1	10 ± 1	13 ± 1	12 ± 2
	R		8 ± 2	15 ± 1	12 ± 1	13 ± 1	12 ± 1	17 ± 3
Rewarm 78-84 Hrs	L		9 ± 1	25 ± 1	23 ± 3	7 ± 2	13 ± 1	15 ± 2
	R		6 ± 1	21 ± 3	16 ± 1	12 ± 1	12 ± 1	21 ± 3
Normo-temp	L	17 ± 4	15 ± 1	26 ± 1	20 ± 0	8 ± 1	12 ± 0	16 ± 1
	R	19 ± 5	11 ± 1	21 ± 0	18 ± 0	12 ± 2	12 ± 1	20 ± 0
Period of Normality? Yes (Y) or No (N)			**YES**	**YES**	**YES**	**YES**	**YES**	**YES**

8.4.5 Total Haemoglobin (HbT) Concentration in the HIE infants

The Total Haemoglobin (HbT) was analysed in a similar way to the HbO and HbR. The HIE infants were compared with the Healthy Controls (HC's) by first comparing against all time/data points, then the data was separated into stages of treatment: during TH, rewarming, and normotemp. The boxplots for these comparisons are displayed in APPENDIX J. The HbT measurement at the commencement of each 2-hour period for each HIE infant (where data were available) was also plotted against the mean HbT (±1SD) of the HC's. The graphs of the HbT in the HIE infants versus the controls for the left and right sensors is displayed in Figure 8-3.

The HbT measurements were then separated into shorter time periods for each HIE infant, and the data from each time period was compared directly with the HC's using the Mann-Whitney-Wilcoxon test. The table indicating the significance of any differences to the HC population is displayed in APPENDIX K. Once again, there were inconsistencies using this method, as the significance of any differences was influenced by the number of data points involved in the comparison. In addition to the statistical anomalies introduced by the number of data points, it would also be more useful clinically if a hard limit could be used for assessment.

Therefore, using the alternative method of comparison introduced earlier, an Upper and Lower Limit was determined for HbT. The Upper Limit was the greater of the left and right-hand mean HbT+1SD, and similarly the Lower Limit for HbT was the lower of left and right-hand mean HbT-1SD. The Upper Limit was determined to be 87μM (mean+1SD in the HC's on the right-hand side), and the Lower Limit similarly determined to be 40μM (mean-1SD in the HC's on the left-hand side). Table 8-6 displays the mean±1SD for each HIE infant by time period, indicating those time periods where there were 4 consecutive 15-min epochs above the Upper or below the Lower Limits.

The patterns evident in HbT concentration in the HIE infants were similar to those in the HbO. HIE13-P and HIE15-P, the infants with the poor outcomes, were the two infants who presented with persistently high HbT measurements. The patterns observed in the HbO were expected to be also discernible in the HbT because HbT is the addition of HbO and HbR; though these patterns are more distinct in the HbO, the addition of the HbR makes those patterns less discernible as the HbR was often significantly lower than in the HC group in those same time periods.

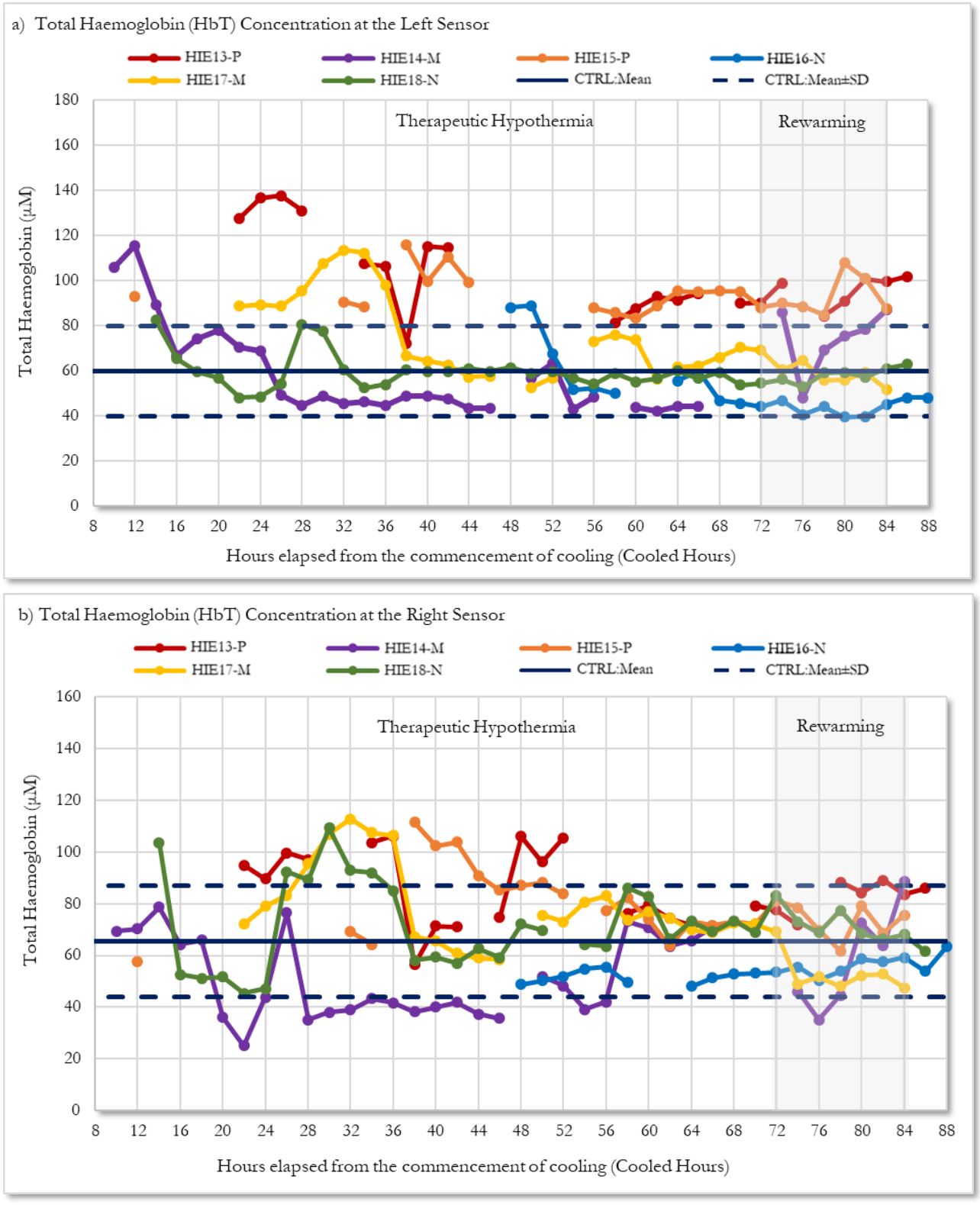


Figure 8-3 Total Haemoglobin (HbT) in HIE Infants at the Left (a) and Right (b) Sensor at commencement of each 2-hour interval.

Total Haemoglobin (HbT) Concentration at the Left (a) and Right (b) Sensor at each 2-hour interval for the HIE infants (where data was available). On the same graphs is plotted a line indicating the mean HbT concentration for the Healthy Controls and the dotted lines indicate ±1SD (standard deviation) from the mean of the Healthy Controls.

Note that all infants are undergoing therapeutic hypothermia treatment from 0-72 Cooled Hours, rewarming occurs between 72-84 hours and infants are at normal temperature subsequent to the rewarming.

Table 8-6 Total Haemoglobin (HbT) in the HIE infants (Mean±SD) by Periods.

Mean HbT for the Periods shown (mean±1SD) for each of the HIE infants. Yellow indicates that there were 4 consecutive 15-min epochs with mean HbT >87μM (Upper Limit, >1SD than mean of Healthy Controls) during that time period. Blue indicates that there were 4 consecutive 15-min epochs with mean HbT <40μM (Lower Limit, <1SD of than the mean HbR in the Healthy Controls), and green otherwise; if there were not 4 or more consecutive 15-min epochs where the mean HbR in the HIE infant was outside of the derived Upper & Lower Limits.

Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L		125 ± 4	66 ± 6	89 ± 5		84 ± 4	61 ± 9
	R		93 ± 3	57 ± 17	58 ± 0		71 ± 4	55 ± 14
24-48 Cooled Hrs	L		121 ± 14	46 ± 2	96 ± 9	87 ± 1	84 ± 20	60 ± 5
	R		89 ± 14	40 ± 3	89 ± 20	49 ± 0	84 ± 21	72 ± 17
48-54 Cooled Hrs	L			55 ± 4		73 ± 15	55 ± 2	60 ± 1
	R			44 ± 7		53 ± 3	68 ± 3	71 ± 1
54-60 Cooled Hrs	L		83 ± 2	43 ± 3	88 ± 3	51 ± 1	77 ± 3	56 ± 1
	R		78 ± 2	41 ± 4	80 ± 3	53 ± 2	82 ± 6	76 ± 10
60-62 Cooled Hrs	L		85 ± 4	43 ± 1	84 ± 0		74 ± 3	55 ± 1
	R		79 ± 4	59 ± 1	76 ± 1		73 ± 2	79 ± 8
62-64 Cooled Hrs	L		91 ± 3	43 ± 1	92 ± 2		59 ± 2	58 ± 2
	R		75 ± 1	57 ± 1	66 ± 4		73 ± 3	72 ± 2
64-66 Cooled Hrs	L		92 ± 2	43 ± 2	96 ± 1	58 ± 1	61 ± 1	60 ± 2
	R		72 ± 2	56 ± 2	72 ± 0	48 ± 2	67 ± 1	71 ± 2
66-68 Cooled Hrs	L		89 ± 4		95 ± 1	51 ± 1	60 ± 1	56 ± 2
	R		69 ± 3		71 ± 1	54 ± 1	64 ±2	69 ± 2
68-70 Cooled Hrs	L		106 ± 3		95 ± 0	48 ± 1	67 ± 2	58 ± 2
	R		79 ± 3		72 ± 1	53 ± 0	70 ± 2	71 ± 3
70-72 Cooled Hrs	L				93 ± 3	45 ± 1	67 ± 3	55 ± 2
	R				71 ± 2	54 ± 1	68 ± 2	69 ± 2
Rewarm 72-78 Hrs	L		95 ± 6	65 ± 12	88 ± 2	44 ± 2	60 ± 3	55 ± 4
	R		74 ± 4	39 ± 5	71 ± 7	54 ± 1	51 ± 5	73 ± 9
Rewarm 78-84 Hrs	L		92 ± 6	78 ± 4	103 ± 5	42 ± 3	57 ± 3	59 ± 4
	R		84 ± 3	61 ± 8	75 ± 4	58 ± 1	50 ± 3	69 ± 5
Normo-temp	L	60 ± 20	100 ± 1	82 ± 2	88 ± 0	48 ± 2	50 ± 1	61 ± 2
	R	66 ± 22	84 ± 2	64 ± 1	77 ± 0	59 ± 7	48 ± 2	63 ± 3
Period of Normality? Yes (Y) or No (N)			**YES**	**YES**	**NO**	**YES**	**YES**	**YES**

8.4.6 Regional Cerebral Oxygen Saturation

The Regional Cerebral Oxygen Saturation ($rScO_2$) at the start of each 2 hour period in each of the 6 HIE infants is displayed in Figure 8-4; a) for the left sensor, and b) for the right-hand sensor. Plotted against the HIE measurements is the mean of the Healthy Controls (HC's) (continuous line) and the mean±1SD shown as dotted lines above and below the mean.

Overall Analysis

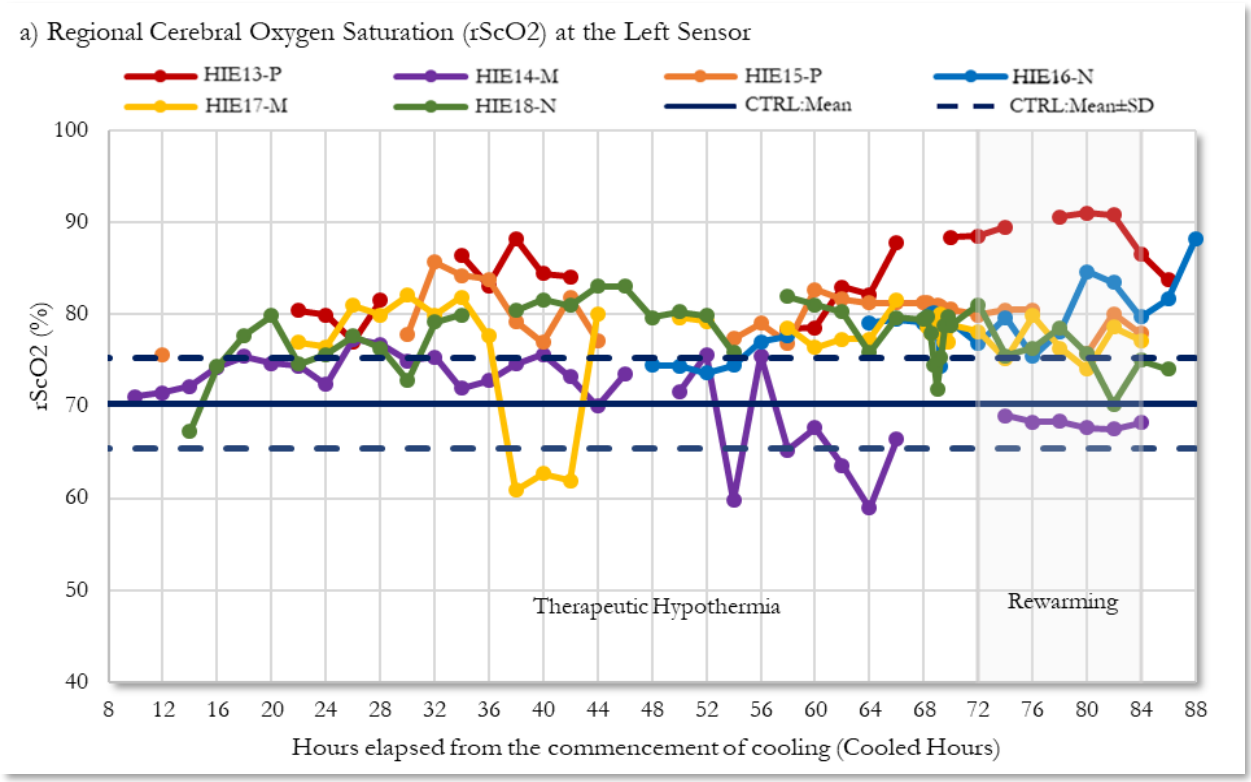
The $rScO_2$ values across all time periods from pooled data of the Control Population were compared with the whole HIE Population and also with each HIE participant. Boxplots for comparisons of each of the HIE infants at each stage of treatment, with indications of significant differences relative to the HC's, may be found in APPENDIX J.

The $rScO_2$ was significantly higher in all HIE participants for all periods except for HIE14-M (all treatment stages), and HIE17-M (left side only) and HIE18-N (both sides) after being returned to normal temperature following rewarming. HIE14-M had an $rScO_2$ that was statistically similar to the Healthy Controls on the left side at all treatment stages and on the right side at normotemp but was significantly lower than the Healthy Controls during TH and rewarming.

The $rScO_2$ is the relative amount of HbO to total Haemoglobin (HbT), which is the addition of HbO and HbR, presented as a percentage. Thus it might follow that a high $rScO_2$ would be due to a high HbO, but this may not always be the case; a high $rScO_2$ may also be due to a lower HbR, alone or in combination with a high HbO, a concurrent decrease in both HbO and HbR, or even a concurrent increase in both HbO and HbT. All of these patterns were observed in the HIE infants in this current study. In contrast the only observation of a lowered $rScO_2$, in HIE14-M, was due to concurrent decreases in HbO and HbR. An overview of the significant differences in HbO and HbR, and the resultant significant differences in $rScO_2$ is presented in APPENDIX L.

Analysis by Time Period

The $rScO_2$ concentration measurements in the HIE infants were also analysed by period, focusing on the 12 hours prior to rewarming in 2-hour periods. The table of the mean $rScO_2$ (±1 SD) for each HIE infant for each of the defined periods at the left and right sensors, indicating any significant differences to the Healthy Controls, is displayed in APPENDIX K. As mentioned previously in the HbO, HbR, and HbT results, the statistical comparison using the Mann-Whitney-Wilcoxon test was influenced by the number of data points used in the comparison. Moreover, it would be more useful to be able to define hard limits for parameters, as these are more useful in a clinical setting. Thus, once again, the alternative method of comparison was utilised.



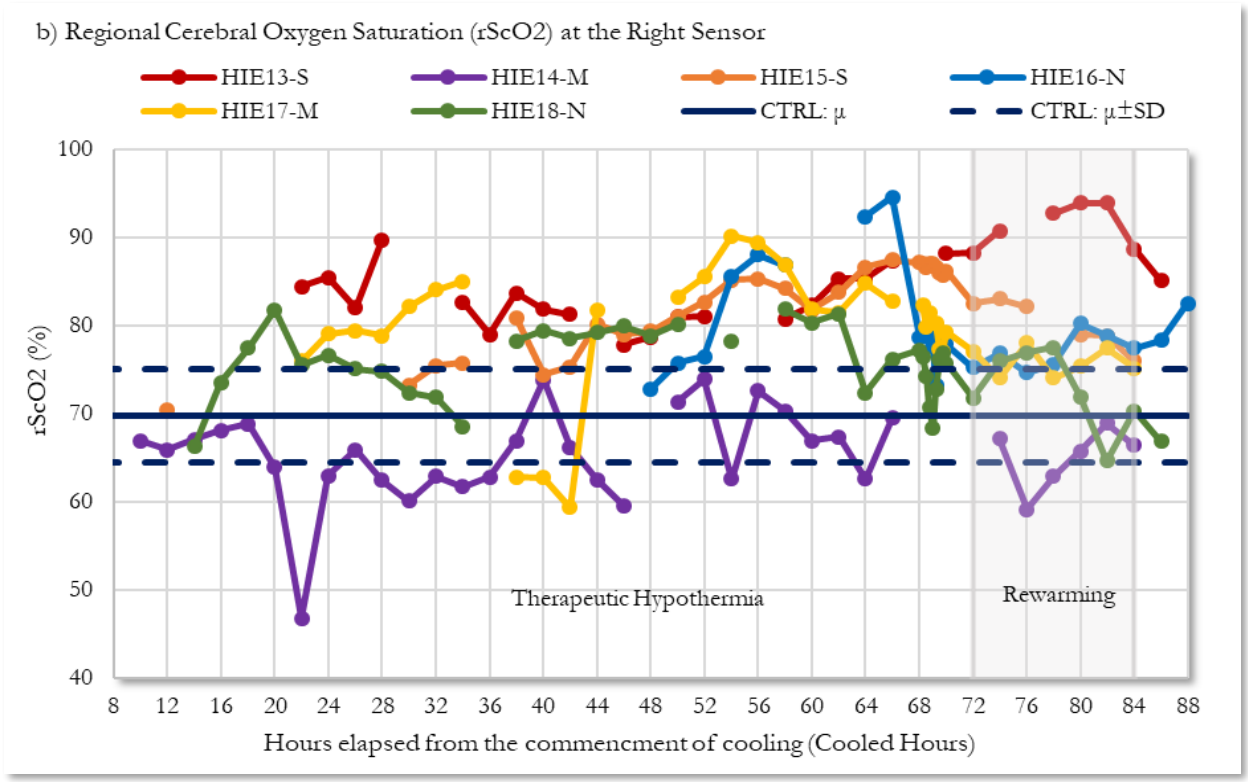


Figure 8-4 The Regional Cerebral Oxygen Saturation ($rScO_2$) in HIE Infants at the Left (a) and Right (b) Sensor at commencement of each 2-hour interval.

The Oxy-Haemoglobin (HbO) Concentration at the Left (a) and Right (b) Sensor for each 2-hour interval for the HIE infants (where data was available). On the same graphs is plotted a line indicating the mean $rScO_2$ concentration for the Healthy Controls and the dotted lines indicate ±1SD (standard deviation) from the mean of the Healthy Controls.

Note that all infants are undergoing therapeutic hypothermia treatment from 0-72 Cooled Hours, rewarming occurs between 72-84 hours and that infants are at normal temperature after the rewarming.

The alternative method for comparison involved deriving an Upper and Lower Limit based on the mean $rScO_2$ ±1SD for the HC's. Although there were differences between the mean±1SD on the left and right-hand sides for HbO, HbR and HbT in the HC's, the mean±1SD in the $rScO_2$ on the left side was the same and the mean-1SD was only 1% different (65% on the left and 64% on the right side). The Upper Limit and Lower Limits were defined as 75% (mean+1SD) and 64% accordingly. Any period where there were 4 consecutive 15-min epochs where the $rScO_2$ was outside the limits was considered statistically abnormal. The $rScO_2$ mean±SD and indications where measurements were outside the limits can be found in Table 8-7.

When comparing directly with the HC's, only HIE14-M had any periods of normality: where both left and right-hand measurements were statistically similar to the HC's on both sides concurrently. Using the alternative method, HIE14-M, HIE18-N and HIE17-M all have periods of normality prior to rewarming, albeit briefly in the latter two. The $rScO_2$ in HIE14-M was lower or within 1SD of the mean in the HC's from 48 hours onwards: whereas HIE 17 only at 24-48 Cooled Hrs, and HIE18-N has a period of normality only at 62-64 Cooled Hr. Only HIE14-M and HIE18-N have consistent $rScO_2$ measurements at normotemp. Otherwise, for all other periods in all HIE infants, except for HIE14-M, the $rScO_2$ was consistently greater than the Upper Limit. At normotemp, the $rScO_2$ was highest in HIE13-P.

8.4.7 Differences between Left and Right Sensors

In this study, differences were observed in HbO and HbR measurements between the left and right sensors in the HIEinfants. There were also some differences between sensors in the HC's, although the difference between left and right-hand HbO measurements was not significant in this population using pairwise comparison. The mean difference between the HbO measurement in the left and right sensors (mean (R-L)) was positive, meaning that the mean right HbO measurement was in general higher than the left mean HbO measurement, albeit not significantly. The HbR in the HC's was significantly different between sensors ($p < 0.05$); the mean right HbR measurement was statistically greater than the mean left HbR measurement.

In an effort to understand whether there was any significance in the differences between sensors, the differences in HbO and HbR in the HIE infants were analysed by pairwise comparison, using the Wilcoxon Signed Rank test, and by determining the mean difference between sensors by time periods. The pairwise comparisons and the mean differences between sensors (±1SD) was tabulated and is displayed in APPENDIX M. Although there were significant differences in the HbO and HbR measurements in the HIE infants at the left and right sensors, a pattern did not emerge.

Table 8-7 Regional Cerebral Oxygen Saturation in the HIE infants (Mean±SD) by Periods.

Mean $rScO_2$ for the Periods shown (mean±1SD) for each of the HIE infants. Yellow indicates that there were 4 consecutive 15-min epochs with mean $rScO_2$ >75% (Upper Limit, >1SD than mean $rScO_2$ of Healthy Controls) during that time period. Blue indicates that there were 4 consecutive 15-min epochs with mean $rScO_2$ <64% (Lower Limit, <1SD of than the mean $rScO_2$ in Healthy Controls), and green otherwise; if there were not 4 or more consecutive 15-min epochs where the mean $rScO_2$ in the HIE infant was outside of derived Upper & Lower Limits.

Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L			74 ± 2	78 ± 3		77 ± 1	76 ± 4
	R			64 ± 6	72 ± 2		77 ± 1	76 ± 4
24-48 Cooled Hrs	L		82 ± 3	74 ± 2	79 ± 4	74 ± 0	75 ± 8	80 ± 3
	R		83 ± 3	63 ± 3	75 ± 2	73 ± 0	76 ± 10	76 ± 3
48-54 Cooled Hrs	L			72 ± 3		75 ± 1	80 ± 1	80 ± 0
	R			70 ± 3		78 ± 4	86 ± 1	80 ± 1
54-60 Cooled Hrs	L		79 ± 1	66 ± 5	78 ± 1	76 ± 1	78 ± 1	79 ± 2
	R		81 ± 0	66 ± 3	85 ± 1	87 ± 1	87 ± 3	81 ± 1
60-62 Cooled Hrs	L		81 ± 2	64 ± 4	83 ± 0		76 ± 0	81 ± 1
	R		84 ± 1	67 ± 2	81 ± 0		83 ± 2	79 ± 1
62-64 Cooled Hrs	L		82 ± 1	64 ±4	81 ± 0		78 ± 1	74 ± 3
	R		85 ± 1	66 ± 3	85 ± 1		83 ± 2	71 ± 5
64-66 Cooled Hrs	L		83 ± 1	63 ± 5	82 ± 1	80 ± 1	79 ± 2	78 ± 2
	R		85 ± 1	65 ± 3	87 ± 1	95 ± 2	83 ± 2	74 ± 2
66-68 Cooled Hrs	L		85 ± 3		81 ± 0	81 ± 0	80 ± 1	79 ± 1
	R		86 ± 1		87 ± 0	79 ± 0	80 ± 2	76 ± 1
68-70 Cooled Hrs	L		88 ± 1		81 ± 0	79 ± 2	78 ± 1	77 ± 3
	R		88 ± 5		87 ± 1	78 ± 2	80 ± 2	74 ± 3
70-72 Cooled Hrs	L				81 ± 0	77 ± 3	79 ± 0	79 ± 2
	R				85 ± 1	76 ± 2	78 ± 1	75 ± 2
Rewarm 72-78 Hrs	L		89 ± 1	70 ± 1	80 ± 1	77 ± 2	78 ± 2	78 ± 2
	R		89 ± 2	61 ± 4	83 ± 1	75 ± 2	76 ± 2	76 ± 2
Rewarm 78-84 Hrs	L		90 ± 1	68 ± 1	78 ± 2	83 ± 3	77 ± 2	74 ± 3
	R		85 ± 1	66 ± 3	79 ± 1	80 ± 2	76 ± 1	70 ± 4
Normo-temp	L	70 ± 5	85 ± 1	68 ± 1	78 ± 0	83 ± 3	77 ± 1	74 ± 1
	R	70 ± 5	87 ± 1	67 ± 1	76 ± 0	80 ± 2	75 ± 0	68 ± 2
Period of Normality? Yes (Y) or No (N)			**NO**	**YES**	**NO**	**YES**	**NO**	**YES**

8.4.8 Relationship between FD-NIRS and DUS parameters

The FD-NIRS parameters: HbO, HbR, HbT and $rScO_2$: and Doppler Ultrasound (DUS) parameters: Peak Systolic Velocity (PSV), End Diastolic Velocity (EDV), Time-Averaged Velocity (TAV), Resistive Index (RI) and Pulsatility Index (PI): were compared to determine whether there were any correlations that would reveal any relationships between the two methods of assessing changes to cerebral perfusion. Although correlations were detected, they were different for every infant indicating that there is not a general, consistent relationship between FD-NIRS and DUS parameters. The correlations discovered are summarised in Table 8-8.

Table 8-8 Summary of Correlations detected between FD-NIRS and DUS parameters in the HIE infants.

Summary of Correlations detected between FD-NIRS NIRS (Frequency-Domain Near Infrared Spectroscopy) and DUS parameters in the HIE Infants. Rho (ρ) value is presented as indication of strength of correlation, asterisks indicate p-value: * - $p<0.05$, ** - $p<0.01$ and ***-$p<0.001$. There were no correlations discovered between FD-NIRS and DUS parameters in HIE14-M. (*S*=Sensor, L=Left, R=Right).

HIE infant	*FD-NIRS Parameters*	*S*	*Doppler US Parameters*				
			PSV	***EDV***	***TAV***	***RI***	***PI***
HIE13-P	**HbO**	**R**	-0.80 **	-0.81 **	-0.80 **		
	HbT	**R**		-0.68 *			
HIE15-P	**rScO2**	**L**				0.86 **	0.89 **
HIE16-N	**HbO**	**L**	-0.64 *				
	HbR	**L**	-0.64 *				
	HbT	**L**	-0.65 *				
HIE17-M	**HbO**	**L**	-0.87 **				
		R	-0.82 **				
	HbR	**L**	-0.86 **	-0.70 *	-0.75 *		
	HbT	**L**	-0.88 **				
		R	-0.80 **				
	$rScO_2$	**R**				0.74 *	0.68 *
HIE18-N	**HbO**	**L**				0.69 *	
	HbR	**L**				0.76 *	0.77 *
	HbT	**L**				0.80 **	0.74 *

8.5 DISCUSSION

Previous studies that have monitored HIE infants with NIRS during cooling have reported higher $rScO_2$ measures in those HIE infants than in their age-matched counterparts (150, 297, 320). An overview of studies of neonates with HIE are presented in APPENDIX I. This current study agrees with Dehaes et al (150), as the Regional Cerebral Oxygen Saturation ($rScO_2$) was significantly higher in all HIE infants undergoing TH compared to the Healthy Controls (HC's), except HIE14-M. Cooling decreases metabolic activity thus less HbO is metabolised to HbR and so an in increase in $rScO_2$ is expected.

The higher $rScO_2$ persisted in most infants including after they were returned to normal temperature. This suggests that the increased $rScO_2$ is due not only to the hypothermia treatment, but also at least partlydue to the HIE itself. Significantly higher $rScO_2$ measurements have been reported previously in HIE infants developing HIE brain injury than those that have not developed brain injury (297, 320).

The 2 infants in this study with poor outcomes at 2 years of age did have significantly higher $rScO_2$ measures to the HC's at both sensors in the period of 24-48 cooled hours, which is in agreement with the Peng et al study (297); though infants HIE17-M and HIE18-N, whose last examinations suggested normal outcomes, also exhibited significantly higher $rScO_2$ measurements in that same period, so this was not definitive for poorer outcome.

The higher $rScO_2$ is could be partly due to the sedation of the infants during TH, or other prescription medications used for seizure control. The infants in this study were sedated using a standard regime of morphine and midazolam: standard protocol for seizure control allows the use of phenobarbital, midazolam, levetiracetam and clonazepam. Sedation has been shown to cause a higher $rScO_2$ in cooled HIE infants (321). The effect of sedation could also explain the persistence of $rScO_2$ through the rewarming process and at normal temperature, as infants were still under the influence of sedatives at the end of the rewarming and when the FD-NIRS sensors were removed.

The greater $rScO_2$ in the HIE infants resulted from several different patterns in the Haemoglobin; a higher HbO, a lower HbR, or a concurrent increase/decrease in HbO and HbR. Therefore, the higher $rScO_2$ did not appear to be the result of the same mechanism in each of the HIE infants and did not provide sufficient differentiation between infants. In calculating the $rScO_2$ as a ratio of HbO to HbO+HbR, some of the power of the individual FD-NIRS parameters was lost.

The effect of hypothermia and sedation to increase the $rScO_2$, by a variety of combinations of both HbR and HbO, and thus obfuscate any increase due to other factors, was the underlying reason that the Frequency-Domain Near Infrared Spectroscopy (FD-NIRS) monitor was selected for this current study. As discussed in Chapter 6. , the FD-NIRS monitor promised absolute measurements of HbO and HbR in addition to the measurement of $rScO_2$. . Where $rScO_2$ is influenced by change in HbR and hence coupled to the metabolism, and therefore hypothermia and sedation, the delivery of HbO is directly linked to CBF which will be greater than metabolic demand in a state of hyper-perfusion. The 2nd hypothesis of this study therefore was that the HbO would be a more powerful indicator than rScO2 of the hyper-perfusion related to HIE

This seond hypothesis was supported by the results of this study; although the $rScO_2$ was significantly higher to the HC's throughout and immediately following TH in 5 out of the 6 infants, the HbO was persistently higher than the HC's through to normal temperature in only the 2 infants with poor outcomes: HIE13-P and HIE15-P. Neither infant had any period of normality, a period whereby HbO was not outside 1SD of the mean HbO in the HC's for more than 4 consecutive measurements at both sensors.

The HbO concentration in HIE13-P was significantly higher in both sensors during TH and rewarming and remained high at normotemp. HIE13-P was found to have significant developmental delays at 2 years of age. HIE15-P had higher HbO measurements throughout TH treatment and rewarming and this remained significantly higher in the left sensor after being returned to normal temperature. Treatment was withdrawn from HIE15 subsequent to the rewarming.

The HbO in HIE16-N, HIE17-M and HIE18-N all had periods of significantly higher HbO up until 60 Cooled Hours. It is feasible that the higher HbO was due to hyper-perfusion resulting from the initial hypoxic insult. All three of these infants consistently demonstrated HbO measurements within the range of mean HbO ±1SD of the Healthy Controls from 60 Cooled Hours onwards. Clinical indications suggest that these infants have had a favourable outcome.

Interestingly, HIE14-M had statistically-similar or lower HbO and $rScO_2$ measurements in both sensors throughout TH, rewarming, and immediately following treatment. The lower HbO also translated into a lower $rScO_2$ throughout the cooling. This author is not aware of any other studies that has observed an $rScO_2$ in HIE infants who is statistically lower than in healthy infants. The hypothesis of this study was that a higher HbO would be indicative of hyper-perfusion and therefore severity of HIE and a poor outcome. Lower HbO and $rScO_2$ does not indicate hyper-perfusion, in fact this suggests that CBF is reduced. Although an MRI on day 5 of life indicated changes consistent

with HIE injury, HIE14 had no significant developmental delay at 2 years of age. An extension of the hypothesis regarding hyper-perfusion could therefore be that HbO within the statistical range of the control population *or lower* is an indication of a good prognosis.

The measurement that was found to be most prognostic given the results of this study is therefore the HbO. The HbO at normal temperature was statistically different to the healthy controls in one or both sensors in the 2 HIE infants who had poor outcomes, and neither infant had a *"period of normality"*, a period in which the HbO did not exceed the mean±1SD of the Healthy Controls for more than 4 consecutive 15-min epochs at both sensors. Perhaps more importantly, HIE13-P and HIE15-P were the 2 infants who had significantly higher HbO in the 12-hour period prior to rewarming.

The 12 hours prior to rewarming is a particular period of interest because an overall aim of this research is to identify a clinical parameter that can be used during TH as an indicator of prognosis. Ultimately it is hoped that the identified parameter can be used to influence treatment in those infants with poor prognoses. If a parameter could reliably predict outcome at an appropriate time prior to rewarming, then the decision to rewarm or delay could be more confidently made.

HbR was the FD-NIRS parameter which was most closely correlated with the number of hours that had elapsed from the commencement of TH, the number of Cooled Hours. As HbR is a reflection of metabolism, a link was expected to be found between HbR and the cooling; given the number of Cooled Hours dictates when an infant is cooled, the correlation of HbR with Cooled Hours is likely to be this link. Though of course the same correlations with Post-Natal Age (PNA) were also observed, as the two methods of recording time are linearly related, with an offset for Cooled Hours. Thus, a correlation with time could also indicate an evolving or resolving condition.

In contrast, the correlations between HbR and the temperature were not as strong. Only 3 of the 6 infants had correlations with temperature, and these correlations were not consistent with type of relationship (direct or inverse), the FD-NIRS parameter or the sensor that was involved. The 3 infants who did not have any correlations with temperature were HIE13-P, HIE15-P and HIE18-N: HIE13-P had strong correlations between all FD-NIRS parameters and Cooled Hrs on at least one side, whereas HIE15-P and HIE18-N did not have correlations between FD-NIRS parameters and Cooled Hours either. These observations suggest that the alterations to the cerebral perfusion and therefore the FD-NIRS parameters cannot be explained by hypothermia alone, further supporting the conjecture that the changes to Haemoglobin in the brain tissue is at least partly due to the hyper-perfusion related to HIE.

HbT is the concentration of Haemoglobin, both HbO and HbR in the interrogated tissue. The amount of HbT in brain tissue directly related to the CBV (317, 318): CBV can even be calculated from HbO and HbR given a small change in SaO_2 (322). The elevated HbT, observed in all except one infant, was more common in the first 54 hours. Given HbT is correlated to CBV then it is possible that the higher HbT was due to an increase in CBV as a result of hyper-perfusion. Interestingly, it was the HbO which was more distinctly elevated until 60 Cooled Hours. An explanation for this is that because the HbR is reduced by an inhibited metabolism (due to TH), the HbT is offset by the reduced HbR, given it is the addition of the HbO and HbR. Thus, the hypothermia obscures the measurement of CBV.

Thus, the HbO may be more reflective of hyper-perfusion, as it heralds a delivery of CBF above and beyond metabolic demand. It is also feasible, therefore, that the higher HbO in the first 60 hours after the commencement of the cooling observed in this study is due to hyper-perfusion related to HIE. The HbO is lowered after 60h, which suggests that the hyper-perfusion resolves. The higher HbO measures persist in the 2 infants with poor outcome, a possible indication that the time it takes for the hyper-perfusion to resolve may be linked to the severity of the HIE.

During the analysis of the FD-NIRS parameters, differences in measurements between sensors were noted. In the Healthy Controls there was no significant difference between the left and right measurements in HbO, HbT and $rScO_2$ by pairwise comparison. In contrast, there was a significant difference between left and right in HbR measurement in the HC's using the same Wilcoxon signed rank test. This suggests that CBF maintains a homogenous HbO concentration in the brain tissue, but the disparity in HbR may indicate varying degrees of brain activity at the two locations.

Similarly, the HIE infants had periods where the HbR at the left and right-hand sensors were statistically different to each other: the HIE infants also had periods where the HbO at the left and right-hand sensors were statistically different to each other. The fact that the HIE infants all exhibited differences in HbO between the two sensors at various times, when the HC's in general did not, suggests that in addition to hyper-perfusion there could be differences between the HC's and the HIE infants also in the homogeneity of the cerebral oxygenation.

Wijbenga et al investigated the lateralisation of $rScO_2$ in premature infants, by measuring $rScO_2$ at 4 locations: left and right, front and back; they concluded that there were no regional differences in $rScO_2$ (101). This may be true in a healthy neonatal population and was true for the Healthy Controls in this study, but the $rScO_2$ was often different between the left and right sensors in the HIE infants. In an investigation by Lemmers et al (323) also found good agreement between the left and right

hemispheres in regional cerebral oxygen saturation, but interestingly discovered that this this lateralisation was stronger when arterial oxygen saturation (SaO_2) was stable; when SaO_2 was not stable, left and right $rScO_2$ could be different up to 10%. The HIE infants in this study are experiencing altered haemodynamics due to the HIE and TH and so the differences between the left and right-hand sensors could be due to unstable systemic oxygenation.

Differences between left and right hemispheres may also be due to lateralisation of brain activity. Chiron et al (324) reported higher CBF's in the right hemisphere, as measured by dynamic single photon emission computed tomography; Chiron's explanation for this was that the right hemisphere develops earlier than the left. Although neither the HbO nor HbT was significantly higher on the right side in the HC's in this study, the HbR was slightly higher on the right side. Perhaps this difference observed in the HC's is due to greater activity on the right side, causing more HbO to be metabolised, and a higher concentration of HbR; which would be in agreement with Chiron's study. Other previously published studies which have reported lateralisation of HbO and HbR concentrations resulting from cerebral haemodynamic responses to stimuli (325-328), also support the possibility of differences in HbO and HbR between hemispheres due to brain activity.

In healthy infants, with intact cerebral autoregulation, the delivery of HbO by CBF meets metabolic demand so that Regional Cerebral Oxygen Saturation ($rScO_2$) is stable (94). Cerebral autoregulation is disturbed in HIE (43, 75, 169, 178, 244, 284, 286) and so CBF may be unduly influenced by changes to MABP. This disturbance causing fluctuations in the CBF could be an alternative explanation not only for increased HbO, but also for differences between the left and right given CBF is not stable. There were no discernible patterns in the differences between left and right in the FD-NIRS parameters, and so although the differences could be due to lateralisation of brain activity or CBF, at this stage the reason for the differences is not clear.

Indeed, as there were only 6 HIE infants continuously monitored with FD-NIRS in this study there was not always a large enough cohort for clear patterns to emerge. The small number of participants was due to: the challenges of a research project of this nature, the limited duration of the project in order to include 2 year development reviews, and the limited number of infants diagnosed with HIE admitted to the Mercy Hospital for Women where the research was conducted, and for which parents gave consent. The results presented here suggest that HbO could be a marker of altered cerebral perfusion in HIE, but with the limited number of infants, a definitive conclusion cannot be drawn. Nevertheless, the results indicate that FD-NIRS parameters warrant further study as cerebral perfusion markers for Hypoxic-Ischemic Encephalopathy.

8.6 HYPOTHESIS 2 OUTCOME

8.6.1 Hypothesis 2

Oxyhaemoglobin (HbO) was the Frequency-Domain NIRS (FD-NIRS) parameter that indicated poor outcome, as the 2 infants with poor outcome had persistently high HbO concentrations; thus HbO could be a marker of cerebral hyper-perfusion in infants with Hypoxic-Ischemic Encephalopathy (HIE) during therapeutic hypothermia (TH). The number of infants involved in the study precludes generalisation, but the results suggest further study is warranted.

8.6.2 Alternative Hypothesis 2

There were infants with Oxy-Haemoglobin (HbO) concentrations significantly higher than the healthy controls at normal temperature after rewarming; this suggests that the greater HbO concentration observed in the HIE infants was not just a consequence of the TH, and could be associated with the hyper-perfusion related to HIE.

8.7 CONCLUSION

The FD-NIRS parameters of HbO, HbR and HbT were investigated as potential cerebral perfusion markers of HIE. A parameter was sought that could indicate the severity of the HIE while the infants were undergoing TH to improve prognostication, and to influence treatment whilst the infants were still cooled.

Higher $rScO_2$ has been reported in infants with HIE treated with TH in previously published studies and has been linked to outcome. The concern with using $rScO_2$ as a marker of cerebral perfusion in cooled infants is that the TH itself induces an increase in $rScO_2$. Hypothermia reduces the metabolism, so that less HbO is metabolised into HbR, and given that $rScO_2$ is the ratio of HbO to HbO+HbR (HbT), that reduction precipitates a rise in $rScO_2$.

The $rScO_2$ was indeed often higher in the HIE infants than their healthy counterparts in this study. $rScO_2$ was not a suitable candidate as a cerebral perfusion marker for the severity of the HIE though, as it was high in all infants during TH regardless of outcome, and in all bar one infant was consistently high in the 12 hours prior to and during rewarming.

The issue with TH causing an increase in the measured $rScO_2$ was the reason that the FD-NIRS monitor, which promised absolute measurements of HbO and HbR, was chosen. Using the FD-NIRS monitor, HbO and HbR was continuously monitored in 6 HIE infants. The parameter which was most highly correlated to outcome was HbO, with the 2 infants with a poor outcome both

exhibiting persistently high HbO measurements. This increase in HbO persisted until after TH treatment had ceased, indicating the increase was not just due to the TH treatment itself. This supported this author's hypothesis that the HbO could indicate hyper-perfusion related to HIE, and not just a higher concentration of HbO due to a reduced metabolism. This is a new finding, which has not been reported elsewhere to the knowledge of this author.

CHAPTER 9. DISCUSSION & FUTURE RESEARCH DIRECTION

9.1 INTRODUCTION

The prevalence of Hypoxic Ischaemic Encephalopathy in Australia and New Zealand in 2017 was 1 out of every 1000 live births, or 2 out of every eligible live births (gestation > 34 weeks) (329). With prevalence rates as high as 26 per 1000 live births in the under-developed world, the global prevalence of HIE is at a higher rate of 8 per 1000 live births (330). The incidence of HIE is not declining and remains the leading cause of neonatal mortality (331).

Therapeutic Hypothermia (TH) has reduced mortality and morbidity in HIE infants; although still approximately 50% of infants treated with TH die or suffer a severe neurological disability (332). Even when TH is accesible, in the first world, the mortality rate for HIE infants is 10% overall and as high as 77% for those infants with severe (stage 3) HIE, and an estimated 26% of all infants with HIE suffer neurodevelopmental impairment (330). Thus, even with the advent of TH, the societal burden of HIE is still high.

The "Summary from the Neurology Group" in 2006 suggested that treatment strategies would likely depend on the severity of the findings during the initial examination (3). The initial examination is clearly important for identification of infants who could benefit from TH, especially when TH must be initiated within 6 hours. The findings from this study suggest that over time there are individual variations in the evolution of the encephalopathy which impact on the cerebral perfusion and oxygenation; these time-dependent variations were observed in the DUS and FD-NIRS parameters. An initial examination is likely not sufficient to describe the evolution of the HIE fully, as it is dependent not only on the initial hypoxic event, the origin of which is not always clear, but also the individual response to that event. Perhaps if the evolution of the change to cerebral perfusion were better understood, then TH treatment could be adapted during the cooling period, rather than pre-determining duration and depth of cooling. Furthermore, there are several adjuvant therapies on the horizon including allopurinol (15), erythropoietin (16) and melatonin (17), that may not have the same brevity of therapeutic window, and so could be prescribed based on real-time assessment of cerebral perfusion during TH.

Characterising the severity of HIE while the affected infant is cooled is challenging for several reasons. Physically the infant must remain cool at a target brain temperature of 33.5°C, requiring the infant to be physically connected to a cooling device. Although Magnetic Resonance Imaging (MRI)

is the gold standard for diagnosing structural HIE injury to the brain, the physical challenge of moving the infant to the MRI and keeping them cool during the imaging is problematic. Moreover, the HIE injury may not be evident in the first few days (333) and may be less predictive in a cooled population; 26% of infants with a normal MRI at 5 days of age still had moderate-severe neurodevelopmental delays in a study by Rollins et al (334). Arterial Spin Labelling MRI (ASL-MRI) offers the ability to measure cerebral blood flow (CBF) non-invasively, and has been used to measure CBF alterations in HIE (21, 320), but suffers from the same physical challenges as conventional MRI. The Sarnat and Sarnat method of staging HIE is not relevant when an infant is sedated during TH, and the aEEG (136) and RI (275) are less predictive during cooling. Similarly, the HbO concentration, as measured by NIRS, is influenced by cooling: the oxygen saturation of the brain tissue increases during TH because less oxygen is metabolised causing a greater ratio of oxy-haemoglobin to total haemoglobin.

The objective of this research study was to identify cerebral perfusion markers that would indicate the severity of the HIE during TH, by which treatment could be guided in the future.

9.2 DISCUSSION OF FINDINGS

9.2.1 Cerebral Blood Flow Velocities and the Resistive Index

This investigative study started with the question of whether the currently accepted indicator of a hypoxic event, the Resistive Index (RI), would be reliable enough to be a valid cerebral perfusion marker. The results from measuring Cerebral Blood Flow Velocities (CBFV's), and subsequently calculating the RI, showed that although the CBFV's and RI were deranged in the HIE infants, neither the RI nor the CBFV's is a reliable indicator of poor prognosis during TH. Furthermore, the CBFV's and RI are determined with Doppler US, so are one-off measurements requiring a skilled operator. The time-dependent progression of the HIE would most likely necessitate to make accurately-timed examinations. Thus, the first part of this study, presented in Chapters 3-5, concluded that the CBFV's and RI are not suitable, at least not independently, for use as markers for the severity of the HIE.

The prognostic power of the CBFV's and the RI could be improved when used in conjunction with qualitative observations that are made during the Cranial Ultrasound examination, because changes resulting from the HIE may be evident on Diagnostic Ultrasound (US). Qualitative changes that can be visualised include increased hyperechoic areas of the parenchyma, loss of boundaries between the grey and white matter, and compression of the lateral ventricles. Changes such as these have been

seen on Ultrasound: in one study these changes were found to be correlated to the HIE injury determined by autopsy (335). Guan et al (270) correlated long-term outcome to US examination, by classifying infants according to Sarnat & Sarnat grading; all infants classified as severe HIE had changes consistent with severe injury to the parenchyma as seen by US. The moderate HIE cases were also well identified with 58 out of 60 of the Moderate HIE group having indications of moderate injury on US examination study.

The difficulty in particular is in quantifying what could be only subtle changes, only 25 out of the 54 infants categorised as mild HIE had any noticeable changes on US. Moreover, that study employed 2 radiologists with more than 10 years of experience to quantify the changes into mild, moderate and severe categories, and did not correlate the categorisation or US findings with long-term outcome. Also, the timing of the examination was not declared. An earlier comparison study by Boo et al of HIE infants versus Controls examined at < 24 hours of age found no correlation between qualitative changes to the parenchyma, basal ganglia or ventricles as seen by Ultrasound and outcomes at 1 year of age (336).

The use of the qualitative analysis of the US image has reportedly improved the PPV value of the RI in a previous study (272). In a study by Jongeling et al, a low (<0.55) RI in combination either with evidence of severe oedema on US or severe encephalopathy by Sarnat & Sarnat grading reportedly improved the PPV to 80%, though because the determination of the presence of oedema was highly subjective, the authors of that study recommended caution in the use of severe oedema as a predictor.

The ability of US examination to detect changes resultant from HIE reliably is also influenced by the timing of when it is conducted. The accuracy, sensitivity, and specificity of the detection of HIE injury using US was 91%, 100% and 33% respectively; that was reduced to 53%, 74%, 19% when the MRI verification examination was not conducted within 2 hours of the US (337).

The expertise required, the subjectiveness and the time dependence of US makes it unsuitable for the purposes of having a reliable, real-time parameter to track the evolution of the HIE. Qualitative US also suffers from the same issues as DUS, requiring a skilled operator and not being a continuous method of monitoring. DUS with or without supplementary US does not offer a reliable, continuous indicator of the severity of the HIE, so other options for a reliable cerebral perfusion marker were considered.

9.2.2 Frequency-Domain Near Infrared Spectroscopy

Frequency-Domain Near-Infrared Spectroscopy (FD-NIRS) offers the promise of quantitative, continuous measures of cerebral oxygenation. FD-NIRS was chosen in particular because of the ability to measure absolute concentrations of Oxy-Haemoglobin (HbO) and Deoxy-Haemoglobin (HbR) in the brain. The addition of HbO and HbR gives the total Haemoglobin (HbT), and Cerebral Oxygen Saturation ($rScO_2$) is the ratio of HbO to HbT.

Total Haemoglobin (HbT) can be used as a surrogate measure for cerebral blood volume (CBV) and so HbT changes reflect changes in CBV (317, 318); though to obtain a quantitative measure of CBV, a 5-10% change in SaO_2 is necessary (322). With hyper-perfusion, as seen in HIE, CBF will exceed metabolic demand and CBV will increase, thus, an increase in HbT could be expected.

In HIE infants undergoing TH, metabolism will also be reduced, and less HbO is metabolised, so there is likely to be less HbR than in a healthy, non-cooled infant. Hence the increase in CBV, which results in a higher concentration of haemoglobin in total, is likely to be more prominent in the HbO.

All the FD-NIRS parameters (HbO, HbR, HbT and $rScO_2$) in the HIE infants were significantly different to those of the Healthy Control population. Analysis of the results revealed that HbO was the most indicative of outcome: the 2 infants who had a poor outcome both had the highest concentrations of HbO, which persisted through TH, rewarming, and immediately following at normal temperature.

9.3 HYPOTHESES CONCLUSIONS

9.3.1 Hypothesis 1 Outcome

The initial aim of this research study was to investigate whether Resistive Index (RI) is a reliable marker of altered cerebral perfusion due to Hypoxic Ischaemic Encephalopathy (HIE). The RI is accepted as proxy measure of the resistance of the cerebral arteries; a lowered resistance, indicated by an $RI < 0.55$, implies that the resistance of the cerebral arteries has been lowered to redirect blood flow preferentially to the brain following a period of hypoxia.

The initial part of this study therefore employed Doppler Ultrasound (DUS) to measure Cerebral Blood Flow Velocities (CBFVs) from which the RI could be determined. Over two sub-studies, the CBFVs and RI were determined in a healthy neonatal population in the first 4 days of life so that a baseline RI could be established. Interestingly an $RI < 0.55$, which could be considered pathological in the HIE population, was observed in some healthy neonates.

In 18 infants with HIE, the CBFVs and RI were then measured periodically during cooling, rewarming and immediately following rewarming in 18 infants with HIE. The CBFVs and RI were deranged in the HIE infants, though the direction (higher or lower) and grade of derangement, and the timing was different for each individual HIE infant. Although severe derangement was indicative, there was no pattern of derangement definitively correlating with poor outcome. Moreover, the best comparison was against a healthy population, whereas derangement of more than 2SDs in CBFV's or RI would indicate the grade of derangement and therefore the likelihood of a poor outcome.

As RI's < 0.55 were observed in the healthy population, and the variation in the HIE infants was not definitively correlated to outcome, the RI was found to be unreliable as a cerebral perfusion marker of HIE. Therefore the results do not support the hypothesis that the RI could be a reliable marker of cerebral perfusion in HIE; the CBFV's were more descriptive of changes to the cerebral perfusion. Although severe derangements in the CBFV's suggested a poor outcome, for this to be useful as a parameter further research would be necessary to determine the normal range of all CBFVs, and establish which pattern of CBFV's are associated with poor outcome. The CBFV's also suffer from being a one-off measurement which require a skilled operator.

9.3.2 Hypothesis 2 Outcome.

The second hypothesis of this research study was that Oxy-Haemoglobin (HbO) as measured by Frequency-Domain NIRS (FD-NIRS) would be a better marker of cerebral hyper-perfusion than the DUS parameters in infants with HIE during TH. This hypothesis was supported by the results because as the HbO was higher in the 2 infants who had a poor outcome throughout cooling, rewarming and through to normal temperature. The number of infants involved in this study was too small for a definitive conclusion to be drawn suggesting further study of HbO as a cerebral perfusion marker of HIE is warranted.

The alternative hypothesis was that any change in Oxy-Haemoglobin (HbO) in infants with Hypoxic-Ischemic Encephalopathy (HIE) undergoing therapeutic hypothermia (TH) would be a consequence of the hypothermia and not correlated to hyper-perfusion related to the HIE. The alternative hypothesis was not supported because the 2 infants with poor outcome still had higher HbO measures (HIE13-P on both sides, and HIE15-P on the left side) after being returned to normal temperature.

Interestingly, the $rScO_2$ was persistently high in 5 out of 6 of the HIE infants studied with FD-NIRS. The high rScO2 also continued through to normal temperature in 4 out of those 5, suggesting that the changes in $rScO_2$ were, at least partly, related to the HIE. The difficulty in using the $rScO_2$ alone

is therefore that the specificity would be low, because infants with a good prognosis exhibited significantly higher $rScO_2$ measures after treatment and at normal temperature.

The HbO was the FD-NIRS parameter that, in the limited number of HIE infants involved in the study, was the best predictor of poor outcome. The HbO persisted through to normal temperature and so was not just a consequence of the TH and was more sensitive than HbT and more specific than $rScO_2$.

9.4 FUTURE WORK

9.4.1 Identification of Cerebral Perfusion Markers for severity of HIE

HbO was found to be the most promising marker of cerebral perfusion in HIE in this study. Unfortunately, due to the limited number of infants monitored with FD-NIRS, this cannot be concluded definitively. A larger study, preferably replicated elsewhere, is required to confirm the link between HbO and the severity of the HIE. That larger study would also allow other patterns in the other FD-NIRS parameters (HbR, HbT and $rScO_2$) to emerge should they exist, and clarify whether there is any correlation between the difference in sensors. A larger study population would also enable the investigation of auxiliary or complementary parameters that might assist with the description of the altered cerebral haemodynamics related to HIE.

The regional cerebral oxygen saturation, $rScO_2$ is a function of cerebral blood flow (CBF), the oxygen content of the blood, and then how much of the delivered oxygen is metabolised by the cells: the amount of oxygen in the blood and thus delivered to the brain tissue depending on the arterial oxygen saturation (SaO_2), haemoglobin (Hb) content, and partial pressure of Oxygen (PaO_2) in the blood. Where cerebral autoregulation and $PaCO_2$ reactivity is intact, CBF is primarily influenced by the partial pressure of carbon dioxide ($PaCO_2$) and pH of the blood (338) to support a constant Regional Cerebral Oxygen Saturation ($rScO_2$). The Regional Cerebral Oxygen Saturation ($rScO_2$) is the ratio of HbO to HbT, where the HbT, the total haemoglobin concentration in the brain tissue, is related to CBV. Figure 9-1 illustrates the inter-relationships between the cerebral haemodynamic parameters that are involved in maintaining $rScO_2$.

There are, therefore, many variables involved in cerebral haemodynamics and so it is likely that the altered cerebral haemodynamics in HIE cannot be described by one parameter alone; a multivariate approach may be more suitable. Thus, it could be beneficial to explore the additional parameters that could enhance the information gained from the HbO. Of most interest in a situation of hyper-

perfusion is CBF and therefore the regulation of cerebral blood flow, the cerebral autoregulation (CA), and those parameters that could affect CBF, including SaO_2, MABP and HR.

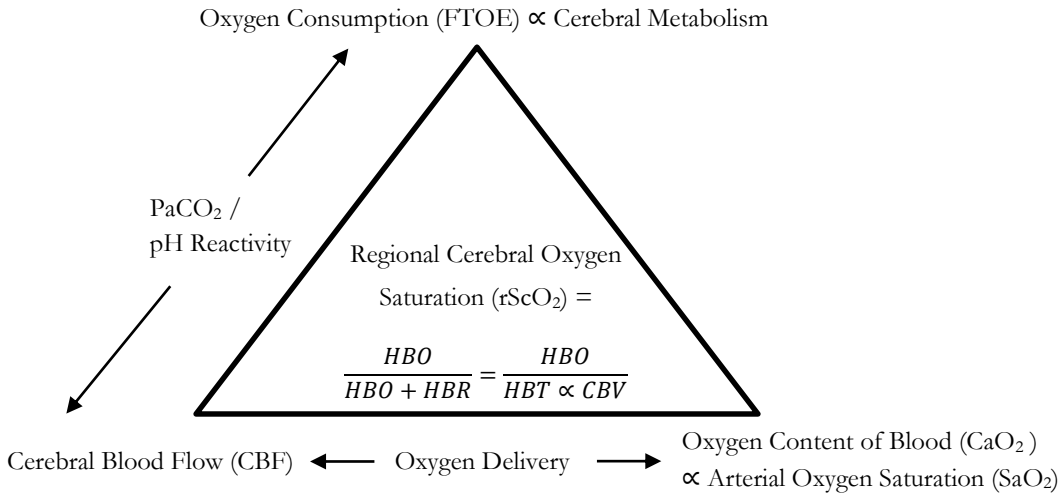


Figure 9-1 Inter-Relationship of Cerebral Haemodynamics parameters involved in maintaining Regional Cerebral Oxygen Saturation ($rScO_2$).

Cerebral Autoregulation (CA)

Hyper-perfusion is cerebral blood flow (CBF) that exceeds metabolic demand, suggesting that the cerebral autoregulation (CA) is impaired. By taking advantage of spontaneous fluctuations in MABP, coherence between MABP and $rScO_2$, as a surrogate for CBF, can be assessed using correlation, cross-spectral wavelet coherence analysis. These techniques were first described in studies of CA in premature neonates (171, 176, 177). Utilising the same techniques the loss of CA, flagged by a greater coherence between MABP and $rScO_2$, has been linked to poor outcome in HIE infants (339, 340). Vesoulis et al (340) reported that hyper-perfusion, determined by a breach of the upper limit of CA, was more prevalent in the infants with HIE injury on the 3rd day of cooling. This would be an appropriate time to identify those infants likely to have a poor outcome if extended cooling were to be considered. The sensitivity was low in that study, with only 54% of the infants with HIE injury exhibiting this hyper-perfusion on day 3, versus 14% in the infants without injury. Furthermore, inotropes were used exclusively in the infants with HIE injury which may have contributed to the hyper-perfusion.

Loss of CA in premature neonates is characterised by pressure passivity, whereas hyper-perfusion related to HIE is a result of vasoparalysis subsequent to a hypoxic event; CBF is increased by vasodilation, due to an increase in $PaCO_2$ or pH or both (338), followed by persistent vasoparalysis.

The increased CBF may not be resultant from changes to MABP so an assessment of CA purely by measures of coherence between MABP and CBF may not discover an impairment to CA.

A multivariate approach to the study of cerebral autoregulation could be better able to characterise the disturbances to cerebral haemodynamics following a hypoxic insult, rather than a focus on the pressure reactivity of CBF, as has been done previously in assessment of CA. That approach could focus on the infant's ability to maintain CBF and brain homeostasis in response to variations in the oxygen, carbon dioxide and pH of the blood, and any other factors that could affect CBF.

Caicedo et al (341) proposed a multivariate method for the analysis of cerebral haemodynamic regulation (CHR) using wavelet regression and oblique sub-space projections (WR-OBP). WR-ORP mathematically models the contributory inputs to the system to describe their contribution to the observed output measurement. The method essentially decomposes the output into the contributory signals from each of the inputs; so that it can be viewed and analysed as a function of the inputs. Caicedo et al used this method to identify periods of coherence between $rScO_2$ and HR, as an indicator of neurogenic activity, $PaCO_2$, MABP and $rScO_2$; their study showed promise in signalling an impending Intra-ventricular Haemorrhage (IVH).

Thus, future work could include using a multivariate approach for analysing the coherence of the FD-NIRS parameters with those variables that influence the CBF. The measurement used for the coherence analysis in all the previously published studies of CA in premature and HIE neonates has been $rScO_2$ (171, 176, 177, 339-341). Given the possible link between the HbO and the severity of the HIE, and the relationship of HbO with CBV, future analysis could include both the coherence of HbO, in addition to $rScO_2$, as the observed, measured outputs. The inputs against which the coherence with HbO and $rScO_2$ could be analysed are those variables that could influence CBF. The continuous variables that are available in standard monitoring, and those parameters which are available in the initial 6 infants, are SaO_2, MABP and PaO_2 / $PaCO_2$ (measured transcutaneously as $TcPCO_2$ and $TcPO_2$). The oxygen content of the blood and therefore SaO_2 both influence the oxygen content of the tissue and therefore NIRS measurements, so including SaO_2 in the coherence analysis will also allow the elimination of those changes to $rScO_2$ caused by SaO_2 alone, effectively decoupling it from the FD-NIRS parameters. CBF is also related to myocardial function, and in particular, Cardiac output (CO), so heart rate (HR) could also be included.

Myocardial Function

Myocardial dysfunction has been observed in HIE infants. This may be decreased cardiac output (CO L/min), left ventricular dysfunction and increased pulmonary artery pressure (PAP) (267, 342).

Although echocardiographic examinations were included in the protocol for the HIE infants in this study, these have not as yet been compared with the cranial DUS, FD-NIRS parameters, or the outcome. Echocardiography could also provide some insight into the pathology of the encephalography, as changes to the heart walls and major vessels may be evident in chronic hypoxia, an indication of an earlier genesis to the birth transition. There is also some modest evidence that the Heart Rate Variability (HRV) is compromised in infants with HIE (343). Cardiovascular dysfunction is a future area of investigation and may offer cardiovascular markers complementary to the FD-NIRS parameters.

Time Series Analysis & Adaptive Control Model

The ability to monitor FD-NIRS parameters continuously is an opportunity to be able to analyse those parameters as a time series. Analysing as a time series will better indicate not only the static measures, but also dynamic changes. In some of the periods the mean did not well describe the haemodynamic changes within that period, and the standard deviation (SD) was large because of significant fluctuations during that period. Analysing as a time sequence would better describe and track the changes in the cerebral haemodynamics.

The physiological control of the vasculature of the blood vessels in the brain has parallels to the physiological control of the respiratory system. Both are complex feedback systems with multiple sensory inputs to the brain. Thus, a suitable model for the cerebral vasculature in the neonate with HIE could be the adaptive control model as described in the paper by Koumoundouros et al (344), where an adaptive control model was used model the airway tone in sheep during bronchial challenges. That model describes the physiological mechanisms involved in a bronchoconstriction reflex; a reflex which triggers changes to the rate and depth of ventilation, and the airway smooth muscle (ASM) tone, which controls the resistance of the airway.

The foetal response to acute hypoxia has similarities to the bronchoconstriction reflex. The carotid chemoreceptors, sensitive to changes in PaO_2, $PaCO_2$ and pH, are principally responsible for the response to acute hypoxia (345). This chemoreflex induces bradycardia primarily via vagal outflow, and sympathetic influences increase peripheral and decrease vascular resistance. Bradycardia allows for increased stroke volume, thereby increasing cardiac output (CO), even with a decreased HR. During an acute hypoxic event, the carotid blood flow can be up to 8 times the femoral blood flow (345).

The initial chemoreflex is a fast mechanism, but like the physiological system of the airways described by Koumoundouros et al (344) there are also slower dynamic mechanisms involved. The release of

catecholamines, vasopressin and angiotensin II maintain peripheral vasoconstriction. Increases in Nitric Oxide (NO), a vasodilator, opposes the vasoconstriction, but its effect is mitigated by reactive oxygen species (ROS). ROS is generated by anaerobic energy production, so the effect of NO is less pronounced in the vasoconstricted peripheral circulation. NO produces vascular oxidant tone in the vasculature, supporting the redirection of blood flow to the brain.

The role of NO in mediating vascular tone was a discovery made in the last 10 years; the role and interaction of all the physiological mechanisms at play in the brain-sparing effect are still under investigation (345). The cerebral and cardiovascular haemodynamics in the period subsequent to both a hypoxic insult and birth transition are also still under investigation, especially with the advent of TH. There is a lack of literature providing commentary for the cerebrovascular haemodynamics observed during this period; this is probably partly due to the disparate DUS and NIRS results as reported in previously published studies which makes any explanation of the many varied observations between HIE infants difficult. The adaptive control model could assist in providing an explanation for the observations.

In the adaptive control model for the cerebral haemodynamics, the depth and rate of ventilation in the airway model could be substituted for HR and CO; with peripheral and cerebral resistances in place of the elastance and conductance of the lungs. The output of this model would be CBF; so, a so a surrogate reference signal would most likely be HbO or HbT.

Once suitable cerebral perfusion markers have been identified, these could be used to implement an adaptive control model of the cerebral hemodynamics of HIE. The development of an adaptive control model would not only improve understanding of the foetal response to a hypoxic insult, but also the subsequent cerebral haemodynamic mechanisms involved in the restoration of the brain homeostasis. This understanding would be beneficial for research into individualising TH treatment and the use of adjuvant treatments including antioxidants such as allopurinol, erthropeitin and melaton which mitigate the effect of Nitric Oxide (NO).

Cerebral Metabolism and Fractional Oxygen Extraction

The foetal defence strategy to acute hypoxia includes reducing the oxygen consumption by decreasing cerebral metabolism and increasing the extraction of oxygen from the Haemoglobin. Although cerebral metabolism is affected by hypothermia, analysis of changes to cerebral metabolism due to spontaneous desaturations have suggested that there is a stronger relationship between SaO_2 and cytochrome oxidase, a marker of metabolism, in infants with poor outcome (332).

Cytochrome oxidase (OxCCO) can be measured by a broadband NIRS system; the OxiplexTS™ used in this study is not capable of OxCCO measurements.

Cerebral fractional oxygen extraction (cFOE) is a measure of the amount of oxygen that is extracted from the Haemoglobin in the brain tissue and is therefore related to cerebral metabolism. cFOE is the ratio of the rate of oxygen consumption to the amount of oxygen delivered; i.e. the ratio of the cerebral metabolic rate of Oxygen ($CMRO_2$) to cerebral oxygen delivery (CD_{O2}), given in Equation 1-8, as the difference between the oxygen content of the arterial and venous blood (183). Measurement the cerebral venous Oxy-Haemoglobin saturation requires partial venous occlusion for 5-10s, and is a one-off, intermittent measurement (346).

A surrogate measure of cFOE is cFTOE, the cerebral fractional tissue oxygen extraction: cFTOE is a reflection of the balance between oxygen delivery and oxygen consumption (190). It is calculated as the ratio of the difference between the oxygen saturation of the blood delivered and that of the tissues (SaO_2-$rScO_2$), a measure of the oxygen consumption, to the oxygen saturation of the blood (SaO_2) as in Equation 1-9. cFTOE can be determined continuously and non-invasively using NIRS (191). Although cFTOE is less accurate than cFOE, it has been validated in a piglet model (182) and does reflect the fractional oxygen extraction. cFTOE also provides a correction for $rScO_2$ caused by changes in SaO_2 (191). Correlations between $tPCO_2$ ($PaCO_2$ measured transcutaneously) and cFTOE have been observed in neonates in the first 4 days, inferring that $PaCO_2$ influences cerebral oxygenation (347). Although SaO_2 measurements were available for the HIE infants in this study, the cFTOE in relation to outcome or its relationship with other variables has not yet been explored, and is a subject for future work.

9.4.2 Clinical Use of Identified Markers of HIE

If a Frequency-Domain Near-infrared Spectroscopy (FD-NIRS) parameter, such as HbO, either independently or in combination with any other parameter, can be found to be a reliable indicator of the severity of the HIE during TH, then it could be used clinically to influence treatment; either by tailoring the TH treatment to the individual infant or targeting the more severe HIE cases for additional adjuvant or supplementary treatments, such as allopurinol, melatonin or erythropoietin.

Longer and deeper cooling has been found to be neuroprotective in preclinical animal models (34, 348-352), and so a large randomised clinical trial was conducted to determine whether the improved outcome observed in animal trials was commutable to neonates with HIE (353). That multi-centre study was halted as longer and/or deeper cooling increased the risk of death, and the preliminary

results suggested there was limited probability of a statistically significant benefit to either longer or deeper cooling.

That clinical trial was randomised; any infant diagnosed with moderate-severe HIE may have been assigned to either or both longer (120h) or deeper (32°C) cooling. Randomisation does not account for the varying nature of the evolution of the HIE, or the individual infants response to the hypoxic insult and the hypothermia treatment. Moreover, the cooling duration was increased from 72h to 120h, almost double the currently accepted duration, whereas in a case study of recooling, only an additional 24h was necessary to avert clinical deterioration during rewarming (354).

Rather than pre-determining treatment on admission, another approach, and the one proposed by this author, is to guide the treatment using a reliable indicator; an indicator that could signal that the cerebral perfusion has stabilised, and the infant has recovered sufficiently to rewarm safely. Rebound seizures and clinical deterioration have been reported during the rewarming previously (354, 355). In the latter of those case studies, the seizures that commenced during rewarming ceased completely on re-establishment of hypothermia, which suggests that the rebound seizures may not have occurred had the infant been allowed more time to recover sufficiently to be able to tolerate the rewarming and return to normal temperature.

Thus, with a reliable indicator, infants could remain cooled only for as long as is required to protect the brain during the recovery from the hypoxia and then rewarmed. Infants with the most severe HIE could perhaps stay cooled longer than the 72 hours, but only until such time as the best outcome can be assured. Infants with mild HIE could perhaps be rewarmed earlier than 72 hours if the indicator could reliably anticipate a good outcome at an earlier stage.

There is some evidence from this research study that HbO as measured by FD-NIRS could be an indicator that does offer a reliable insight into the recovery from hyper-perfusion. This has not been reported previously, and the results from this study suggest it is worthy of future research.

CHAPTER 10. CONCLUSION

Hypoxic-Ischemic Encephalopathy (HIE) is still a leading cause of neonatal mortality and morbidity. Despite Therapeutic Hypothermia, around 50% of the 1 in 1000 infants affected in the developed suffer from a disability such as cerebral palsy, or die, with significant burden to the community. Treatment thus far has been based on the determination of HIE on the initial examination; infants with indications of HIE need cooling to commence within 6 hours of birth. The TH treatment is then the same for each individual infant; 72 hours at 33.5° followed by gradual rewarming over 12h.

Ongoing real-time assessment of cerebral haemodynamics could allow TH treatment to be individually tailored to the infants condition or for adjuvant treatments to be commenced during TH. To be able to guide treatment based on the severity of the encephalopathy, a measure of that severity is required. As HIE is characterised by hyper-perfusion, caused at least in part by vasoparalysis, markers of cerebral perfusion were sought in this research study; the main objective was to find a marker of hyper-perfusion.

The Resistive Index (RI) is an accepted method for assessing whether an infant has experienced a period of hypoxia; an RI < 0.55 is a dichotomous indicator of such an event. The RI was investigated as a marker of hyper-perfusion: a lowered RI being suggestive of increased cerebral blood flow (CBF) and possibly hyper-perfusion. Thus, the first hypothesis of this research study was that the RI would not be a reliable marker of cerebral perfusion, and therefore of the severity of the HIE.

Derangements in the RI, and also the cerebral blood flow velocities (CBFV's) from which the RI was determined, were observed in the HIE infants relative to a Healthy Control population. Although severe derangements in CBFV's and/or RI were indicative of a poor outcome, a decreased RI was also seen in healthy infants and infants with HIE who subsequently had a normal outcome. Similarly, normal RI's were observed in some infants with HIE who had a poor outcome. Neither the RI nor the CBFV's were considered reliable indicators of the severity of the HIE. Moreover, CBFV's are also unsuitable because they are one-off measurements by Doppler US that require a skilled operator and require baseline measurements from a healthy population against which to compare. The CBFV's and RI are therefore not suitable as cerebral perfusion markers upon which to base treatment decisions.

Cerebral perfusion is the delivery of oxygen, as bound to Oxy-Haemoglobin (HbO), to the brain by a cerebral blood flow (CBF). In a healthy infant with intact cerebral autoregulation (CA) the CBF, and HbO delivery, matches metabolic demand. After a hypoxic-ischaemic event, CA is compromised; the brain-sparing effect initially increases CBF by reducing the resistance of the

cerebral arteries, preferentially redicting blood flow to the brain, but consequent vasoparalysis leads to CBF that is greater than metabolic demand causing hyper-perfusion. A cerebral perfusion marker that could identify this hyper-perfusion was desirable.

Near-Infrared Spectroscopy uses absorption of light in the Near-Infrared Spectrum to measure concentrations of Oxy-Haemglobin (HbO) and Deoxy-Haemoglobin (HbR) in the brain tissue. Cerebral Regional Oxygen Saturation ($rScO_2$) is a measure of the percentage of Haemoglobin in the brain tissue that is oxygenated, by taking the ratio of HbO to the total haemoglobin (HbT). Continuous-wave NIRS (CW-NIRS) does not provide absolute measurements of HbO and HbR in the brain tissue. Moveover differences in HbO, HbR and $rScO_2$ have been reported between the different CW-NIRS devices and sensors; there are even differences when re-siting sensors. Frequency-Domain NIRS (FD-NIRS) frequency modulates the light and so, by modelling the light as a photon density wave, the absorption and scattering co-efficient can be directly calculated. From the absorption and scattering coefficients, the absolute concentrations of HbO and HbR can be determined. FD-NIRS allowed the measurement of HbO, HbR, and the subsequent calculation of HbT and $rScO_2$. It was due to the capability of FD-NIRS to measure absolute HbO and HbR, and therefore HbT and $rScO_2$, that the OxiplexTS™ FD-NIRS monitor was chosen for the second part of this research study.

Hyper-perfusion is CBF exceeding metabolic demand; thus, a delivery of blood and particularly HbO exceeding cerebral metabolic demand. HbO was therefore forecast to be increased in the HIE infants, and the magnitude of increase would be related to the extent of the hyper-perfusion; which in turn would be associated with the severity of the HIE. This formed the second hypothesis that HbO as measured by FD-NIRS would be an indicator of the hyper-perfusion that is related to and linked to the severity of the HIE.

First a study was conducted involving 40 healthy neonates with no known pathology to establish a baseline of the FD-NIRS parameters: HbO, HbR, HbT and $rScO_2$. The healthy neonates were examined with FD-NIRS in the first 4 days of life to match the post-natal age of the HIE infants. The baseline was established in the healthy population for comparison with the HIE infants, as there were limited studies of healthy infants in the first 4 days using FD-NIRS. Six infants with HIE were monitored continuously with the FD-NIRS system and HbO, HbR, HbT and $rScO_2$ were all recorded and analysed. Comparisons with the healthy population indicated derangements in all the FD-NIRS parameters; in general, a higher HbO, HbT and $rScO_2$, and lower HbR. The TH was expected to be reflected in the higher $rScO_2$, as an increased $rScO_2$ has been previously been reported

in HIE infants; but as hypothermia inhibits the metabolism, this may at least been due partly to the TH.

The advantage of the FD-NIRS monitor was that it provided the ability to measure the absolute HbO concentration. HbO was considered to be a surrogate measure of CBF, without any dependence on HbR, the concentration of which would be most affected by the reduced metabolism. The two infants with a poor outcome had significantly higher HbO measures during TH, rewarming which persisted through to normal temperature. All the infants with an expected normal outcome had "normal" HbO concentration measurements, similar to the healthy controls, before rewarming. This observation supported the second hypothesis that the hyper-perfusion related to HIE would be expressed in a higher HbO concentration than in the healthy controls. Due to the limited number of HIE infants enrolled in the latter part of the study, and the lack of other published studies of HbO in HIE infants, a definitive conclusion cannot be drawn from these results; but the results suggest that HbO is worthy of further study in HIE infants.

The results from this study are encouraging, though a larger, multi-centre study is necessary to confirm the findings. In addition, it would be beneficial to use a multivariate approach and investigate auxiliary or complementary parameters that could improve any prognostic power of the HbO. Parameters for further investigation would include those that are recorded as part of the standard protocol: SaO_2, MABP, $TcPO_2$ and $TcPCO_2$, as well as the calculation of the fractional tissue oxygen extraction (FTOE) as a measure of the cerebral metabolism. Cerebral autoregulation alterations could also be explored by the analysis of the interrelationships between the FD-NIRS parameters and those parameters, SaO_2, MABP, $TcPO_2$ and $TcPCO_2$, that influence CBF using correlation and coherence analysis techniques. The observations from those additional investigations would be useful in improving the understanding of the altered cerebral haemodynamics in HIE for the future and assist in the development of a model of cerebral haemodynamics after hypoxia.

Should it be possible to confirm that HbO, or a combination of HbO with complementary parameters, within normal range before rewarming is indicative of a good prognosis then clinicians may be better placed to inform parents. The cooled infants with HIE that do not have the chosen parameters within a normal range might be most appropriate to target for modified TH treatment, remaining cool until the HbO or other parameters "normalise" or adjuvant therapies such as allopurinol, melatonin or erythropoietin.

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APPENDIX A. COMPARISON OF THE RESISTIVE INDEX (RI) FROM PROMINENT STUDIES.

Gestation and Resistive Index (RI) quoted with one standard deviation (SD) where available.

Study	Cohort Size	Post-natal Age (Hrs†)	Gestation (Weeks)	Artery	RI	Correlations
Ando et al, 1983 (244)	43	< 6	Term	ACA	0.76 ± 0.05	▪ RI & EDV inversely correlated to post-natal age (ductal closure) ▪ No correlation with delivery type
		> 6			0.68 ± 0.07	
		72			0.69 ± 0.04	
Wright et al, 1988 (77)	10	< 8	39.6 ± 1.3	ACA	0.74 ± 0.08	▪ RI inversely correlated to EDV and post-natal age (ductal closure) ▪ Systemic blood pressure influence on RI, not vice versa
				MCA	0.78 ± 0.07	
		24-30		ACA	0.65 ± 0.07	
				MCA	0.70 ± 0.07	
Bode et al, 1988 (248)	25	Day 1	Preterm & Term	MCA	0.69	▪ Positively correlated to post-natal age
		Day 20		MCA	0.72	

Deeg et al, 1989(249)	121	Corrected gestational age – Post-natal age not known.	29-45 weeks	ACA	0.73±0.08	▪ Positively correlated to post-natal age
Seibert et al, 1989 (245)	57	< 7 days 	< 33 > 34 Pre-term & Term	ACA/MCA/ICA‡	0.77 ± 0.09 0.71 ± 0.07 0.75 ± 0.10	▪ RI inversely correlated to post-natal age. ▪ RI inversely correlated to gestation
Connors et al, 1992 (76)	16	4.6 ± 0.2 23.9 ± 0.3 47.5 ± 0.3	41.0 ± 0.1	ACA	0.75 ± 0.01 0.66 ± 0.02 0.65 ± 0.02	▪ RI correlated to post-natal age, due to vascular re-modelling for impedance matching rather than ductal shunting
Allison et al, 2000 (356)	40	< 24	38-42	ACA/MCA/ICA‡	0.73 ± 0.06	
Couture et al, 2001 (68)	491	Not known	32-43	ACA	≈0.75-0.80§	▪ CBFV correlated to gestation & post-natal age (progressive increase in CBFV from 32 weeks to 8 months)
Pezzati et al, 2002 (242)	30	2-8	38-41	ACA MCA	0.78 ± 0.07 0.81 ± 0.07	▪ CBFV correlated to birth weight & post-natal age. ▪ RI correlated with gestational age.

Monteiro et al, 2012 (140)	256	10-48	Term - (Feeding)	ACA	0.67	▪ RI inversely correlated to breastfeeding, associated with increased CBFV
			Term - (Rest)	ACA	0.71	
Zamora et al, 2014 (247)	63	7.1 ± 13.6 days	> 37	ACA	0.66 ± 0.07	▪ RI inversely correlated to gestational age.
This Study	38	5-71	37.0-41.6 weeks	ACA	0.67 ± 0.06	▪ Inverse correlation to gestation & postnatal age. ▪ Correlation with post-prandial time as binomial with pivot as 25 minutes
				MCA	0.68 ± 0.07	

† Hours unless otherwise specified

‡ Overall mean of all interrogated vessels, ICA=Internal Carotid Artery

§ Value approximated from graph – RI reported as unchanged from 32-40 weeks

APPENDIX B. CEREBRAL BLOOD FLOW VELOCITIES IN THE ACA AND MCA AS REPORTED IN THE FOLLOWING TRIALS (MEAN ± SD)

Study	Cohort Size	Gestation (Mean±SD,)	Post-natal Age (Hours)†	ACA (Mean - cm/s)			MCA (Mean - cm/s)		
				PSV	EDV	TAV	PSV	EDV	TAV
Wright et al, 1988 (77)	10 (Longitudinal)	39.6 ± 1.3	< 8	24.9 ± 6.9	6.6 ± 2.9	-	39.9 ± 9.5	8.7 ± 2.7	-
			24-30	28.2 ± 5.4	9.3 ± 2.4	-	50.5 ± 5.0	15.3 ± 2.0	-
Bode, 1988 (248)	25 (Longitudinal)	Preterm & Term	Day 1	32 ± 7	11 ± 5	17 ± 5	42 ± 10	13 ± 4	22 ± 6
			Day 5	42 ± 7	13 ± 3	21 ± 6	54 ± 10	15 ± 4	26 ± 6
Deeg, 1989 (249)	121	29-45 weeks	Corrected gestational age – Post-natal age not known.	54	13	25	-	-	-
Low, 1993 (252)	95	Term	1	-	-	-	43.2	9.3	-
Maesel, 1994 (253)	18	38-41	Pre-cord cut	-	-	-	67.2 ± 13	26.3 ± 10	44 ± 10
			Post-cord cut				48.3 ± 10	14.6 ± 6	29.1 ± 8
			1				39.3 ± 4	7.9 ± 4	20.6 ± 4
			Day 1				44.2 ± 7	15.6 ± 5	26.7 ± 6

Couture et al, 2001 (68)	491	32 40	Corrected gestational age – Post-natal age not known.	43.1 52.9	9.6 13.2	21.9 28.7	-	-	-
Pezzati et al, 2002 (242)	120	38-41	< 8	24	4.7	12	31	5.7	16
Aranha et al, 2009 (243)	50	37-41 (mean 38.94)	12-72	44.4	15.4	26.1	47.9	15.1	26.4
Current study conducted 2014	38	39.4±1.2	12-72	36.3 ± 6.6	12.4 ± 3.9	22.1 ± 4.0	41.5 ± 13.2	13.2 ± 5.5	26.7 ± 7.9

† Hours unless otherwise specified

APPENDIX C. OVERVIEW OF THE SHORT- AND MEDIUM-TERM OUTCOMES FOR THE HIE INFANTS ENROLLED IN THE STUDY

The following table summarises the formal, reported assessments for each of the infants with Hypoxic-Ischemic Encephalopathy enrolled in the study. The assessments include Apgar scores at 1 min and 5 min, the Sarnet & Sarnat score as determined by the attending clinician, the formal report of the Cranial Ultrasound as conducted by a sonographer, the summary of the MRI report and the report including the Bayley scale of infant development (Bayley-III) as reported by the Paediatrician at the 2 year review (where attended). The probable diagnosis is based on the available data.

	Apgar Scores					2 Yr Developmental Review		
ID	**1 min**	**5 min**	**Sarnat Score**	**Formal CUS Summary**	**MRI Summary**	**Bayley-III Scale of Infant Development**	**Comments**	**Summary**
HIE1-N	5	7	2	Subtle regions of increased echogenicity are noted in the watershed territories of the deep white matter suspicious for early HIE. No evidence of germinal matrix haemorrhage. Anterior cerebral artery RI is within normal limits.	Small acute left cerebellar stroke, IVH grade 2 bilateral, small subdural haemorrhage in posterior fossa without mass effect, bilateral cephalhaematoma.	Cognitive 63rd percentile; Language 42nd percentile; Motor 99th percentile	Generally developing well; referred audiology and speech therapy due to expressive language delay.	Normal (Not HIE injury)

HIE2-M	2	4	3	Diffusely increased echogenicity of the white matter is seen, consistent with oedema. RI of the anterior cerebral artery is decreased at 0.56.	No definite intracranial abnormality, tiny focus of DWI signal inferiorly in left basal ganglia may represent an acute lacunar infarct but may be artefactual	Cog 84th percentile; Language 79th percentile; Motor 95th percentile	Developing very well across all domains assessed	Normal (Mild HIE injury)
HIE3-N	1	2	1	Note is made of incidental choroid plexus cysts bilaterally. No structural abnormality, MCA RI is 0.7 and this is within normal limits.	Unremarkable	Lost contact with family.		Normal
HIE4-P	1	3	2	No structural abnormalities, RI low and varied from .55 to .62.	Ischaemic change involving basal ganglia, thalamus and peri Rolandic cortex	Unable to comply with formal administration, unable to be assessed		Severe developmental delay.
HIE5-P	4	1	Not stated.	Low RI on CRUSS (0.5), mild cerebral oedema.	Extensive signal abnormality in basal ganglia and deep white matter in keeping with HIE	Ceased at Paediatrician's request. Diagnosed with Mod-severe CP, Epilepsy, PEG in situ. Not talking just making sounds.		Cerebral Palsy, Severe developmental delay.
HIE6-N	0	5	2	No formal report.	Unremarkable.	Cog 75th percentile; Language 79th percentile; Motor 79th percentile	Developing very well across all domains assessed	Normal

HIE7-N	1	4	2	Normal, no evidence of HIE.	No evidence of HIE injury with small right temporal lobe lesion.	Lost contact with family.	Normal
HIE8-P	1	3	3	Day 2 - Normal CUS. No structural/physiological abnormality, The RI is normal at 0.7. Day 4 - Parenchyma somewhat echogenic suggesting mild cerebral oedema. The RI is .67.	Severe widespread hypoxic injury.	N/A	Treatment withdrawn - Deceased.
HIE9-P	1	3	3	Bilateral abnormally echogenic periventricular white matter and possibly also caudate nuclei, suggestive of ischaemic brain injury.	N/A	N/A	Treatment withdrawn - Deceased.

HIE10-N	5	6	Not stated	The ventricles are slit-like, which can be a normal finding in the first few days of life. No subependymal, periventricular or intra-parenchymal abnormality has been demonstrated.	Multiple small foci involving left basal ganglia & multiple left cortical locations, consistent with coagulant necrosis, in keeping with acute ischaemic changes, all within the left internal carotid territory. Overall suggests embolic cause rather than HIE.	Lost contact with family.		Normal
HIE11-N	1	5	1	The ventricles are slit-like which can be a normal finding in the first few days of life. The brain is otherwise normal in appearance with normal parenchyma, normal midline structures and normal posterior fossa structures	Normal. Gyral pattern and myelination appear age appropriate. Midline structures appear normal. The ventricular system is of normal size. No evidence of intracranial haemorrhage. No diffusion restriction or other evidence of infarction is identified. Multivoxel spectroscopy at the level of the basal ganglia demonstrates no abnormality.	Cognitive 99th = 34 M, language 99th = 40M, Motor 95th, Fine motor 38 M, Gross motor 24M	Happy, verbally advanced child	Normal.

HIE12-N	0	3	1	Normal CUS, RI 0.8.	MRI Day 6 (verbal report): essentially normal with movement artefact but no convincing evidence of changes associated with HIE	Cognitive 50th centile = 29 months, Language 50th centile = 29 months, Expressive 32 M, Receptive 26M, Motor 27th centile Fine M = 31 M, Gm = 21 M		Normal
HIE13-P	5	7	2	There is generalised echogenicity throughout the cranium in keeping with cerebral oedema. The RI is reduced at 0.50-0.55 in keeping with this. There is generalised cerebral oedema. No focal change.	Patent falcine sinus and prominent of the superior sagittal, straight, transverse and sigmoid sinuses noted, not associated with HIE	Uncooperative, Cognitive 9th centile = 19M, language 1st centile = 14 months, motor 1st centile = 20 months	Bilateral sensorineural hearing deficit. R cochlear implant June 2017, L strabismus being patched, Speech therapy needs overall early intervention	Severe developmental delay, may not be related to HIE
HIE14-M	0	3	2	Slight periventricular echogenicity in some areas. Normal structural examination other than possible early PVL. The RI is normal at .65.	Probable coagulative necrosis involving bilateral basal ganglia compatible with hypoxic ischaemic injury.	Cognitive 91st centile = 29 M, Language 34th centile. Receptive 22 M, Expressive 22M, Motor 79th centile Fine M = 31 M, Gross M = 21 M	Referred for speech pathology	Normal

HIE15-P	1	2	3	There is diffuse loss of parenchymal differentiation and overall increased echogenicity, particularly of the periventricular deep white matter in keeping with ischaemic change. No focal hyperechoic germinal matrix or periventricular lesion is seen to haemorrhage. The ACA RI is abnormal at 0.51. Diffuse increased echogenicity of the brain in keeping with HIE, with associated abnormal ACA RI traces.	Abnormal - changes consistent with severe global hypoxic injury with extensive involvement of both cerebral hemispheres, the basal ganglia, corpus callosum and the dorsal brainstem tracts.			Deceased
HIE16-N	2	6	2	No echogenicity/No oedema. The RI is normal at 0.88.	Normal	Lost contact with family.		Normal

HIE17-M	1	3	Not Stated	There is a small subtle focus of increased periventricular echogenic within the right fronto-parietal white matter. This may be artefactual or relate to HIE. The RI of the ACA is 0.8 (within normal limits)	Tiny foci of ischaemic injury involving the cortex and subcortical white matter as described above, compatible with mild HIE. (peritrogonal region of both lateral ventricles, and cortical posterior left temporal lobe).	Appointment with Paediatrician declined.	Mild HIE injury/Normal
HIE18-N	3	Ex-CM[1]	2	No echogenicity/evidence of oedema, Normal RI (RI = 0.7)	No MRI (transferred prior to MRI).	Lost contact with family	Normal

[1] External Cardiac Massage

APPENDIX D. SIGNIFICANT DIFFERENCES IN CEREBRAL BLOOD FLOW VELOCITIES (CBFV'S) AND THE RESISTIVE INDEX (RI) AS REPORTED IN HIE INFANTS IN PREVIOUS STUDIES.

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Bada et al, 1979 (75) [CC][2]	12 normal term AGA infants, 12 term AGA infants with asphyxia (Apgar$^{1\ min}$ < 5, requiring resuscitation)	N	Not known	ACA	Increased EDV	Decreased RI related to HIE	• Increased EDV → Decreased RI • Increased EDV indicates vasodilation most likely the result of hypoxia, acidosis or hypercarbia, the biochemical changes of asphyxia.
Perlman et al, 1983 (284) [CC]	12 premature infants: HIE n=3, IVH, n=5, meningitis n=1, hypernatraemia n=1, possible hypernatraemia n=1, unknown n=1 GA 26-34 wks (mean 30 ± 3 wks) (Normal mean RI=0.66±0.06, derived from 50 patients ranging in gestational age from 30-40 weeks.)	N	D1 or on admission, then daily.	ACA	Marked increase in EDV during seizures, followed by marked decrease following seizures. No change in PSV in 10 of 12, 2 had slight increase in PSV.	RI decreased with seizures in every case (duration 1-5 mins), RI increased with cessation of seizure within 5 minutes to initial values (prior to seizure).	• Decreases of RI during seizures and subsequent increases statistically significant ($p < 0.001$) • 10 of 12 infants had only subtle seizures, all 12 received mechanical ventilation at time of seizures • Increase in EDV concurrent with sharp increase in MABP and ICP (and HR). • No consistent or marked changes in ABG or pH were observed in the minutes following seizures.

[1] TH: Therapeutic Hypothermia (Yes or No), RI: Resistive Index, PI: Pulsatility Index

[2] CC: Case-control Study, PO: Prospective Observational Study

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Yukinori et al, 1983 (244) [CC]	15 PA[1] term neonates (Apgar < 7, 5 with anoxic encephalopathy: unconsciousness, seizures or hyperirritability), Controls: 34 term neonates with Apgar scores ≥8 and no abnormal neurological signs.	N	3-72	ACA	Low EDV (n=1), High EDV (n=4)	High RI high n=1 (SDD[2]), Low RI n=4 (2 died, 1 SDD, 1 normal at 2 months)	• RI values of most mild asphyxic infants were within the normal range. • Infants with anoxic encephalopathy (n=5) showed high or low RI values indicating poor prognosis.
Archer et al, 1986 (357) [PO]	43 PAE (post-asphyxial encephalopathy), 25 normal outcome and 18 poor outcomes (death or disability at 18 months)	N	Daily scans	ACA	N/A	Low RI < 62 h, SE[3] 100%, SP[3] 81%, 86% PPV	• None with normal RI had poor outcome. • Median age of abnormal result was 28 hrs PNA (range 3-62 hrs) • No correlation with MABP or ICP
Greisen et al, 1986 (169) [CC]	1 infant with severe birth asphyxia, no heart action for the first 8 minutes of life, and pH in umbilical arterial blood was 6.89	N		ACA	TAV increased during 2 seizures (corresponding major, spontaneous rises in in MABP and HR).	RI decreased during seizures.	• Electrical model of the systemic arterial system is presented and used to demonstrate that the RI is likely to be severely affected by changes in cerebro-arterial compliance, peripheral resistance, duration of systole as a fraction of heart cycle and patency of the arterial ductus.

[1] PA: Post-Asphyxial

[2] SDD: Severe Developmental Delay

[3] SE: Sensitivity, SP: Specificity

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Van Bel et al, 1987 (263) [CC]	17 term newborns with perinatal asphyxia (GA 38.9 ± 1.8), 17 healthy matched controls (GA 38.9 ± 1.8)	N	8h & repeated every 8 hrs until D3, then every 12 hrs to D7	ACA	Increased CBFV's: - Mean EDV higher until 96h, except at 40, 48 and 84h of age, max @ 32-56h - Mean TAV higher from birth until 96 h of age except at 48h	RI stable in first 24 hrs, decreased until 48 hrs, then increased gradually to normal values on D5.	• Haemodynamic changes coincided with cerebral oedema and neurological abnormalities • Mean PSV of both groups were not consistently different during the study period. • By D5, differences compared to the control group were no longer significant.
Levene et al, 1989 (46) [CC]	Full term (37-42 wks), 126 healthy infants studied between 4 hrs-7 days PNA, 34 infants with moderate-severe HIE (HIE stage II n=7, HIE stage III n=27).	N	3-8 exams starting at median age of 6 hrs (2-48 hrs)	ACA	21 of 34 abnormal: - - 4 low TAV (≤ 2SD below normal mean), always present on 1 exam, mean 21h (4-31h) - 17 higher TAV (≥ 2SD above normal mean), present at mean 26h (4-133h)	RI > 0.55 for all infants with low CBFV's RI < 0.55 common when CBFV high	• 2 abnormal patterns: low TAV (<2SD, n=4) or high TAV (≥2 SD, n=17), all had severe PAE[1] • 3 of 4 with low TAV died, no infant with TAV >3 SD survived without severe impairment • PPV of CBFV measurements < 2 SD or > 3 SD for death or SDD is 94%, compared with 83% for low RI • No correlation with PaCO2 or MABP

[1] PAE: Post-asphyxial Encephalopathy

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Gray et al, 1993 (273) [PO]	26 term infants with HIE (Diagnosis of asphyxia indicated by presence of at least 2 of the following: (1) documentation of intrapartum foetal distress on foetal heart-rate monitoring (2) $Apgar^{5min}$ <6, and/or foetal or neonatal pH < 7.15 (3) required resus	N	Between D2 and D5 on 25 infants, D7 on 1 infant	ACA & MCA	High ACA-TAV: SE 59%, SP 100%, PPV 100%, NPV 56% MCA-TAV: not correlated to outcome.	RI < 0.55: ACA-RI SE 88% SP 89%, PPV 94%, NPV 80% * MCA-RI SE 82%, SP 89%, PPV 93%, NPV 73%	• Low RI in ACA & MCA associated with adverse outcome • ACA-TAV had higher PPV than the RI of either the ACA or MCA but lower SE (TAV underestimated number of infants with poor outcome). • MCA-TAV > 40cm/s & ACA-TAV >33cm/s considered abnormal.
Low et al, 1994 (283) [CC]	26 term newborns with intrapartum foetal asphyxia (BD > 12mmol/l) compared to 59 normal newborns	N	<24 hrs post-delivery & >24 hrs.	MCA	Increased MCA-PSV and MCA-EDV after 24 hrs: 73% abnormal CBFV's in asphyxia group and 25% abnormal CBFV's in normal group	N/A	• Diastolic BP[1] significantly greater in study group & correlated to EDV in all (study & normal) < 24h & >24h • No evidence of abnormal perfusion pressures or ABG's[2] with CBFV increases (>24h) • BD and Apgar scores but not metabolic acidosis associated with abnormal CBFV's • Correlation between diastolic BP and EDV for both normal and asphyxiated infants, before and after 24 hrs

[1] BP: Blood Pressure,

[2] ABG: Arterial Blood Gases

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Mires et al, 1994 (274) [CC]	PVCL (n=3), foetal metabolic acidosis (pH<7.2, BD>8 mmol/L, normal CUS[1], 33-34 wks n=5, ≥35wks n=10); Compared to reference ranges of ACA-RI & MCA-RI at 24 hrs PNA from normal pre-term infants	N	1, 12, 24, 48, 72, 144, 168, 240 hrs PNA (as available).	ACA & MCA	N/A	MCA-RI fell at 48-72 hrs PNA, ACA-RI no difference	• Both infants with evidence of metabolic acidosis at delivery and who developed PVCL had significant fall in MCA-RI between 48-72 hrs of life.
Stark et al, 1994 (261) [PO]	16 term neonates, evidence of asphyxia (low Apgar scores) and on the first day of life demonstrated RI < 0.6 and high EDV	N	< 24 hours	ACA & ICA	High EDV associated with low RI	• Low RI < 0.60, associated with an adverse outcome.	• 16 infants: 2 died, 10 SDD with profound handicaps @ 3-32 mths, 3 normal outcomes @ 8mths-1 yr, 1 lost • Only 50% of these patients demonstrated abnormal sonographic imaging.
Eken et al, 1995 (139) [PO]	34 infants GA 37-42 weeks, with evidence of intrauterine asphyxia, foetal metabolic acidosis pH <7.10, required resus, $Apgar^{5min}$ < 5. Classified as mild, moderate or severe.	N	< 6 hrs, daily during wk1, twice weekly until discharge	MCA	* <6h: Increased EDV (2 of 32), absent EDV associated with PaCO2 < 30 mmHg (1 of 32), reversed EDV (1 of 32)	* < 6 h: RI < 0.55 (2 of 32) associated with high EDV * > 6 h: RI < 0.55 (9 of 12 survivors)	• All 4 infants with abnormal RI died, of the other 28: 15 normal, 1 major handicap, 1 minor handicap, 11 died. • No ultrasound abnormalities were noted in 20 cases. • Ultrasonography and RI were of little value, as they were almost always normal at < 6h

[1] CUS: Cranial Ultrasound

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Liao et al, 1997 (262) [CC]	47 infants with perinatal asphyxia Apgar^{1min} < 5 vs 30 Controls	N	< 24 hours	ACA	EDV & TAV significantly higher, PSV not significantly different	RI significantly lower	RI < 0.55, 9% normal outcome, 0.55≤RI<0.6, 42% normal outcome, RI≥0.6, 91% normal outcome.
Bennhagen et al, 1998 (264) [CC]	18 HIE (requirement for resus, Apgar$^{5\ min}$ ≤ 6, disturbed neurological function graded as HIE-1 (mild), HIE-2 (moderate), HIE-3 (severe) and 28 normal controls.	N	D1-D3	ICA & ACA	Initially Lower CBFV's, CBFV's increase over 85h	Higher RI & PI until 24 hours, RI falls at 30-85 h PNA in severe HIE	• CBFV values were constantly higher in the internal carotid than in the anterior cerebral artery • From 30-85 h after birth, resistance indices were lower in infants with severe HIE.
Ilves et al, 1998 (45) [CC & PO]	39 asphyxiated (Apgar^{1min} < 4 & Apgar^{5min} < 7) and 35 healthy term newborn infants, GA 37-42 weeks	N	12±2 h, 24-36 h, 36-72 h, 72-120 h	ACA & MCA	**12±2 h:** ACA-TAV lower in mild and moderate HIE, MCA_TAV lower in moderate & higher in Severe-HIE **36+ hrs**: Severe HIE persistent increase in CBFV's	Not reported.	• 4 out of 6 infants with severe-HIE and TAV of +3 SD above the mean for normal infants at the age of 12 h died and 2 developed multicystic encephalopathy • Mild-HIE (n=24), Moderate-HIE (n=7), Severe HIE (n=8)

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Jongling et al, 2002 (272) [PO]	212 infants eligible (Seizures or any 2 of: abnormal consciousness, difficulty maintaining respiration, difficulty feeding, abnormal tone and reflexes). 181 with CUS; 125 within 72 hours, 104 had an RI (Retrospective)	N	< 72 hrs	ACA & ICA	N/A	RI < 0.56; SE 33% (10 of 30), SP 95% (4 of 74), PPV 71%, NPV 78%, odds ratio 8.8	▪ 46 of 212 (22%) died or developed CP by 2 yrs of age, 104 had an RI and 30 (29%) of these infants had an adverse outcome ▪ The presence of two or more abnormal results (low resistive index, severe oedema, severe encephalopathy) increased PPV to 80%. ▪ Infants only had scans when indicated by Consultant, including all infants PPV dropped to 58%
			< 24 hours		N/A	RI <0.56; SE 53%, (9 of 17), SP 95% (21 of 22), PPV 90%, NPV 95%, odds ratio 23.6	▪ 23.6 times more likely to develop moderate to severe HIE if low RI in < 24 hours
	39 with signs of possible intrapartum hypoxia		< 72 hours		N/A	RI < 0.56; SE 47% (7 of 15), SP 92%, (22 of 24). NPV 73%, PPV 78%,	▪ Odds ratio 9.6

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Kirimi et al, 2002 (282) [CC]	23 HIE; GA 39.7 ± 1.2 (Apgar^{5min} < 6, pH < 7.2, no medications (dopamine, adrenalin or phenobarbital), no vent, 20 control infants; GA 39.8 ± 1.4	N	< 12 hours	ACA & MCA	Lower PSV and EDV compared to controls	Higher RI compared to controls	10 neonates with poor prognosis had significantly lower PSV/EDV and higher RI than those with good prognosis.
Ilves et al, 2004 (44) [CC & PO]	81 healthy; mean GA 39.8 (37.6-42.0 wks), and 60 asphyxiated term infants; 42 HIE stage I or II with mean GA 39.7 (37.1-42.4 wks), and 18 with HIE grade III, mean GA 40.4 (37.5-43.4)	N	2-6, 12±2, 24-36, 36-72, 72-120 hrs	ACA, MCA, ICA & basilar artery	12-24h: Lower CBFV's in ACA in mild-moderate & normal outcome 12h+: CBFV's higher in ACA, MCA and basilar artery in Severe/poor outcome	Severe HIE/poor outcome: Lower RI in: ▪ 2-6h basilar artery ▪ 12±2h in MCA/ICA and ▪ 24h+ in all arteries	• 2-6h: no significant difference in CBFV's • Increased CBFV's > 3SD showed early onset of severe vasoparalysis, associated with severe HIE and adverse outcomes • Lower CBFV's in mild HIE/normal outcome, higher CBFV's in severe HIE/poor outcome • Mild-mod HIE and normal outcome had no changes in RI in first 5 days
Fukuda et al, 2005 (358) [PO]	127 infants with low Apgar scores (less than 7 points), low pH (less than 7.25), low base excess (less than –5.0 mmol/L), and elevations of serum creatinine kinase levels (greater than IU/L). 72 developed symptoms of HIE.	N	D1 to D3	ICA & MCA (l & R)	ICA-TAV & MCA-TAV higher in infants with HIE & CP at 1 yr, MCA-TAV lower in infants without HIE & CP at 1 yr	N/A	▪ ICA-TAV & MCA-TAV higher in HIE infants, especially those developing CP by 1 yr. ▪ MCA-TAV lowest in infants with CP at 1 yr without HIE (not significant)

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Daneman et al, 2006 (265)	42 asphyxiated infants - Population data not published. Study authors unpublished experience.	N	Not known	Not known	Increased EDV associated with low RI (some infants with higher RI, had decreased EDV).	Decreased RI - degree of decrease (below 0.60) and duration (up to several day) correlated to severity.	▪ Some full-term neonates with significant asphyxia did not show this decreased RI and may instead have an increased RI that is due to a relative decrease in EDV ▪ Large fluctuations in RI observed (Some infants with increased RI)
Liu et al, 2007 (266) [CC]	40 term neonates with PAE (perinatal asphyxia, metabolic acidemia cause by hypoxia, neurologic manifestations, increased ICP) GA 38.6 ± 0.74 (37.3–39.9), 30 healthy controls	N	< 24 hours	MCA (L & R)	Markedly increased or decreased CBFV's depending on degree of haemodynamic disturbance	RI < 0.5 or RI > 0.9 in severe HIE, RI > 1.0 associated with later brain death	▪ HIE haemodynamics characterised by either: Elevated RI with decreased CBFV's, especially EDV, CBFV's < normal mean -2SD and/or RI>0.75, or ▪ RI<0.55 with either significantly decreased CBFV suggestive of hypoperfusion mostly seen in mild HIE or with significantly increased CBFV (> normal mean value +2SD) suggestive of hyper-perfusion mostly seen in moderate-to-severe patients
Liu et al, 2007 (267) [CC]	40 term infants with PAE (perinatal asphyxia, metabolic acidemia cause by hypoxia, neurologic manifestations), 30 healthy controls, GA 37–40 weeks	N	D1, D3, D7, D12, D14	MCA (L or R)	Lower CBFV's at < 24h compared to controls and 3, 7 and 12-14 days	Higher RI on D1 compared to controls and 3, 7 and 12-14 days	▪ CO and left ventricular function decreased - CBFV's lower and RI higher in those with CO and LVF disturbances ▪ Higher RI and Lower CBFV's only at <24 hours, then normalised.

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Epelman et al, 2010 (278) [PO]	76, 43 suspected HIE & others requiring MRI evaluation (congenital (n= 19) or metabolic abnormality (n=8) & infection n=6. GA 37.8 (29- 42 wks).	N	1 to 44 days (mean = 9.8 days)	ACA	More variation in PSV in HII+[1] (by MRI) infants. No difference in EDV.	More variation in RI in HII+ (by MRI)	▪ Doppler spectral analysis evaluation was performed in only 53 infants. ▪ The ROC curves for the RI, PSV and EDV showed low SE and SP for each measurement (all less than 76%).
Elstad et al, 2011 (42) [PO]	125 infants with HIE (clinical evidence of PA, $Apgar^{10min}$ <5, prolonged resus, acidosis (pH<7.0 or base deficit <16mml/L), moderate or severe clinical encephalopathy, abnormal aEEG or seizures)	Y	24-72 hrs	ACA	N/A	Low RI (<0.55): PPV 60%, NPV 78%, SE 63%, SP 76% (poor outcome) RI lowest at 48 hrs SE 58%, SP 83%, PPV 64% and NPV 79%, greater discrimination between good and poor outcomes	▪ Low RI is less predictive of poor outcome during hypothermia than normothermia. ▪ PPV during hypothermia is significantly lower than the pooled PPV of 84% (95% CI 73%, 91%) from previous published studies of RI at normothermia. ▪ RI < 0.55 at or after 24 h significantly differentiated between good and poor outcome for hypothermia-treated infants

[1] HII: Hypoxic-Ischaemic Injury, +positive or - negative

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Simon et al, 2011 (268) [CC]	21 asphyxiated newborns (meeting WHO, AAP and ACOG criteria for PA) with GA 38.2 ±1.6 wks, 200 healthy newborns (controls) GA 38.9±1.4 wks	N	< 72 hrs (12, 24, 48 & 72 hrs)	ACA	CBFV's initially lower EDV increased after 12 hours and remained persistently higher	RI decreased and lower than controls after 12 hrs and remained low (with associated increase in EDV).	▪ SV and EDV normalised in HIE stage I infants in first 48 hrs, HIE II infants had not normalised within 72 hrs. ▪ Changes in RI from very high to very low values were found in severe HIE ▪ Significant direct correlation between pCO_2 and PSV/EDV after 24 hrs and inverse correlation between PaO_2 and PSV/EDV. ▪ PSV and MABP strongly correlated. ▪ Correlation between severity of HIE and EDV after 48 hrs, subsequent inverse correlation with RI.
Kudreviciene et al, 2014 (276) [PO]	78 HIE (PA or asphyxia requiring resus, $Apgar^{5min}$ ≤7, foetal acidosis (pH < 7.2) or neonatal acidosis (pH < 7.3) infants (GA 40 ± 1.1) & 47 Controls, GA 39 ± 1.2	N	Daily scans for first 5 days	ACA and MCA	Increased ACA & MCA EDV associated with poor outcome.	Lower RI MCA (D2/D3) & ACA (D3); RI ≤ 0.55 on D1–5 associated with HI injury (as evaluated by CUS).	▪ In subjects with spastic quadriparesis, mean EDV was significantly higher, and mean RI were significantly lower. ▪ ACA-EDV > 20 cm/s correlated to CUS exam on D1 and HIE injury. ▪ No significant difference in PSV (D1-D5) for neuromotor and mental development groups (ACA & MCA).

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Skranes et al, 2014 (279) [PO]	45 infants with NE (GA ≥ 36 weeks and at least one of the following: (a) Apgar10^{min} < 5, need for respiratory support 10 min after birth, or pH <7.00 and/or BE≤16 mmol/L and moderate to severe encephalopathy	Y	Early (median 11h) & late (median 62h) cooling & after rewarming (median 89h).	ACA	N/A	RI did not differentiate between good & poor outcome during cooling. RI < 0.55 after rewarm: PPV 100%	▪ RI ≤ 0.55 PPV 43% during late cooling and PPV 100% after rewarming. ▪ RI > 0.55 predicted good outcome in 86% during late cooling and in 89% after rewarming.
Barseem et al, 2015 (277) [CC]	40 full-term HIE neonates (GA≥ 37 wks) with PA, required resus, Apgar$^{5\ min}$ after birth ≤6, foetal acidosis (pH < 7.2) or neonatal acidosis (pH < 7.3). Control group: 25 healthy full-term neonates.	N	12h	ACA & MCA	Lower EDV & PSV compared to Controls and to HIE infants with good prognosis	Higher RI compared to Controls and to HIE infants with good prognosis	
Gerner et al, 2016 (271) [PO]	28 infants pre-cooling, 21 infants post-cooling - GA 38.31 ± 1.970 with diagnosis of neonatal HIE, treatment with TH, availability of pre- and post-cooling RI values and participation in neurodevelopmental follow-up	Y	Prior to (<6h) and following cooling (>72h)	ACA	N/A	RI < 0.60 pre-cooling associated with poor outcome. Post-cooling not significant	Relationship found between post-cooling RI and gross motor outcome, but no relationship between pre-cooling RI and gross motor outcomes.

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Guan et al, 2016 (270) [PO]	158 neonates with HIE (clinical evaluation of consciousness, neuromuscular control, complex reflexes, autonomic function and presence of seizures): 54 mild, 60 moderate and 44 severe HIE. 120 normal controls.	N	< 72 h	ACA & MCA	Mostly lower CBFV's (n=43), 1 with higher CBFV's	Mostly increased RI (n=43), 1 with low RI (associated with higher CBFV's)	Abnormal ultrasound findings of brain parenchyma were found in 25/54 (46.3%) neonates with mild HIE whereas they were found in 58/60 (96.7%) neonates with moderate HIE and 44/44 (100%) neonates with severe HIE.
Peeples et al, 2018 (291) [CC]	Umbilical cord clamped for 18-20 mins, delivered and resuscitated (n=6) with 3 undergoing TH and 3 no TH, no umbilical cord occlusion (n=3)	TH vs no-TH	Day 0-1, D4	ACA	N/A	Decreased RI in ACA Day 0-1 (on average 0.08 lower) in neonates with poor outcome	▪ Study in non-human primates ▪ No difference in RI between groups or outcomes in either ACA or Thalamus at D4
				Thala-mus	N/A	Increased RI in occl-with-TH during cooling (<36h), and on D0-1 in neonates with poor outcome	

Study	Study Population & Criteria	TH[1]	PNA	Artery	CBFV's	RI / PI[1]	Conclusion / Comments
Pikala et al, 2018 (287)	138 High Risk (Evidence of asphyxia, seizures, severe hyperbilirubinemia requiring phototherapy, sepsis, and premature neonates ≥34 weeks GA) neonates enrolled including 22 (21%) asphyxiated infants.	N	< 72 hrs	ACA & MCA	N/A	RI < 0.60 in ACA and MCA associated with abnormal outcome at 6 mths (ACA-RI - 83%, MCA-RI - 50%).	▪ Diffuse cerebral oedema was detected by neurosonogram in 20 (19.2%) of high-risk neonates, associated with abnormal outcome at 6 mths ▪ RI < 0.6 was observed in 32 (30.8%) in ACA and 47 (45.3%) in MCA. ▪ The positive likelihood ratio is more for low RI of MCA (18) as compared to neurosonogram.

APPENDIX E. DEGREE OF DERANGEMENT OF CBFV'S AND RESISTIVE INDEX (RI) IN THE INFANTS WITH HYPOXIC-ISCHAEMIC ENCEPHALOPATHY (HIE)

a) Infants with Normal Outcome – HIE1-N, HIE3-N & HIE6-N

	HIE1-N				HIE3-N				HIE6-N			
	RI	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**
0-12h												
12-24h	>3SD	<1SD	<1SD	N	=+1SD	N	N	N	<2SD / <0.55	<1SD	<3SD	<5SD
D2	>2SD	N	>1SD	N	>2SD	N	N	N	N	>2SD	>2SD	>3SD
D3									>1SD	N	N	N
72-74 C-h	>3SD	<1SD	<1SD	N	>1SD	N	N	N	N	N	>1SD	>2SD
74-76 C-h	>3SD	<1SD	<1SD	N	>2SD	N	N	N	>1SD	N	N	>1SD
76-78 C-h	>3SD	<1SD	N	N	>3SD	N	N	N	>3SD	<1SD	N	N
78-80 C-h	=5SD / =0.9	<1SD	<1SD	N	>3SD	<1SD	N	>1SD	>1SD	N	N	N
80-82 C-h	>3SD	N	N	>1SD	>2SD	N	N	N	>2SD	N	N	N
82-84 C-h	>3SD	<1SD	N	N					>2SD	N	N	N
84-86 C-h	>4SD	<1SD	N	N					>3SD	N	N	>1SD
Day 5												
Day 6												
Day 7												

a) **Infants with Normal Outcome – HIE7-N, HIE10-N & HIE11-N**

	HIE7-N				HIE10-N				HIE11-N			
	RI	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**
0-12h												
12-24h	<3SD / <0.55	N	<2SD	<2SD								
D2	>1SD	N	N	N	>2SD	<2SD	<2SD	<2SD	>2SD	N	N	N
D3	=+2SD	<1SD	N	N								
72-74 C-h	N	N	N	<1SD	>1SD	N	N	N	>2SD	N	N	N
74-76 C-h	=+4SD	<1SD	<1SD	<1SD	=+2SD	N	N	N	=+3SD	<1SD	N	N
76-78 C-h	>3SD	<1SD	<1SD	<1SD	>3SD	<1SD	<1SD	N	>2SD	N	N	N
78-80 C-h	>3SD	<1SD	<1SD	<1SD	>2SD	<1SD	<1SD	N	>1SD	N	N	N
80-82 C-h	>4SD	<1SD	<2SD	<1SD	>3SD	<1SD	<1SD	N	>3SD	<1SD	N	N
82-84 C-h	>5SD	<2SD	<2SD	<1SD	=+1SD	N	N	N	>2SD	N	N	N
84-86 C-h	>4SD	<2SD	<2SD	<1SD	>4SD	<1SD	<1SD	N	>3SD	<1SD	N	N
Day 5												
Day 6	>1SD	N	N	<1SD								
Day 7					>2SD	<1SD	<1SD	<1SD				

a) **Infants with Normal Outcome – HIE12-N, HIE16-N & HIE18-N**

	HIE12-N				HIE16-N				HIE18-N			
	RI	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**
0-12h												
12-24h									N	<1SD	<2SD	<3SD
D2	=+1SD	N	N	N	>1SD	<1SD	<1SD	<1SD	N	N	>1SD	N
D3	>2SD	<1SD	N	N	N	<1SD	<1SD	<2SD	N	>1SD	>1SD	N
72-74 C-h	>2SD	=+1SD	N	N	=+3SD	<1SD	<1SD	<1SD	<3SD / <0.55	>4SD	>1SD	N
74-76 C-h	>2SD	<1SD	<1SD	N	=+3SD	<1SD	<1SD	<2SD	<3SD / <0.55	>1SD	>1SD	N
76-78 C-h	>4SD	<1SD	<1SD	N	>2SD	<1SD	<1SD	<1SD	<1SD / <0.55	>1SD	N	N
78-80 C-h	>3SD	<1SD	N	N	N	N	N	N	N	>1SD	>1SD	N
80-82 C-h	>1SD	N	N	N	>1SD	N	N	<1SD				
82-84 C-h	>2SD	N	N	N	>1SD	<1SD	<1SD	<1SD				
84-86 C-h	>2SD	N	>1SD	N	N	N	N	N	N	N	N	N
86-88 C-h					>1SD	N	N	N				
Day 5									N	>1SD	>1SD	>1SD
Day 6												
Day 7												

b) Infants with Mild-Moderate HIE (Normal Outcome) – HIE2-M, HIE14-M, HIE17-M

	HIE2-M				HIE14-M				HIE17-M			
	RI	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**
0-12h												
12-24h	<4SD / <0.55	<1SD	<2SD	<4SD	>2SD	<1SD	N	N				
D2	<1SD	>2SD	>2SD	>2SD	N	N	>4SD	>1SD	=+3SD	<1SD	<1SD	N
D3	<2SD / <0.55	>5SD	>5SD	>5SD					>1SD	N	N	N
72-74 C-h	<3SD / <0.55	>4SD	>3SD	>3SD	N	N	>3SD	N	>5SD / > 0.9	<2SD / <2cm/s	<2SD	<1SD
74-76 C-h	<2SD / <0.55	>5SD	>4SD	>4SD	>3SD	<1SD	>2SD	N	>6SD / > 0.9	<2SD / <1cm/s	<2SD	<1SD
76-78 C-h	<2SD / <0.55	>6SD	>6SD	>6SD	>1SD	N	>3SD	N	>5SD / > 0.9	<1SD / <2cm/s	<2SD	<1SD
78-80 C-h	N	>1SD	>2SD	>1SD	>2SD	N	>2SD	N	>3SD	<1SD	<2SD	N
80-82 C-h	N	>2SD	>3SD	>3SD	>2SD	N	>3SD	N	>4SD	<1SD	<1SD	N
82-84 C-h	N	>3SD	>4SD	>2SD	=+1SD	N	>5SD	>1SD	>4SD	<1SD	<1SD	N
84-86 C-h	N	>1SD	>2SD	>2SD	=+2SD	N	>4SD	N	>5SD / > 0.9	<2SD	<1SD	N
Day 5	N	>2SD	>2SD	>3SD								
Day 6	N	>1SD	>2SD	>3SD								

c) Infants with Poor Outcome – HIE4-P, HIE5-P and HIE8-P

	HIE4-P				HIE5-P				HIE8-P			
	RI	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**
0-12h	<2SD	N	<2SD	<3SD					>2SD	<1SD	N	>3SD
12-24h					N	<1SD	<2SD	<2SD	>2SD	N	N	>2SD
D2	N	>2SD	>2SD	N	>2SD	<2SD	<1SD	<1SD	>2SD	N	>5SD	>4SD
D3	N	N	<1SD	<2SD	>1SD	N	N	N	N	N	N	N
72-74 C-h					=-2SD / <0.55	>1SD	>2SD	>1SD				
74-76 C-h					<2SD / <0.55	>2SD	>3SD	>2SD				
76-78 C-h					<1SD	>3SD	>3SD	>3SD				
78-80 C-h	>1SD	< 1SD	<1SD	N	<1SD	N	>1SD	N				
80-82 C-h	>3SD	<1SD	N	N	<1SD	>3SD	>4SD	>4SD				
82-84 C-h	N	N	N	N	<1SD	>2SD	>3SD	>4SD				
84-86 C-h	N	N	N	N	<1SD	>1SD	>2SD	>2SD				
86-88 C-h					<2SD / <0.55	>2SD	>2SD	>2SD				
Day 4					<1SD	>2SD	>2SD	>2SD				
Day 5					<2SD / <0.55	>2SD	>4SD	>4SD				
Day 6					>1SD	N	N	N				
Day 9	<1SD	N	N	N								

c) Infants with Poor Outcome – HIE9-P, HIE13-P and HIE15-P

	HIE9-P				HIE13-P				HIE15-P			
	RI	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**	**RI**	**EDV**	**TAV**	**PSV**
0-12h	N	N	N	N					<3SD / <0.55	>9SD	>3SD	>2SD
12-24h	>1SD	>1SD	>1SD	>1SD	N	N	N	N				
D2	>4SD	<3SD	<1SD	N	<1SD / <0.55	N	N	N	=-1SD	>1SD	>2SD	>1SD
D3					N	N	>1SD	>2SD	=-1SD / <0.55	>3SD	>3SD	>3SD
72-74 C-h					N	>1SD	>2SD	>1SD	<1SD	>3SD	>4SD	>4SD
74-76 C-h					<1SD	>2SD	>2SD	>1SD	<2SD / <0.55	>4SD	>3SD	>3SD
76-78 C-h					<2SD / <0.55	>2SD	>2SD	>1SD	<1SD	>3SD	>4SD	>4SD
78-80 C-h					=-2SD / <0.55	>1SD	>1SD	>1SD	=-2SD / <0.55	>4SD	>4SD	>4SD
80-82 C-h					=-2SD / <0.55	>2SD	>2SD	>1SD	=-2SD / <0.55	>4SD	>4SD	>4SD
82-84 C-h					<2SD / <0.55	>1SD	>2SD	N	=-2SD / <0.55	>3SD	>4SD	>4SD
84-86 C-h					=-2SD / <0.55	N	N	N				
86-88 C-h					<1SD	N	N	N				
Day 4					N	N	N	>1SD				
Day 5					N	N		N				
Day 6									<3SD / <0.55	>6SD	>6SD	>6SD
Day 9												

APPENDIX F. OVERVIEW OF THE PATTERNS OF DERANGEMENT IN RI AND CBFV'S IN THE HIE INFANTS

	Initial Exam		Prior to Rewarm		During Rewarm		Changes
HIE Infant	**RI**	**CBFV's**	**RI**	**CBFV's**	**RI**	**CBFV's**	
HIE6-N	↓	↓ PSV/TAV	N	↑ ALL	↑	N	RI - Low to High, CBFV's initially low, then high, then normal
HIE7-N	↓	↓ PSV/TAV	N	N	↑	↓ EDV/TAV	RI - Low to High, PSV/TAV low initially, later EDV/TAV low
HIE-3-N	N	N	↑	N	↑	N	Normal RI, increasing prior to rewarming - Normal CBFV's throughout
HIE12-N	N	N	N	↓ PSV	↑	↓ PSV	Normal RI, increased during rewarming due to low PSV
HIE16-N	N	N	N	↓ PSV	↑	↓ PSV	Normal RI, increased during rewarming due to low PSV
HIE18-N	N	↓ PSV/TAV	N	N	↓	↓ EDV	Normal RI, low PSV - RI increased during rewarming due to high EDV
HIE1-N	↑	N	↑	N	↑	N	High RI and normal CBFV's throughout
HIE10-N	↑	↓ ALL			↑	N	High RI throughout, initially all CBFV's low, then normalised
HIE11-N	↑	N			↑	N	High RI, normal CBFV's throughout
HIE2-M	↓	↓ PSV/TAV	↓	↑ ALL	↓	↑ ALL	Low RI due to low PSV, stayed low, all CBFV's increased
HIE14-M	↑	N	N	↑ TAV	↑	↑ TAV	High RI with normal CBFV's then high TAV
HIE17-M	↑	N	N	N	↑	↓ EDV/TAV	High RI with normal CBFV's then low EDV/TAV
HIE4-P	↓	↓ PSV/TAV	N	↑ EDV/TAV	↑	N	Low RI with low PSV, then normal RI with high EDV, then high RI with normal CBFV's
HIE15-P	↓	↑ ALL	↓	↑ ALL	↓	↑ ALL	Low RI with all CBFV's increased
HIE5-P	N	↓ PSV/TAV	↑	↓ EDV	↓	↑ ALL	Normal RI with low PSV, RI higher with decreased EDV, then lower with all CBFV's increased
HIE9-P	N	N	↑	↓ EDV			Normal RI initially increasing with decreasing EDV
HIE13-P	N	N	N	↑ PSV	↓	↑ EDV/TAV	Normal RI initially, PSV increasing initially then EDV/TAV resulting in decreased RI
HIE8-P	↑	↑ PSV	↑	↑ PSV/TAV			Increased RI with increased PSV throughout

Key: Low RI with - Low CBFV's: - High CBFV's: - Normal CBFV's:
High RI with - Low CBFV's: - High CBFV's: - Normal CBFV's:
Normal RI with - Low CBFV's: - High CBFV's: - Normal CBFV's:

APPENDIX G. TABLE OF THE MAXIMUM PERMISSIBLE EXPOSURE (MPE) FOR SKIN TO LASER RADIATION.

Reproduced from AS/NZS IEC 60825.14:2011.

Table 7 – Maximum permissible exposure (MPE) of skin to laser radiation

<table>
<tr><th rowspan="2">Wavelength
λ in nm</th><th colspan="6">Exposure time t in seconds (s)</th></tr>
<tr><th><10⁻⁹</th><th>10⁻⁹ to 10⁻⁷</th><th>10⁻⁷ to 10⁻³</th><th>10⁻³ to 10</th><th>10 to 10³</th><th>10³ to 3×10⁴</th></tr>
<tr><td>180 to 302,5</td><td rowspan="3">3×10¹⁰ W·m⁻²</td><td colspan="2"></td><td colspan="2">30 J·m⁻²</td><td colspan="2"></td></tr>
<tr><td>302,5 to 315</td><td colspan="3">C₁ J·m⁻² (t > T₁)
C₂ J·m⁻² (t > T₁)</td><td colspan="2">C₂ J·m⁻²</td></tr>
<tr><td>315 to 400</td><td colspan="3">C₁ J·m⁻²</td><td>10⁴ J·m⁻²</td><td>10 W·m⁻²</td></tr>
<tr><td>400 to 700</td><td>2×10¹¹ W·m⁻²</td><td colspan="2">200 J·m⁻²</td><td>1,1×10⁴ t⁰,²⁵ J·m⁻²</td><td colspan="2">2 000 W·m⁻²</td></tr>
<tr><td>700 to 1 400</td><td>2×10¹¹ C₄ W·m⁻²</td><td colspan="2">200 C₄ J·m⁻²</td><td>1,1×10⁴ C₄ t⁰,²⁵ J·m⁻²</td><td colspan="2">2 000 C₄ W·m⁻²</td></tr>
<tr><td>1 400 to 1 500</td><td>10¹² W·m⁻²</td><td colspan="2">10³ J·m⁻²</td><td>5 600 t⁰,²⁵ J·m⁻²</td><td colspan="2" rowspan="4">1 000 W·m⁻² [a]</td></tr>
<tr><td>1 500 to 1 800</td><td>10¹³ W·m⁻²</td><td colspan="3">10⁴ J·m⁻²</td></tr>
<tr><td>1 800 to 2 600</td><td>10¹² W·m⁻²</td><td colspan="2">10³ J·m⁻²</td><td>5 600 t⁰,²⁵ J·m⁻²</td></tr>
<tr><td>2 600 to 10⁶</td><td>10¹¹ W·m⁻²</td><td>100 J·m⁻²</td><td colspan="3">5 600 t⁰,²⁵ J·m⁻²</td></tr>
<tr><td colspan="7">NOTE 1 For correction factors and units see Table 8.
NOTE 2 There is only limited evidence about effects for exposures of less than 10⁻⁹ s. The MPEs for these exposure times have been derived by maintaining the irradiance applying at 10⁻⁹ s.
[a] For exposed skin areas greater than 0,1 m², the MPE is reduced to 100 W·m⁻².
Between 0,01 m² and 0,1 m², the MPE varies inversely proportional to the irradiated skin area.</td></tr>
</table>

Table 8 – Correction Factors for MPEs

Parameter	Spectral region nm
$C_1 = 5{,}6 \times 10^3\ t^{0,25}$	302,5 to 400
$T_1 = 10^{0,8(\lambda - 295)} \times 10^{-15}$ s	302,5 to 315
$C_2 = 10^{0,2(\lambda - 295)}$	302,5 to 315
$T_2 = 10 \times 10^{[(\alpha - \alpha min)/98,5]}$ s[a]	400 to 1 400
$C_3 = 1{,}0$	400 to 450
$C_3 = 10^{0,02(\lambda - 450)}$	450 to 600
$C_4 = 10^{0,002(\lambda - 700)}$	700 to 1 050
$C_4 = 5$	1 050 to 1 400
$C_5 = N^{-1/4}$ [b]	400 to 10^6
$C_6 = 1$ for $\alpha \leq \alpha_{min}$	400 to 1 400
$C_6 = \alpha/\alpha_{min}$ for $\alpha_{min} < \alpha \leq \alpha_{max}$	400 to 1 400
$C_6 = \alpha_{max}/\alpha_{min} = 66{,}7$ for $\alpha > \alpha_{max}$[d]	400 to 1 400
$C_7 = 1$	700 to 1 150
$C_7 = 10^{0,018(\lambda - 1150)}$	1 150 to 1 200
$C_7 = 8$	1 200 to 1 400

[a] $T_2 = 10$ s for $\alpha < 1{,}5$ mrad and $T_2 = 100$ s for $\alpha > 100$ mrad

[b] C_5 is only applicable to pulse durations shorter than 0,25 s

[c] C_6 is only applicable to pulsed lasers and to CW lasers where thermal injury dominates

[d] The limiting angle of acceptance γ shall be equal to α_{max}

$\alpha_{min} = 1{,}5$ mrad

$\alpha_{max} = 100$ mrad

N is the number of pulses contained within the applicable duration (see 5.2)

NOTE 1 There is only limited evidence about effects for exposures of less than 10^{-9} s for wavelengths less than 400 nm and greater than 1 400 nm.

NOTE 2 Correction factors C_1 to C_7 and breakpoints T_1 and T_2 used in Tables 5 to 7 are defined in the following expressions.

NOTE 3 See Table 2 for limiting apertures.

NOTE 4 In the formulae in Tables 5 to 7 and in these notes, the wavelength has to be expressed in nanometres, the emission duration t has to be expressed in seconds, and α has to be expressed in milliradians

APPENDIX H. OVERVIEW OF ALL KNOWN NIRS STUDIES OF HEALTHY NEONATES

Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Cooper et al, 1996 (319)	19 neonates were studied; gest age 23-38 wks	Post-conceptual age mean 38.1 ±3.43	CCD spectrometer system:	Optode distance 4.9cm, wavelength range 650-1000 nm, temporal frontal aspect	HbR = 14.6 ± 4.0 μM,	Used 2nd differential NIRS of water to determine the mean DPF of neonatal brain, HbR concentration determined by ratio of 2nd differential of HbR to water
Buchvald et al, 1999 (359)	11 healthy term newborns: 38-41 wks, BW 2670-4720g)	3 or 4 days old, breast fed within 30 mins and asleep	Radiometer (Copenhagen, Denmark)	Forehead, 4 wavelengths, interoptode distance 3 cm	▪ $rScO_2$ =64.12 ± 4.6% ▪ $rScO_2$ Range 57-87%	
Isobe et al, 2000 (309)	7 term neonates: 36-41 wks (BW 1962g (36 wks) to 3334g) (3 infants inhaled oxygen, 4 did not)	Immediately after birth	IMUC-7000 (Otsuka Electronics, Osaka, Japan) (fs-NIRS, full spectrum NIRS)	Interoptode distance 20mm, left or right forehead	▪ $rScO_2$ = 69±5% ▪ 18% @ 1.5 mins ▪ 55% @ 5-6 mins ▪ 69±5% @ 15 mins ▪ HbO increased 2–3 min after birth, transient increase in HbT, after which decreased with HbR	▪ HbO, HbR and HbT in arbitrary units, trends only ▪ fs-NIRS: variations in optical path length due to light scattering causes variations in shape of the spectrum - apply diffusion correction equation to normalise the optical path length across wavelengths

Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Isobe et al, 2002 (310)	26 healthy term neonates, gest 37-41 wks	Immediately after birth	IMUC-7000 (Otsuka Electronics, Osaka, Japan)	Interoptode distance 20mm, left or right forehead	▪ Mean $rScO_2$ = 64±8%. ▪ VD: 29±17% @ 2 min to 68±6% @ 8.5 min, followed by an almost constant value (66±7% at 15 min). ▪ CD: Increased rapidly until 8 mins then decreased to 15 min. 40±11% @ 2 min to 68±4 % @ 8.5 min then decreased to 57±5%.	▪ Mode of delivery has a marked influence on cerebral oxygenation immediately after birth. ▪ VD=Vaginal Delivery ▪ CD=Caesarean Delivery
Grossauer et al, 2009 (360)	20 term & preterm infants (gest > 29 wks & BW >1200g). All infants >2000g at time of measurement.	Within first 8 weeks	NIRO-300 (Hamamatsu, Japan)	Left frontoparietal sensor with interoptode distance 4 cm	▪ $rScO_{2\ 70.4}$% (6.7) [Mean (SD)]	
Urlesberger et al, 2010 (312)	61 infants of gest ≥37 wks delivered by Caesarean (CD)	Birth Transition	INVOS 5100 (Somanetics, Troy, Michigan)	Left frontoparietal forehead	▪ Mean rScO2 increased rapidly from 44% (3 mins) to 76% (7 mins), thereafter no significant change.	

Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Urlesberger et al, 2011 (313)	63 VD vs 44 CD term (>37 wks) infants	First 10 mins after delivery	INVOS 5100 (Somanetics, Troy, Michigan)	Left frontoparietal region of the forehead	▪ $rScO_2$ increased from around 50% at 3 mins, to 60% at 4 mins, 70% at min 5, around 80% at min 8-10	▪ From 4 to 8 mins SpO2 values lower for CD than for VD ▪ No difference between $rScO_2$ VD and CD values
Fauchére et al, 2010 (314)	20 healthy neonates born by elective CS (gest mean $38^{1/7}$ wks (range $36^{6/7}$ -$40^{2/7}$ wks), BW mean 3200g (2300-4190g	First 15 mins of life	NIRO 300; (Hamamatsu Photonics, Hamamatsu, Japan)	Interoptode distance 50mm	▪ HbO and $rScO_2$ increased rapidly within first minutes of life & HbR decreased - all reached a plateau by 8 mins. ▪ Mean $rScO_2$ @ 3 mins 52% (47-58%) increased to between 65-70% (range 55-80%).	▪ CW-NIRS: HbO, HbR and HbT measures trends only
Almaazmi et al, 2012 (308)	46 health term infants (VD n=20, CD n=22, assisted VD=4)	First 10 mins of life	FORE-SIGHT (CASMED, Branford, Conn., USA)	Right forehead, 4 wavelength, interoptode distance 5 cm	▪ Median (IQ) $rScO_2$ @ 2 min after birth: VD: 42% (39–46%) CD: 42% (30–52%), assisted VD: 36% (20–53%) Steady state @ 8 min after birth: 73% (62–77%)	▪ No significant difference between groups

Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Bokiniec et al, 2012 (318)	42 normal full-term health neonates (gest 38-42 wks), birthweight mean 3359g (2560-4700g).	D1 and D28 (End of neonatal period)	Freq-Domain / OxiplexTS™ (ISS Inc., Champaign, Illinois, U.S)	5 sensors: Frontal (F) and Temporal (T) on Left and Right and Occipital	Frontal HbT ≈ 40μM, Temporal, HbT ≈ 50μM, Occipital ≈ 67μM	
Noori et al, 2012 (311)	Twenty term neonates (mean gest age, 39.1± 1.3 wks; mean BW: 3449 ± 392 g)	First 20 mins of life and for 3-5 minutes on the follow-up examination at 24-48 hours after birth.	INVOS™ (Somanetics, Troy, Michigan)	Forehead	$rScO_2$ increased from 47% at 1 min to 83% at 8 mins, then decreased progressively to 73% at 20 mins. Post-transitional median age of 26h (range, 18-57h): rScO2=78±7%	rScO2 increases as SaO_2 rises during the first 8 mins, subsequently decreases due to a drop in CBF despite a further increase in SaO_2.
Pichler et al, 2013 (361)	381 neonates: 82 term VD (gest 40±1.3wks, BW 3386±444g), 272 term CD (39±0.9, BW 3343±458g), 27 pre-term CD (34.9±1.4wks, 2259±572g). [VD - vaginal delivery, CD - Caesarean delivery, BW – Birthweight]	First 15 mins of life.	INVOS™ 5100 (Somanetics Corp, Troy, Michigan)	Left fronto-parietal forehead	41% (23-64) @ 2 mins, 68% (45-85) @ 5 mins, 79% (65-90) @ 10 mins, & 77% (63-89) @ 15 mins VD_{TERM}: 2 min - 40.8%, 5 mins - 72%, 10 mins - 78.3%, 15 mins - 75% CD_{TERM}: 2 min - 40.8%, 5 mins - 66.8%, 10 mins - 79.5% & 15 mins - 78%	▪ $rScO_2$ in all neonates, median (10th-90th percentiles) ▪ rScO2 of VD_{TERM} group significantly higher at min 4 and min 5 compared to CD_{TERM} group, higher $rScO_2$ in pre-terms to term infants

Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Baik et al, 2015 (315)	140 term neonates	First 15 mins of life	NIRO-200NX™ (NIRO, Hamamatsu, Japan)	Right forehead	$rScO_2$: 56% (39-75%) @ 2 min, 66% (50-78%) @ 5 mins, 75% (62-85%) @ 10 min and 73% (61-84%) @ 15 mins after birth.	
Montaldo et al, 2015	61 term infants, delivered by elective caesarean section	First 9 hrs	EQUANOX 7600 (Nonin, Amsterdam, Netherlands)	Forehead	Approx. from graph: mean $rScO_2$ 77% (range 72-82%)	▪ Mean CrSO2 increased quickly to 7 minutes, with no further changes ▪ CrSO2 did not change significantly during feeding

APPENDIX I. OVERVIEW OF ALL KNOWN NIRS STUDIES OF NEONATES WITH HIE

Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Van Bel et al, 1993 (317)	31 neonates (gest >34 wks): Gp I: healthy newborns (n=13), Gp II, moderate HIE but neurologically normal in first 24h (n=9), severe HIE with abnormal neurologic behaviour (n=9)	2-12h (Gp I n=8, Gp II n=6, Gp III n=5) & 12-24h (Gp I n=5, Gp II n=3, GP III n=4)	Radiometer, Copenhagen, Denmark) (4 wavelengths)	Neonatal sensors, interoptode distance 3cm	▪ 2-12 h: CBV levels in groups I and II were stable. In group III CBV decreased in all infants. This decrease in CBV was associated with a drop in both HbO and HbR. ▪ 12-24h: all grps stable CBV, positive relation between PcO_2 &CBV in gps II & III.	▪ Positive relationship between CBV and MABP in groups II & III. ▪ CBV, HbO & HbR decreased in the first 12h of life in severely asphyxiated neonates who subsequently developed neurologic abnormalities
Shellhaas et al, 2015 (362)	21 infants enrolled - outcomes available for 18 (3 lost to follow-up)	From time of enrolment on study to end of cooling (72h cooling period)	INVOS™ 5100c (Somanetics, Troy, MI)	Bilateral parietal regions & 1 sensor over the quadriceps for systemic measurements	▪ No relationship between cerebral $rScO_2$ or outcome ($p > .05$) at all time-points	▪ $rScO_2$ did not differentiate between those with favourable (n = 13) vs adverse (n = 5) 18-mth outcomes. ▪ Systemic rSO2 variability was higher during hours 48-72 of cooling among those with favourable outcomes ($.02 < P < .03$).

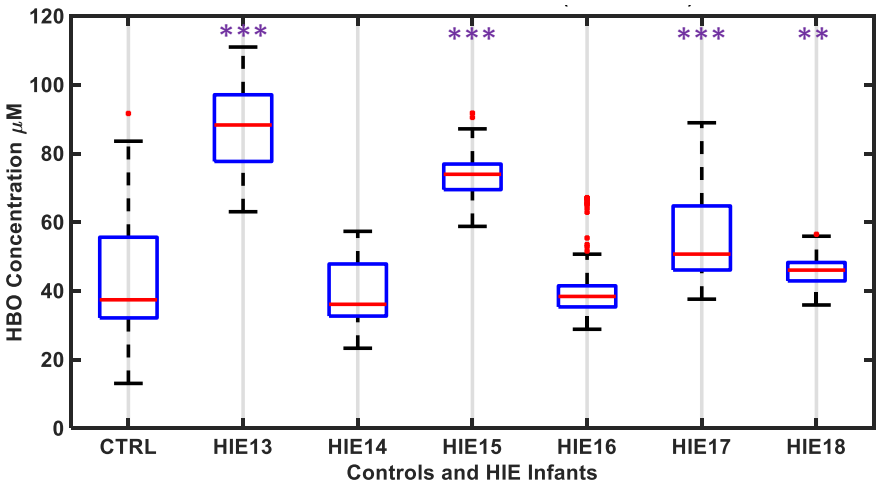
Study	Study Population & Criteria	PNA	NIRS Device	Sensors	NIRS parameters	Conclusion / Comments
Peng et al, 2015 (297)	18 asphyxiated newborns treated with hypothermia were enrolled	72-hour duration of the hypothermia treatment	FORE-SIGHT™ (Cas Medical Systems, Branford, CT)	Neonatal sensors placed on each side of forehead over the area of the frontal lobes	▪ $rScO_2$ was higher in the asphyxiated newborns who developed later brain injury	▪ Sensitivity within the first 10 hours of hypothermia treatment for an adverse outcome was 100% (95% confidence interval [CI], 70–100%) and specificity was 83% (95% CI, 36–99%).
Dehaes et al, 2015 (150)	10 neonates undergoing TH for HIE and 17 healthy controls	During TH (6–78 h), rewarming period (78–90h), & post-TH (106–305h)	OxiplexTS™ (ISS, Inc., Champaign, IL, U.S)	Up to 3 locations: left, middle, and right frontal regions.	▪ $rScO_2$ significantly higher in neonates with HIE during TH compared with age-matched control neonates ▪ $rScO_2$ not significantly different after the therapy	▪ Normal controls: around 70%, range ≈ 57-81%

APPENDIX J. BOXPLOTS OF FD-NIRS PARAMETERS IN THE HIE INFANTS VERSUS THE HEALTHY CONTROLS OVERALL AND AT EACH STAGE OF TREATMENT

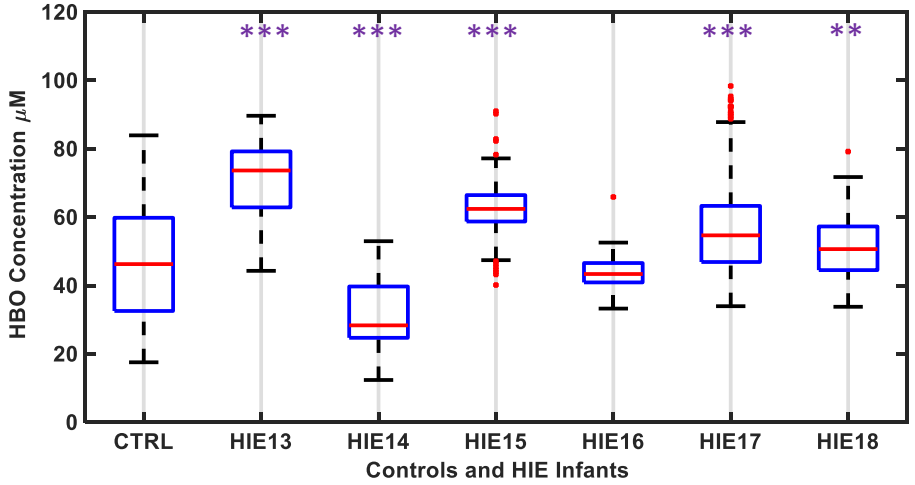
Figure J-1 – Boxplots of Oxy-Haemoglobin (HbO) Concentration (μM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls for all time periods.

Boxplots indicating the Mean, quartiles and outliers of the HbO Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **across all time periods** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor



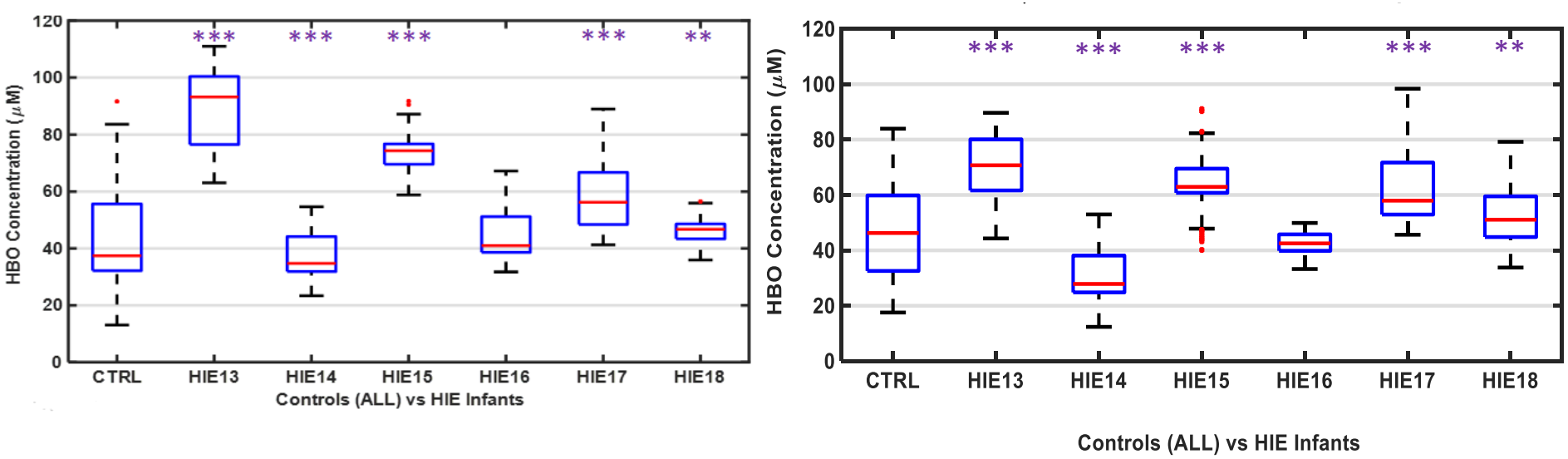
Note: Significant differences between the Healthy Controls and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

Figure J-2 Boxplots of Oxy-Haemoglobin (HbO) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls while undergoing therapeutic hypothermia.

Boxplots indicating the Mean, quartiles and outliers of the HbO Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL) compared to each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18) **while undergoing therapeutic hypothermia.**

a) Left Sensor

b) Right Sensor

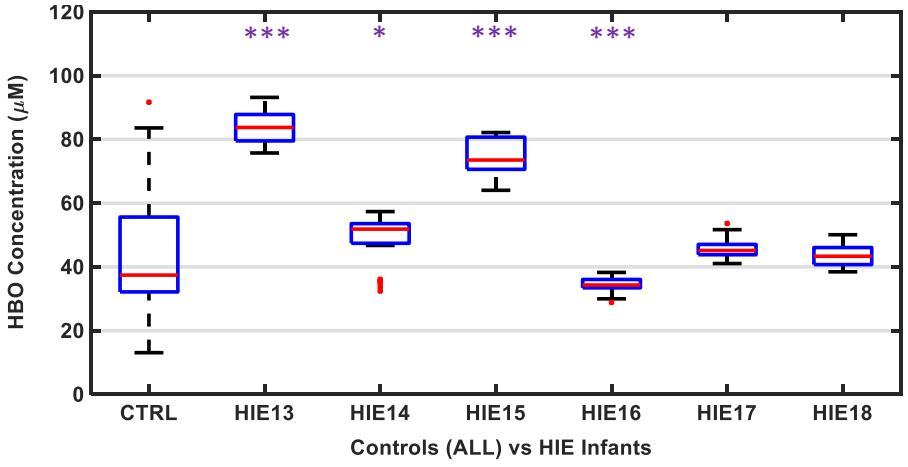


Note: Significant differences between the Healthy Controls and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

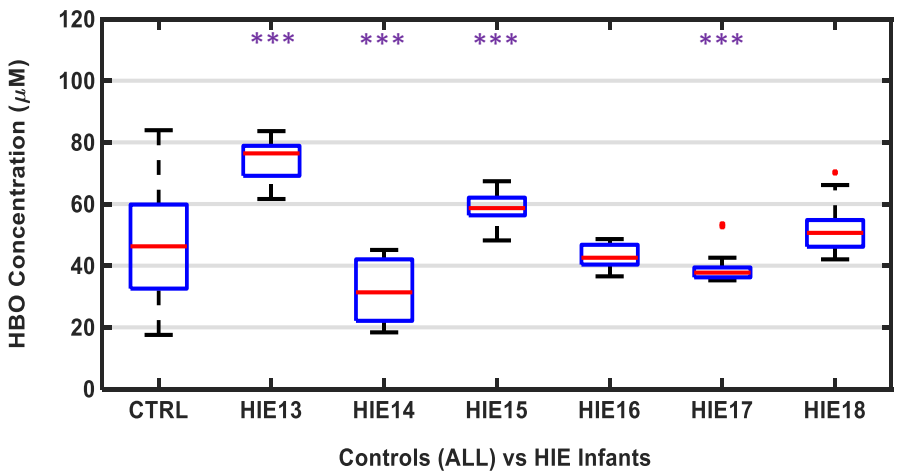
Figure J-3 Boxplots of Oxy-Haemoglobin (HbO) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls during rewarming.

Boxplots indicating the Mean, quartiles and outliers of the HbO Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL) compared to each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18) **during rewarming.**

a) Left Sensor



b) Right Sensor

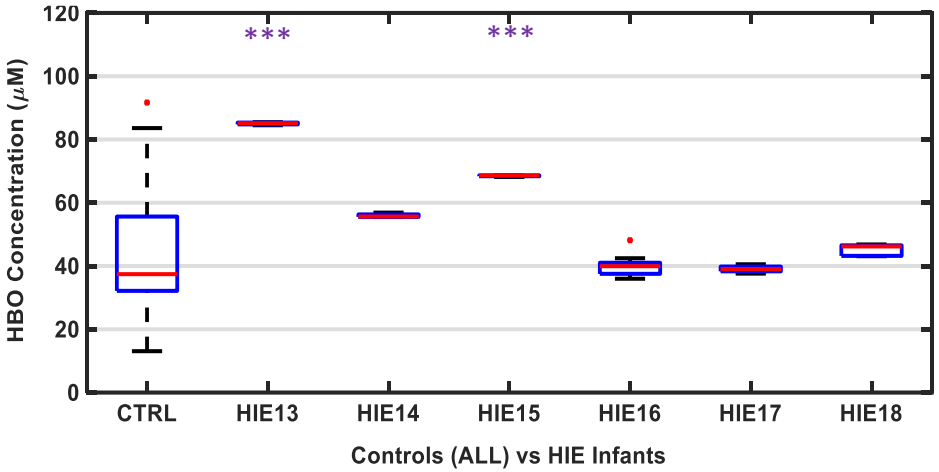


Note: Significant differences between the Healthy Controls and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

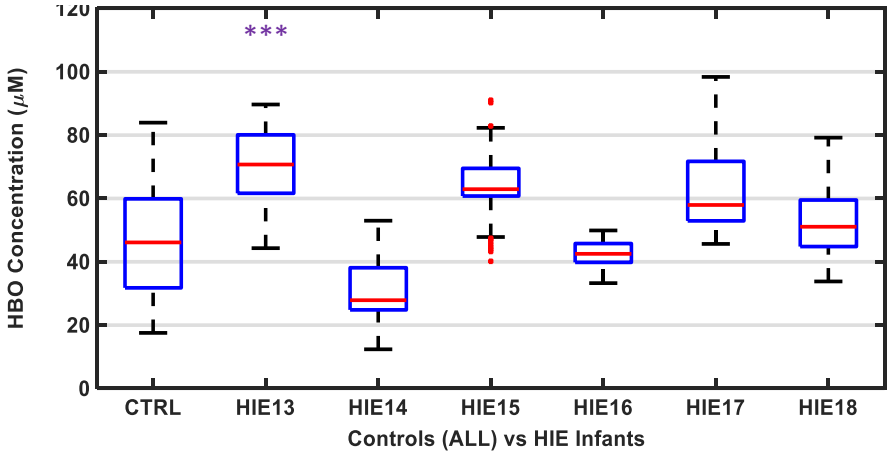
Figure J-4 Boxplots of Oxy-Haemoglobin (HbO) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls at normal temperature immediately following rewarming.

Boxplots indicating the Mean, quartiles and outliers of the HbO Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL) compared to each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18) **at normal temperature immediately following rewarming**.

a) Left Sensor



b) Right Sensor

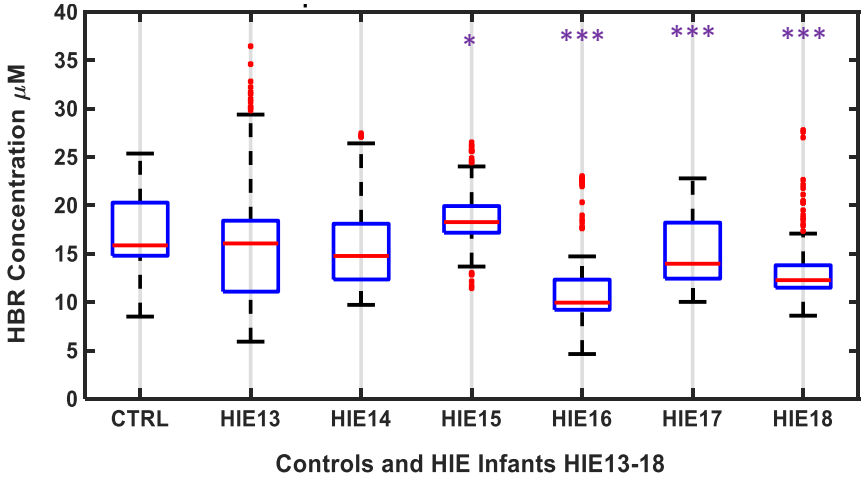


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

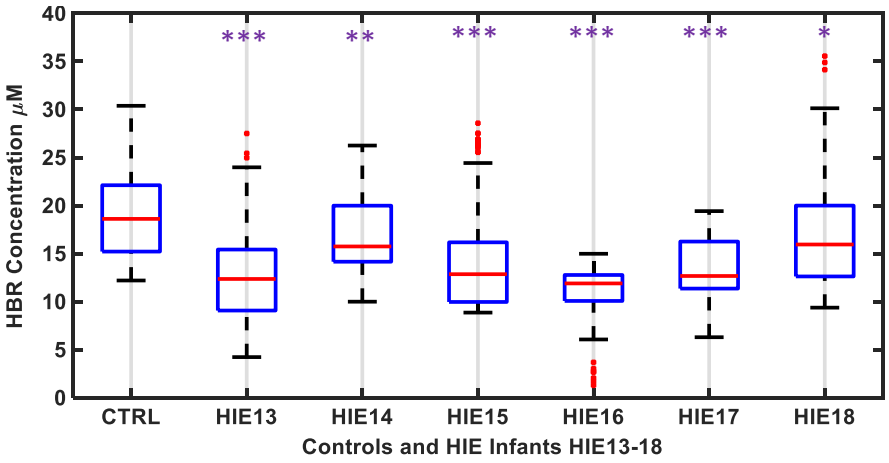
Figure J-5 Boxplots of Deoxy-Haemoglobin (HbR) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls for all time periods.

Boxplots indicating the Mean, quartiles and outliers of the HbR Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **across all time periods** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

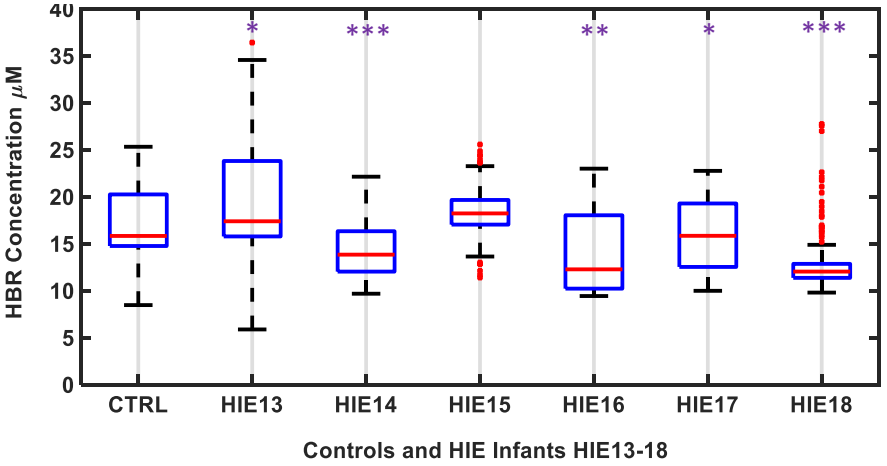


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

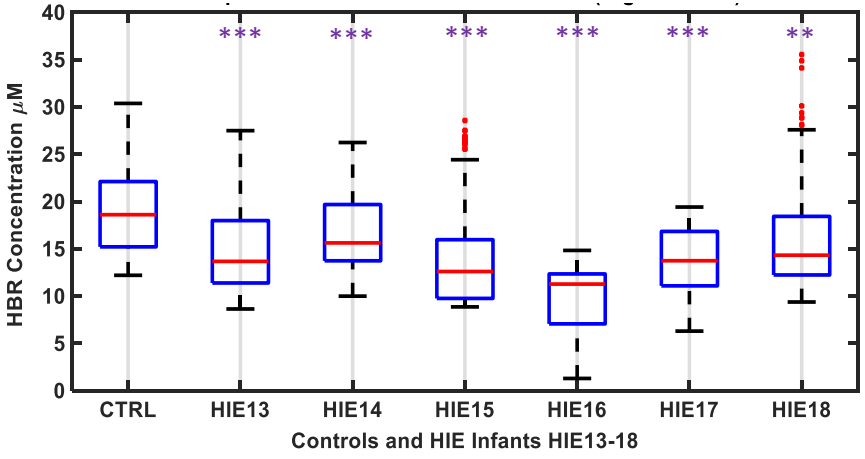
Figure J-6 Boxplots of Deoxy-Haemoglobin (HbR) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls while undergoing therapeutic hypothermia.

Boxplots indicating the Mean, quartiles and outliers of the HbR Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **while undergoing therapeutic hypothermia** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

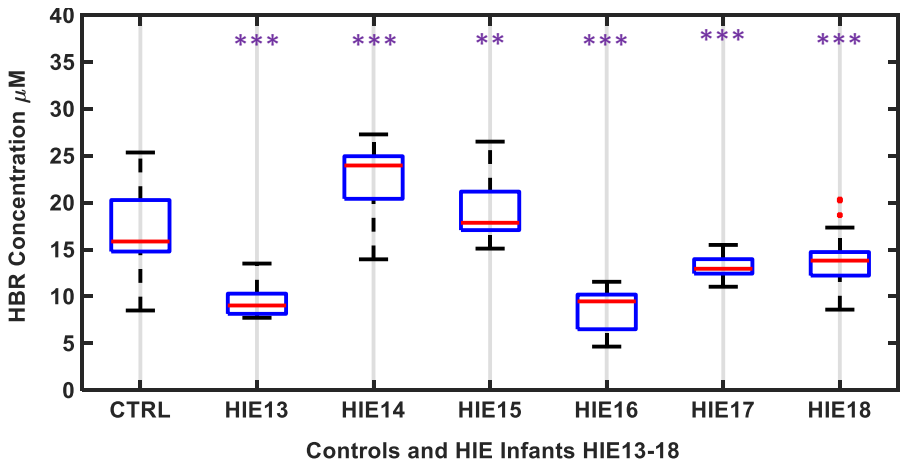


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

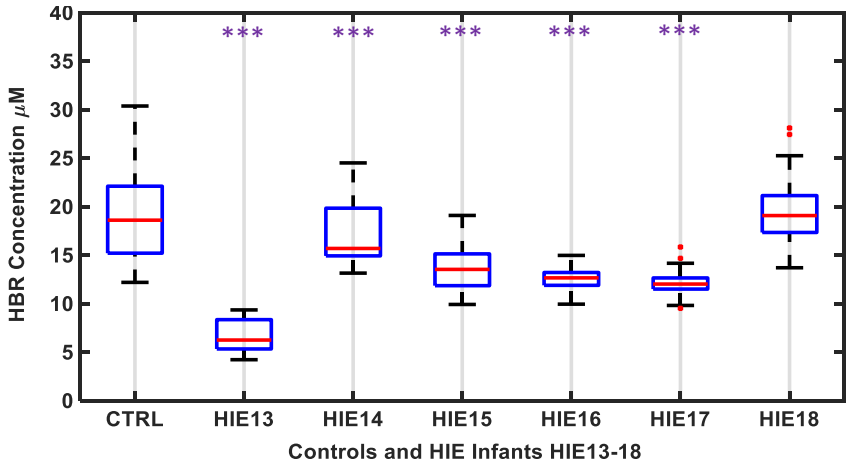
Figure J-7 Boxplots of Deoxy-Haemoglobin (HbR) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls infant during rewarming.

Boxplots indicating the Mean, quartiles and outliers of the HbR Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **during rewarming** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor



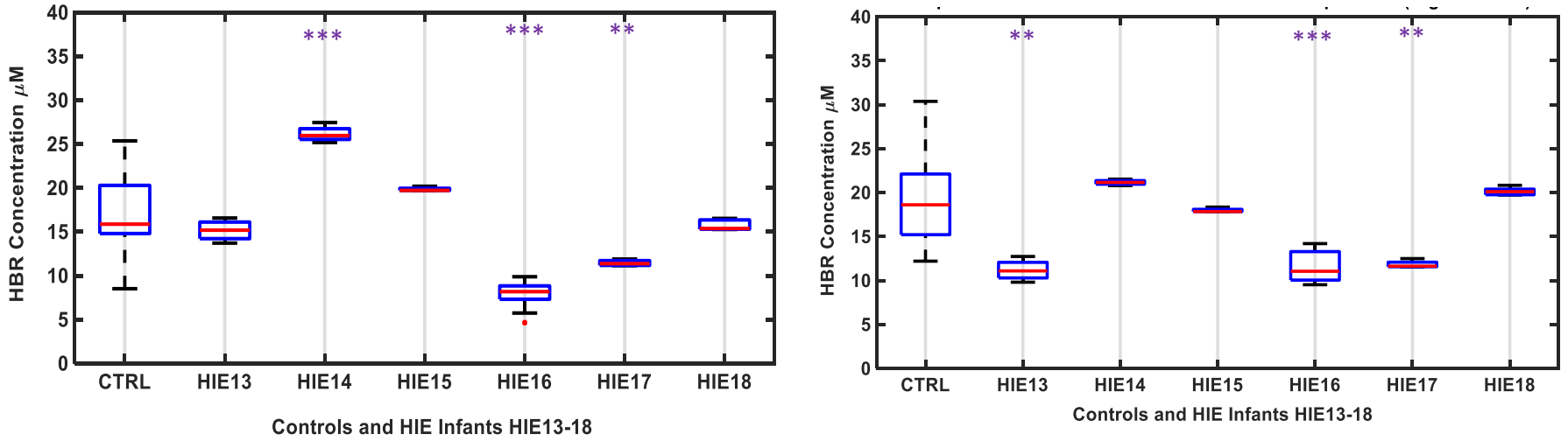
Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

Figure J-8 Boxplots of Deoxy-Haemoglobin (HbR) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls at normal temperature immediately following therapeutic hypothermia.

Boxplots indicating the Mean, quartiles and outliers of the HbR Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data at normal temperature **immediately following therapeutic hypothermia** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor

b) Right Sensor

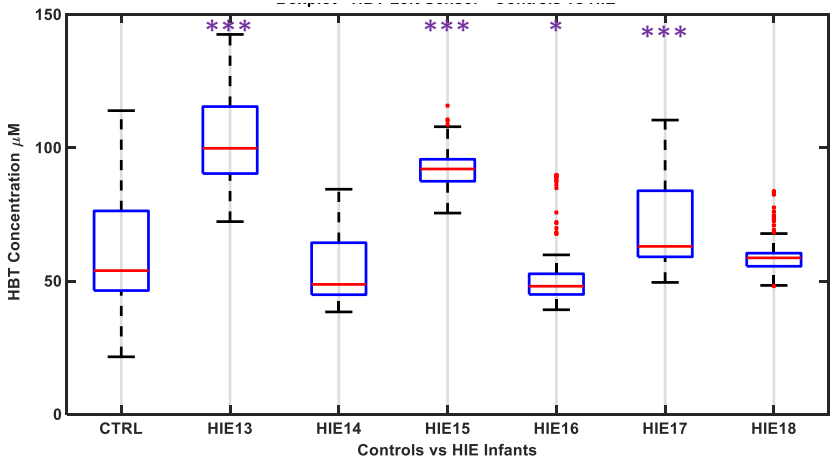


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

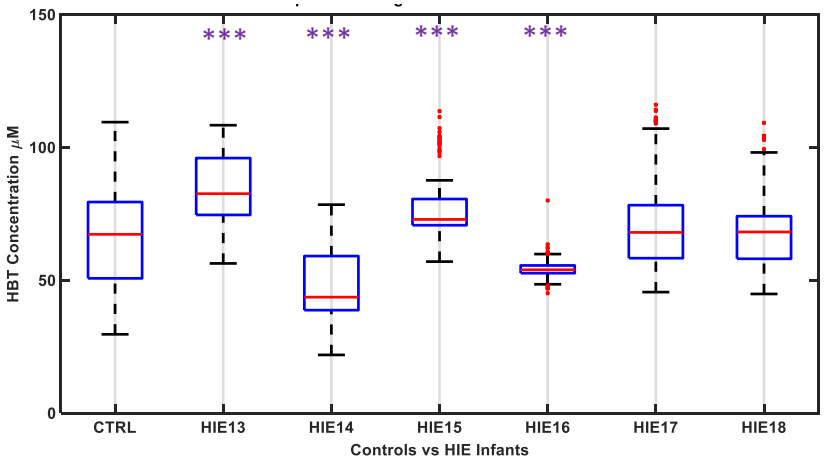
Figure J-9 Boxplots of Total Haemoglobin (HbT) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls including data for all time periods.

Boxplots indicating the Mean, quartiles and outliers of the HbT Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **across all time periods** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

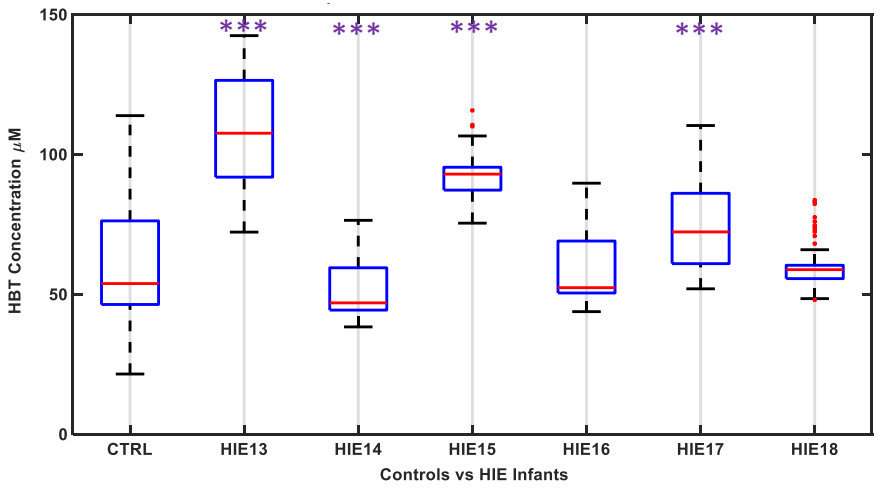


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

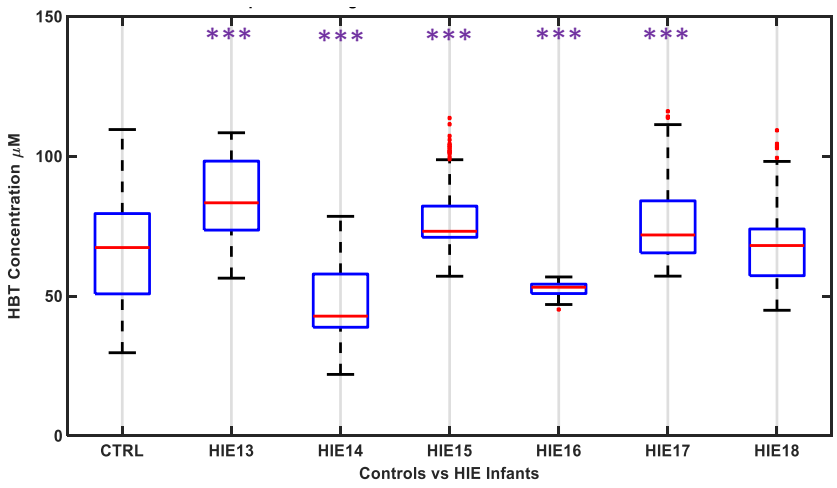
Figure J-10 Boxplots of Total Haemoglobin (HbT) Concentration (µM) at a) Left and b) Right sensors of for each of the HIE Infants vs the Healthy Controls during Therapeutic Hypothermia

Boxplots indicating the Mean, quartiles and outliers of the HbT Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **during Therapeutic Hypothermia** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

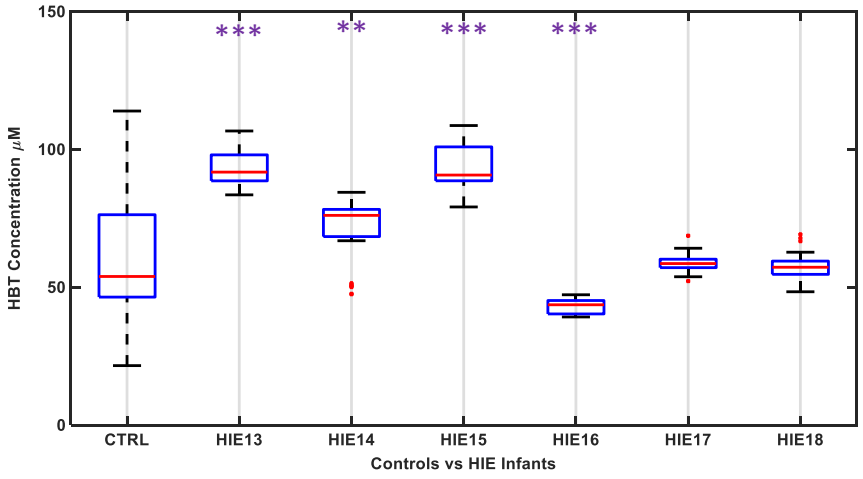


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

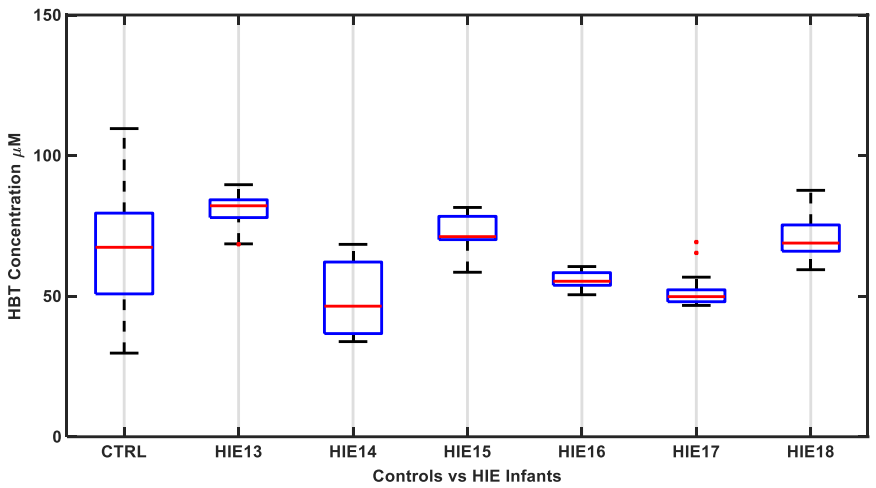
Figure J-11 Boxplots of Total Haemoglobin (HbT) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls including data during rewarming.

Boxplots indicating the Mean, quartiles and outliers of the HbT Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **during rewarming** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

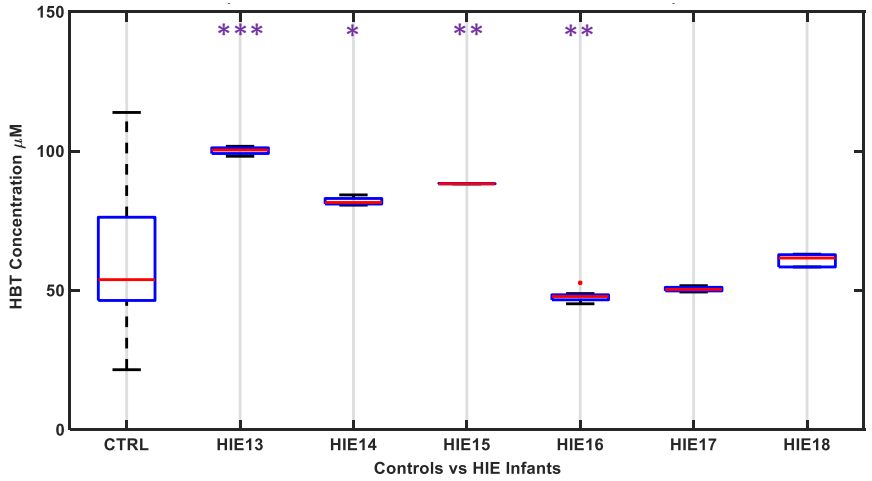


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

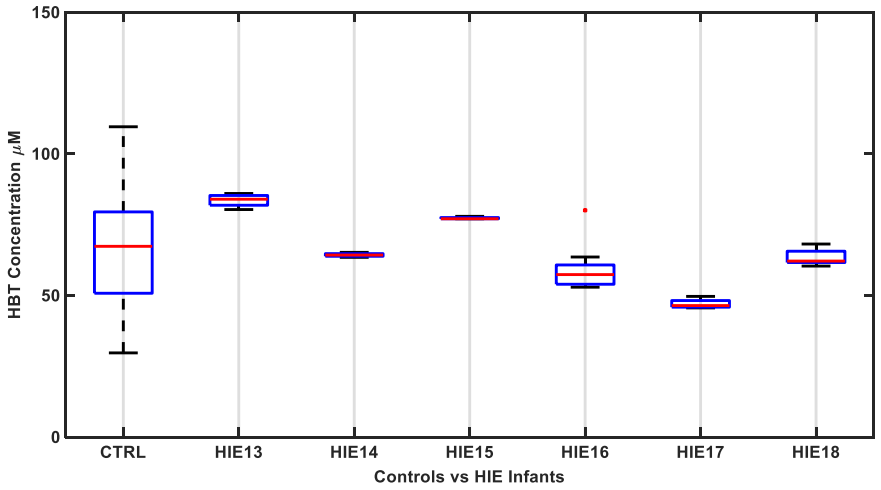
Figure J-12 Boxplots of Total Haemoglobin (HbT) Concentration (µM) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls at normotemp immediately following rewarming.

Boxplots indicating the Mean, quartiles and outliers of the HbT Concentration Measurements at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **at normotemp immediately following rewarming** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

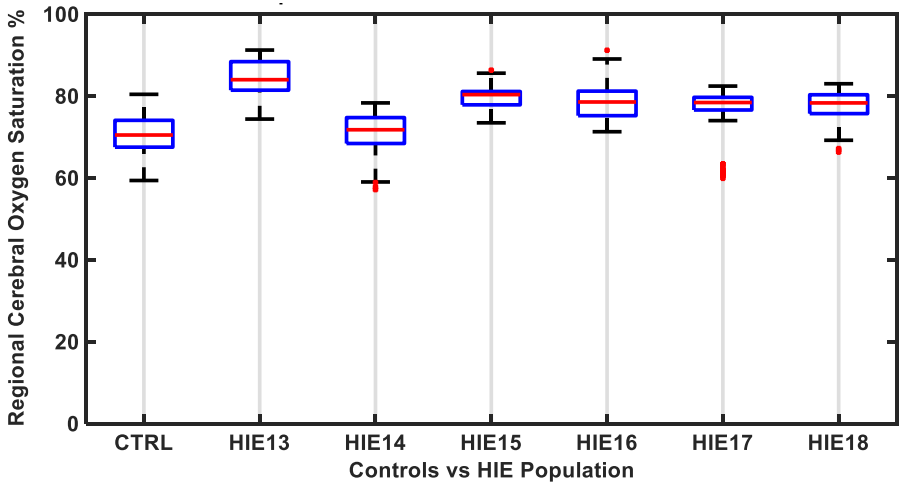


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

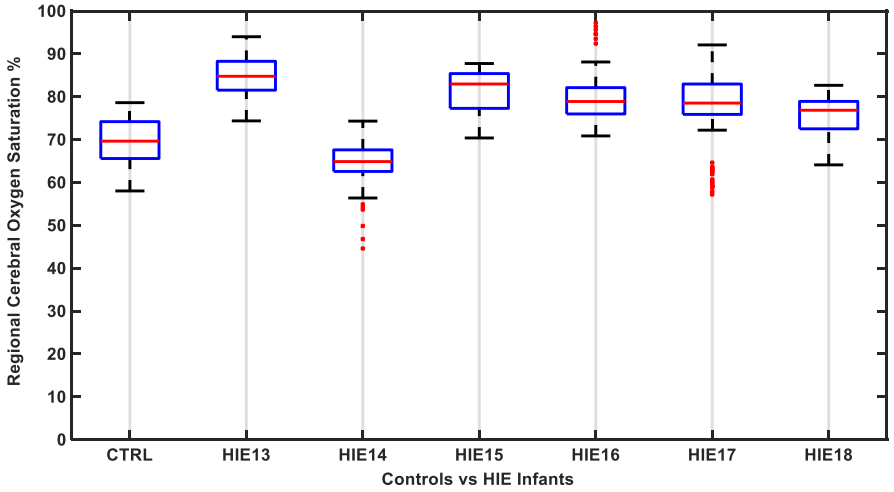
Figure J-13 Boxplots of Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls for all time periods.

Boxplots indicating the Mean, quartiles and outliers of the Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **across all time periods** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

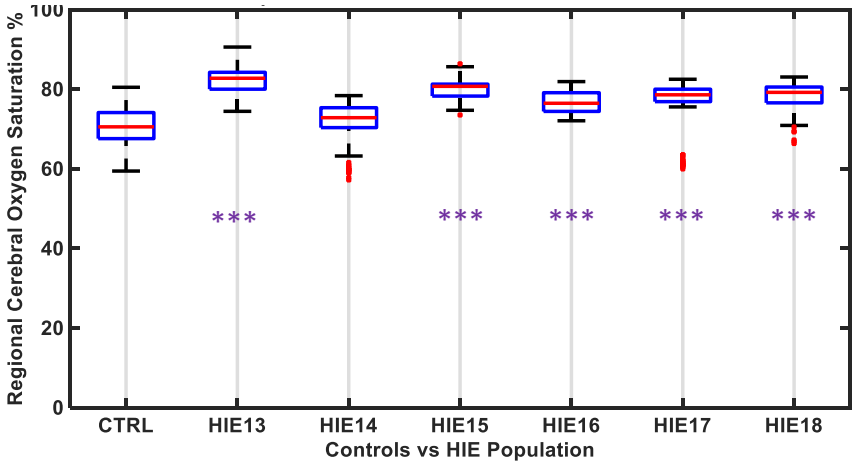


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

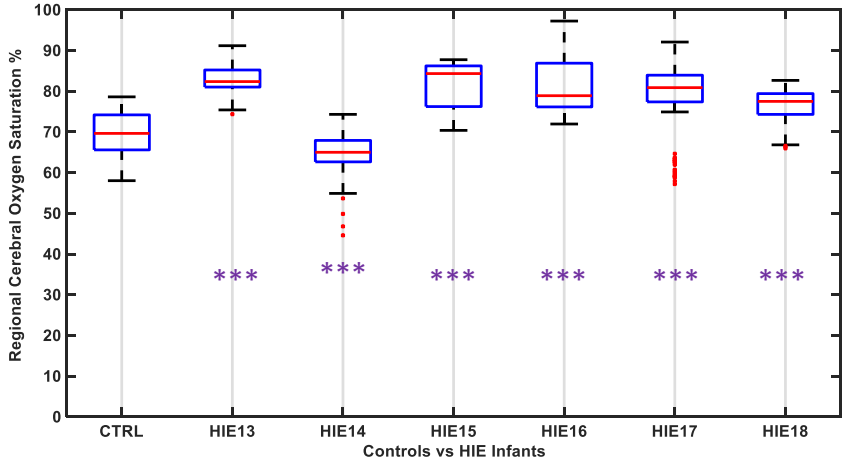
Figure J-14 Boxplots of Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls during Therapeutic Hypothermia

Boxplots indicating the Mean, quartiles and outliers of the Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **during Therapeutic Hypothermia** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

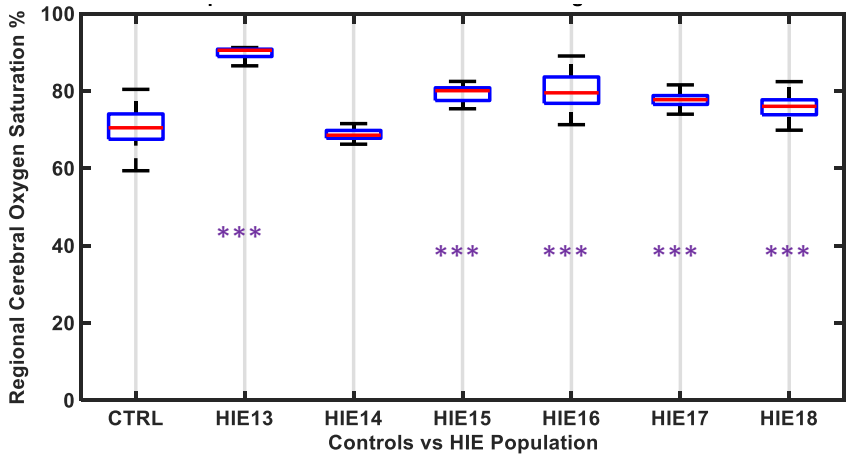


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

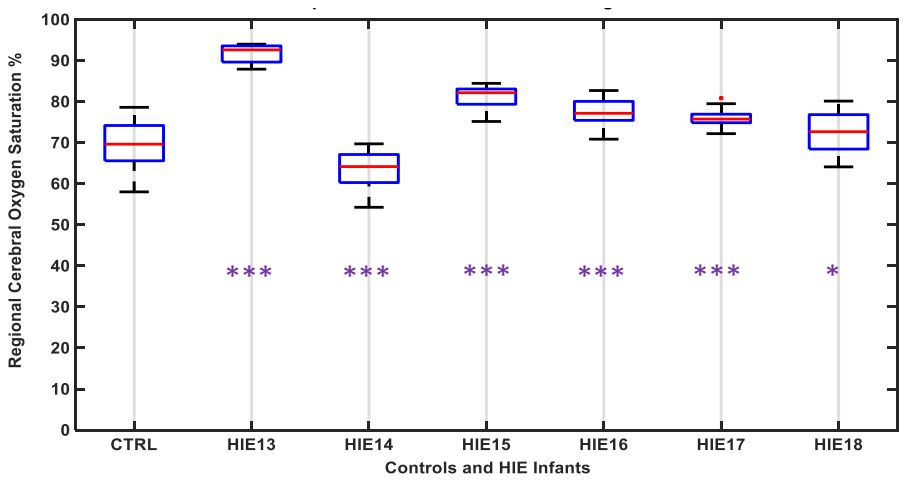
Figure J-15 Boxplots of Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls including data during rewarming.

Boxplots indicating the Mean, quartiles and outliers of the Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **during rewarming** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor

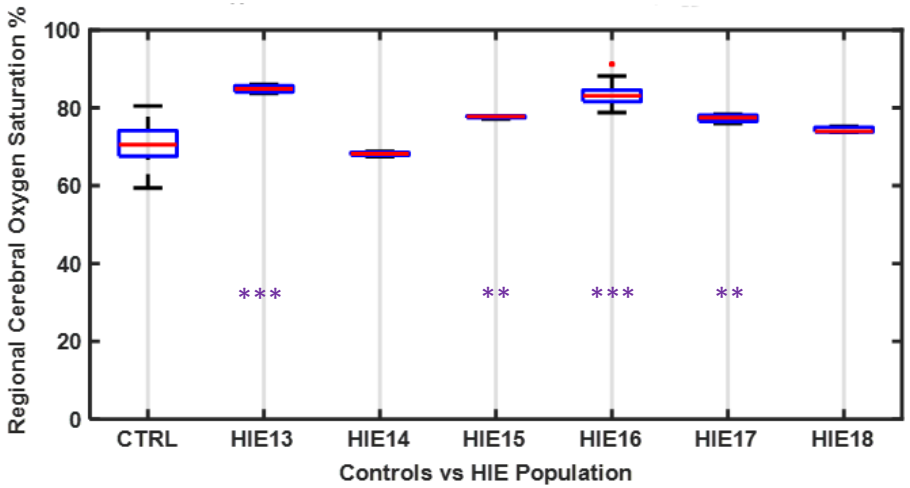


Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

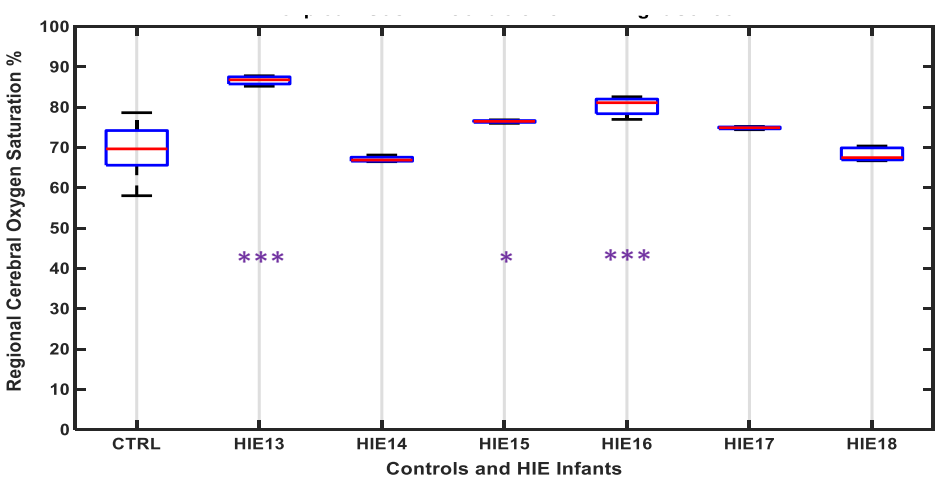
Figure J-16 Boxplots of Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at a) Left and b) Right sensors for each of the HIE Infants vs the Healthy Controls at normotemp immediately following rewarming.

Boxplots indicating the Mean, quartiles and outliers of the Regional Cerebral Oxygen Saturation, $rScO_2$ (%) at the Left Sensor (a) and Right Sensor (b) for the pooled data of all the Healthy Controls (CTRL), against the pooled data **at normotemp immediately following rewarming** for each of the HIE participants (HIE13, HIE14, HIE15, HIE16, HIE17, HIE18).

a) Left Sensor



b) Right Sensor



Note: Significant differences between the Healthy population and an individual HIE infant is indicated by * ($p < 0.05$), ** ($p < 0.01$) or *** ($p < 0.001$).

APPENDIX K. TABLES COMPARING FD-NIRS PARAMETERS FOR EACH HIE INFANT AGAINST THE HEALTHY CONTROLS

Table K-1 Oxy-Haemoglobin (HbO, μM) in HIE infants compared to Healthy Controls with indication of significant differences.

Mean HbO ±1SD (Standard Deviation) for each HIE infant for each analysed period against Healthy Controls (HC's) at the Left and Right Sensors. A period of normality is defined for the $rScO_2$ analysis as a period during which the $rScO_2$ is significantly similar or lower than the mean of the HC's.

Time Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L		101 ± 3***	48 ± 3**	69 ± 1*		64 ± 2***	46 ± 5*
	R		78 ± 3***	38 ± 13*	41 ± 2		55 ± 3	41 ± 8
24-48 Cooled Hrs	L		99 ± 8***	34 ± 2**	76 ± 7***	65 ± 1*	67 ± 16***	48 ± 3**
	R		73 ± 11***	25 ± 2***	67 ± 15***	36 ± 0	69 ± 18***	54 ± 10*
48-54 Cooled Hrs	L			39 ± 4		54 ± 11*	44 ± 2	48 ± 1
	R			32 ± 4***		41 ± 4	59 ± 2*	57 ± 1
54-60 Cooled Hrs	L		65 ± 2 **	29 ± 4***	69 ± 3***	39 ± 1	60 ± 3***	44 ± 3
	R		63 ± 2*	27 ± 2***	68 ± 2***	46 ± 2	71 ± 7***	62 ± 8**
60-66 Cooled Hrs	L		73 ± 4***	27 ± 2***	74 ± 4***		48 ± 3*	45 ± 2
	R		64 ± 3***	38 ± 2	60 ± 4***		58 ± 3**	55 ± 6*
64-66 Cooled Hrs	L		76 ± 2***	27 ± 2***	78 ± 1***	46 ± 1	48 ± 1	47 ± 1
	R		61 ± 1**	37 ± 2	63 ± 1**	46 ± 2	55 ± 1	53 ± 1
66-72 Cooled Hrs	L		83 ± 10***		76 ± 2***	38 ± 3	51 ± 3**	44 ± 2
	R		63 ± 6***		62 ± 2***	42 ± 1	53 ± 3	52 ± 2
Rewarm 72-78 Hrs	L		84 ± 5***	45 ± 8	71 ± 2***	34 ± 2*	47 ± 3*	43 ± 3
	R		67 ± 3***	24 ± 4***	59 ± 6**	40 ± 2	39 ± 5	56 ± 7*
Rewarm 78-84 Hrs	L		84 ± 5***	53 ± 3**	80 ± 4***	35 ± 2	44 ± 3	44 ± 3
	R		78 ± 3***	41 ± 5	60 ± 4**	47 ± 2	38 ± 3	48 ± 5
Normotemp	L	43 ± 16	85 ± 1***	56 ± 1	69 ± 0**	40 ± 3	39 ± 1	45 ± 2
	R	47 ± 18	72 ± 2***	43 ± 1	59 ± 0	47 ± 5	36 ± 1	43 ± 3
Period of Normality? Yes or No			**NO**	**YES**	**NO**	**YES**	**YES**	**YES**

Key:

- and * indicates statistically and significantly higher than Controls p < 0.05
- and ** indicates statistically and significantly higher than Controls p < 0.01
- and *** indicates statistically and significantly higher than Controls p < 0.001
- and * indicates statistically and significantly lower than Controls p < 0.05
- and ** indicates statistically and significantly lower than Controls p < 0.01
- and *** indicates statistically and significantly lower than Controls p < 0.001
- indicates measurement not statistically different to Control population

Table K-2 Deoxy-Haemoglobin (HbR, μM) in HIE infants compared to Healthy Controls with indication of significant differences.

Mean HbR ±1SD (Standard Deviation) for each HIE infant for each analysed period against Healthy Controls at the Left and Right Sensors. Statistical significance levels of $p < 0.001$, $p < 0.01$ and $p < 0.05$ when compared to Control population are indicated. A period of normality is defined for the $rScO_2$ analysis as a period during which the $rScO_2$ is significantly similar or lower than the mean of the Healthy Controls.

Time Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L			17 ± 3	20 ± 4		19 ± 2*	15 ± 4***
	R			20 ± 4	16 ± 2		16 ± 1*	13 ± 6***
24-48 Cooled Hrs	L		22 ± 6***	12 ± 1***	20 ± 5	22 ± 0	17 ± 4	12 ± 3***
	R		15 ± 3***	15 ± 2***	22 ± 5**	13 ± 1*	15 ± 3***	18 ± 7**
48-54 Cooled Hrs	L			16 ± 2		19 ± 4	11 ± 1***	12 ± 0***
	R			14 ± 3***		11 ± 2***	9 ± 1***	14 ± 1***
54-60 Cooled Hrs	L		18 ± 0	15 ± 2	19 ± 1*	12 ± 1***	17 ± 1	12 ± 1***
	R		15 ± 0	14 ± 2***	12 ± 1***	7 ± 1***	10 ± 2***	15 ± 2***
60-66 Cooled Hrs	L		16 ± 1	16 ± 2	17 ± 2		13 ± 1***	13 ± 2***
	R		12 ± 1***	19 ± 2	11 ± 2***		12 ± 1***	19 ± 3
64-66 Cooled Hrs	L		16 ± 1	16 ± 2	18 ± 1	12 ± 1***	13 ± 1***	13 ± 1***
	R		11 ± 1***	19 ± 2	9 ± 0***	2 ± 1***	12 ± 1***	18 ± 1
66-72 Cooled Hrs	L		13 ± 2***		18 ± 0	10 ± 1***	14 ± 1***	12 ± 1***
	R		9 ± 1***		10 ± 1***	12 ± 1***	14 ± 2***	17 ± 2
Rewarm 72-78 Hrs	L		11 ± 1***	20 ± 4	17 ± 1	10 ± 1***	13 ± 1***	12 ± 2***
	R		8 ± 2***	15 ± 1***	12 ± 1***	13 ± 1***	12 ± 1***	17 ± 3
Rewarm 78-84 Hrs	L		9 ± 1***	25 ± 1***	23 ± 3***	7 ± 2***	13 ± 1***	15 ± 2**
	R		6 ± 1***	21 ± 3	16 ± 1*	12 ± 1***	12 ± 1***	21 ± 3
Normotemp	L	17 ± 4	15 ± 1***	26 ± 1***	20 ± 0	8 ± 1***	12 ± 0*	16 ± 1
	R	19 ± 5	11 ± 1***	21 ± 0	18 ± 0	12 ± 2***	12 ± 1**	20 ± 0
Period of Normality? Yes or No			**YES**	**YES**	**YES**	**NO**	**NO**	**YES**

Key:

- and * indicates statistically and significantly lower than Controls $p < 0.05$
- and ** indicates statistically and significantly lower than Controls $p < 0.01$
- and *** indicates statistically and significantly lower than Controls $p < 0.001$
- and * indicates statistically and significantly higher than Controls $p < 0.05$
- and ** indicates statistically and significantly higher than Controls $p < 0.01$
- and *** indicates statistically and significantly higher than Controls $p < 0.001$
- indicates measurement not statistically different to Control population

Table K-3 Total Haemoglobin (HbT, μM) in HIE infants compared to Healthy Controls with indication of significant differences.

Mean HbT ±1SD (Standard Deviation) for each HIE infant for each analysed period against Healthy Controls at the Left and Right Sensors. Statistical significance levels of $p < 0.001$, $p < 0.01$ and $p < 0.05$ when compared to Control population are indicated. A period of normality is defined for the $rScO_2$ analysis as a time period during which the $rScO_2$ is significantly similar or lower than the mean of the Healthy Controls.

Time Period	Sensor	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs	L			66 ± 6**	89 ± 5*		84 ± 4***	61 ± 9
	R			57 ± 17	58 ± 0		71 ± 4	55 ± 14**
24-48 Cooled Hrs	L		121 ± 14***	46 ± 2***	96 ± 9***	87 ± 1*	84 ± 20***	60 ± 5
	R		89 ± 14***	40 ± 3***	89 ± 20***	49 ± 0	84 ± 21***	72 ± 17
48-54 Cooled Hrs	L			55 ± 4		73 ± 15**	55 ± 2	60 ± 1
	R			44 ± 7***		53 ± 3*	68 ± 3	71 ± 1
54-60 Cooled Hrs	L		83 ± 2	43 ± 3***	88 ± 3***	51 ± 1	77 ± 3**	56 ± 1
	R		78 ± 2*	41 ± 4***	80 ± 3***	53 ± 2	82 ± 6**	76 ± 10*
60-66 Cooled Hrs	L		89 ± 5*	43 ± 1***	90 ± 6***		61 ± 1	58 ± 2
	R		75 ± 4***	57 ± 2	71 ± 5		70 ± 4**	74 ± 6
64-66 Cooled Hrs	L		92 ± 2	43 ± 2***	96 ± 1***	58 ± 1	61 ± 1	60 ± 2
	R		72 ± 2***	56 ± 2	72 ± 0	48 ± 2*	67 ± 1	71 ± 2
66-72 Cooled Hrs	L		96 ± 9		94 ± 2***	48 ± 3*	65 ± 4*	56 ± 2
	R		73 ± 6***		71 ± 2	54 ± 1	67 ± 3	70 ± 2
Rewarm 72-78 Hrs	L		95 ± 6	65 ± 12	88 ± 2***	44 ± 2***	60 ± 3	55 ± 4
	R		74 ± 4***	39 ± 5***	71 ± 7	54 ± 1	51 ± 5	73 ± 9
Rewarm 78-84 Hrs	L		92 ± 6***	78 ± 4***	103 ± 5***	42 ± 3***	57 ± 3	59 ± 4
	R		84 ± 3***	61 ± 8	75 ± 4	58 ± 1	50 ± 3***	69 ± 5
Normotemp	L	60 ± 20	100 ± 1**	82 ± 2*	88 ± 0*	48 ± 2*	50 ± 1	61 ± 2
	R	66 ± 22	84 ± 2***	64 ± 1	77 ± 0	59 ± 7	48 ± 2	63 ± 3
Period of Normality? Yes (Y) or No (N)			**NO**	**YES**	**NO**	**YES**	**YES**	**YES**

Key:

- and * indicates statistically and significantly higher than Controls $p < 0.05$
- and ** indicates statistically and significantly higher than Controls $p < 0.01$
- and *** indicates statistically and significantly higher than Controls $p < 0.001$
- and * indicates statistically and significantly lower than Controls $p < 0.05$
- and ** indicates statistically and significantly lower than Controls $p < 0.01$
- and *** indicates statistically and significantly lower than Controls $p < 0.001$
- indicates measurement not statistically different to Control population

Table K-4 Regional Cerebral Oxygen Saturation ($rScO_2$, %) in HIE infants compared to Healthy Controls with indication of significant differences.

Mean $rScO_2$ ±1SD (Standard Deviation) for each HIE infant for each analysed time period against Healthy Controls at the Left and Right Sensors. Statistical significance levels of $p < 0.001$, $p < 0.01$ and $p < 0.05$ when compared to Control population are indicated. A period of normality is defined for the $rScO_2$ analysis as a time period during which the $rScO_2$ is significantly similar or lower than the mean of the Healthy Controls.

Time Period	Sensor	CTRL	HIE13	HIE14	HIE15	HIE16	HIE17	HIE18
0-24 Cooled Hrs	L			$74 \pm 2^{***}$	$78 \pm 3^{*}$		$77 \pm 1^{***}$	$76 \pm 4^{***}$
	R			$64 \pm 6^{***}$	72 ± 2		$77 \pm 1^{***}$	$76 \pm 4^{***}$
24-48 Cooled Hrs	L		$82 \pm 3^{***}$	$74 \pm 2^{***}$	$79 \pm 4^{***}$	74 ± 0	$75 \pm 8^{***}$	$80 \pm 3^{***}$
	R		$83 \pm 3^{***}$	$63 \pm 3^{***}$	$75 \pm 2^{***}$	73 ± 0	$76 \pm 10^{***}$	$76 \pm 3^{***}$
48-54 Cooled Hrs	L			72 ± 3		$75 \pm 1^{***}$	$80 \pm 1^{***}$	$80 \pm 0^{***}$
	R			70 ± 3		$78 \pm 4^{***}$	$86 \pm 1^{***}$	$80 \pm 1^{***}$
54-60 Cooled Hrs	L		$79 \pm 1^{**}$	$66 \pm 5^{**}$	$78 \pm 1^{***}$	$76 \pm 1^{***}$	$78 \pm 1^{***}$	$79 \pm 2^{***}$
	R		$81 \pm 0^{***}$	$66 \pm 3^{*}$	$85 \pm 1^{***}$	$87 \pm 1^{***}$	$87 \pm 3^{***}$	$81 \pm 1^{***}$
60-66 Cooled Hrs	L		$82 \pm 1^{***}$	$63 \pm 4^{***}$	$82 \pm 1^{***}$		$78 \pm 2^{***}$	$78 \pm 4^{***}$
	R		$85 \pm 1^{***}$	$66 \pm 1^{**}$	$85 \pm 1^{***}$		$83 \pm 2^{***}$	$75 \pm 5^{***}$
64-66 Cooled Hrs	L		$83 \pm 1^{***}$	$63 \pm 5^{***}$	$82 \pm 1^{***}$	$80 \pm 1^{***}$	$79 \pm 2^{***}$	$78 \pm 2^{***}$
	R		$85 \pm 1^{***}$	$65 \pm 3^{*}$	$87 \pm 1^{***}$	$95 \pm 2^{***}$	$83 \pm 2^{***}$	$74 \pm 2^{*}$
66-72 Cooled Hrs	L		$86 \pm 3^{***}$		$81 \pm 0^{***}$	$79 \pm 2^{***}$	$79 \pm 1^{***}$	$78 \pm 2^{***}$
	R		$87 \pm 1^{***}$		$86 \pm 1^{***}$	$78 \pm 2^{***}$	$79 \pm 2^{***}$	$75 \pm 2^{***}$
Rewarm 72-78 Hrs	L		$89 \pm 1^{***}$	70 ± 1	$80 \pm 1^{***}$	$77 \pm 2^{***}$	$78 \pm 2^{***}$	$78 \pm 2^{***}$
	R		$89 \pm 2^{***}$	$61 \pm 4^{***}$	$83 \pm 1^{***}$	$75 \pm 2^{***}$	$76 \pm 2^{***}$	$76 \pm 2^{***}$
Rewarm 78-84 Hrs	L		$90 \pm 1^{***}$	$68 \pm 1^{**}$	$78 \pm 2^{***}$	$83 \pm 3^{***}$	$77 \pm 2^{***}$	$74 \pm 3^{***}$
	R		$85 \pm 1^{***}$	$66 \pm 3^{**}$	$79 \pm 1^{***}$	$80 \pm 2^{***}$	$76 \pm 1^{***}$	70 ± 4
Normotemp	L	70 ± 5	$85 \pm 1^{***}$	68 ± 1	$78 \pm 0^{**}$	$83 \pm 3^{***}$	$77 \pm 1^{*}$	$74 \pm 1^{*}$
	R	70 ± 5	$87 \pm 1^{***}$	67 ± 1	$76 \pm 0^{*}$	$80 \pm 2^{***}$	75 ± 0	68 ± 2
Period of Normality? Yes (Y) or No (N)			**NO**	**YES**	**NO**	**YES**	**NO**	**NO**

Key:

- and * indicates statistically and significantly higher than Controls $p < 0.05$
- and ** indicates statistically and significantly higher than Controls $p < 0.01$
- and *** indicates statistically and significantly higher than Controls $p < 0.001$
- and * indicates statistically and significantly lower than Controls $p < 0.05$
- and ** indicates statistically and significantly lower than Controls $p < 0.01$
- and *** indicates statistically and significantly lower than Controls $p < 0.001$
- indicates measurement not statistically different to Control population

APPENDIX L. SIGNIFICANT DIFFERENCES IN HBO, HBR, HBT AND RSCO$_2$ IN HIE INFANTS BY TREATMENT STAGE & OVERALL RELATIVE TO HEALTHY CONTROLS

Table M-1 Significant differences in HbO, HbR, HbT and rScO$_2$ in the HIE infants at different stages of treatment and overall at the a) Left Sensor and b) Right Sensor

Significant differences in FD-NIRS parameters of Oxy-Haemoglobin (HbO), Deoxy-Haemoglobin (HbR), Total Haemoglobin (HbT) and Regional Cerebral Oxygen Saturation (rScO$_2$) at the a) Left Sensor and b) Right Sensor for each of the HIE infants relative to Healthy Controls; higher (yellow with up arrow), lower (blue with down arrow) or no difference (green).

a) Left Sensor

LEFT SENSOR		HbO		HbR		HbT		rScO$_2$	
HIE13-P	COOL	↑	$p < 0.001$	↑	$p < 0.05$	↑	$p < 0.001$	↑	$p < 0.001$
	REWARM	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
	NORMOTEMP	↑	$p < 0.001$			↑	$p < 0.001$	↑	$p < 0.001$
	ALL	↑	$p < 0.001$			↑	$p < 0.001$	↑	$p < 0.001$
HIE14-M	COOL	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$		
	REWARM	↑	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.01$		
	NORMOTEMP			↑	$p < 0.001$	↑	$p < 0.05$		
	ALL								
HIE15-P	COOL	↑	$p < 0.001$			↑	$p < 0.001$	↑	$p < 0.001$
	REWARM	↑	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
	NORMOTEMP	↑	$p < 0.001$			↑	$p < 0.01$	↑	$p < 0.01$
	ALL	↑	$p < 0.001$	↑	$p < 0.05$	↑	$p < 0.001$	↑	$p < 0.001$
HIE16-N	COOL			↓	$p < 0.01$			↑	$p < 0.001$
	REWARM	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$
	NORMOTEMP			↓	$p < 0.001$	↓	$p < 0.01$	↑	$p < 0.001$
	ALL			↓	$p < 0.001$	↓	$p < 0.05$	↑	$p < 0.001$
HIE17-M	COOL	↑	$p < 0.001$	↓	$p < 0.05$	↑	$p < 0.001$	↑	$p < 0.001$
	REWARM			↓	$p < 0.001$			↑	$p < 0.001$
	NORMOTEMP			↓	$p < 0.001$			↑	$p < 0.001$
	ALL	↑	$p < 0.001$	↓	$p < 0.01$	↑	$p < 0.001$	↑	$p < 0.01$
HIE18-N	COOL	↑	$p < 0.01$	↓	$p < 0.001$			↑	$p < 0.001$
	REWARM			↓	$p < 0.001$			↑	$p < 0.001$
	NORMOTEMP								
	ALL	↑	$p < 0.05$	↓	$p < 0.01$			↑	$p < 0.001$

b) Right Sensor

RIGHT SENSOR		HbO		HbR		HbT		$rScO_2$	
HIE13-P	COOL	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
	REWARM	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
	NORMOTEMP	↑	$p < 0.01$	↓	$p < 0.01$			↑	$p < 0.001$
	OVERALL	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
HIE14-M	COOL	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$
	REWARM	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$
	NORMOTEMP								
	OVERALL	↓	$p < 0.001$	↓	$p < 0.01$	↓	$p < 0.001$	↓	$p < 0.001$
HIE15-P	COOL	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
	REWARM	↑	$p < 0.001$	↓	$p < 0.001$			↑	$p < 0.001$
	NORMOTEMP							↑	$p < 0.05$
	OVERALL	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
HIE16-N	COOL			↓	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$
	REWARM			↓	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$
	NORMOTEMP			↓	$p < 0.001$			↑	$p < 0.001$
	OVERALL			↓	$p < 0.001$			↑	$p < 0.001$
HIE17-M	COOL	↑	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$	↑	$p < 0.001$
	REWARM	↓	$p < 0.001$	↓	$p < 0.001$	↓	$p < 0.001$	↑	$p < 0.001$
	NORMOTEMP			↓	$p < 0.01$				
	OVERALL	↑	$p < 0.001$	↓	$p < 0.001$			↑	$p < 0.001$
HIE18-N	COOL	↑	$p < 0.01$	↓	$p < 0.01$			↑	$p < 0.001$
	REWARM							↑	$p < 0.05$
	NORMOTEMP								
	OVERALL	↑	$p < 0.01$	↓	$p < 0.05$			↑	$p < 0.001$

Key:

HIE Infant statistically similar to Healthy Controls (HC's) overall during that stage of treatment or overall

HIE Infant statistically higher than HC's overall during that stage of treatment or overall

HIE Infant statistically lower than HC's overall during that stage of treatment or overall

APPENDIX M. DIFFERENCES BETWEEN SENSORS IN OXY- (HBO) AND DEOXYHAEMOGLOBIN (HBR), AND REGIONAL CEREBRAL OXYGEN SATURATION ($RSCO_2$) IN HIE INFANTS.

LvR is the comparison of the mean of the Right (R) sensor versus the mean of the Left (L) sensor; significance indicates whether the L and R sensor measurements are statistically similar to each other as determined by the Wilcoxon signed rank test. The measure (Meas.) of ΔLR is the difference between L and R calculated as (R-L) where R=measurement at the right sensor and L=Measurement at the left sensor.

Table N-1 Difference between sensors for Oxy-Haemoglobin (HbO)

Time Period	Meas	CTRL	HIE13	HIE14	HIE15	HIE16	HIE17	HIE18
0-24 Cooled Hrs	LvR			L > R***	L = R		L > R***	L > R***
	ΔLR			-11 ± 13***	-28 ± 3		-10 ± 2***	-5 ± 6**
24-48 Cooled Hrs	LvR		L > R***	L > R***	L > R**	L = R	R > L**	R > L*
	ΔLR		-25 ±12***	-9 ± 3***	-9 ± 13**	-29 ± 1**	1 ± 4	7 ± 11
48-54 Cooled Hrs	LvR			L > R***		L > R***	R > L***	R > L**
	ΔLR			-7 ± 3**		-13 ± 13***	16 ± 4	9 ± 2
54-60 Cooled Hrs	LvR		L = R	L = R	L = R	R > L***	R > L***	R > L***
	ΔLR		-2 ± 0	-1 ± 4	-1 ± 4	7 ± 2	11 ± 6	17 ± 6***
60-66 Cooled Hrs	LvR		L > R***	R > L***	L > R***		R > L***	R > L***
	ΔLR		-9 ± 6***	11 ± 2*	-14 ± 5***		10 ± 4*	10 ± 6*
64-66 Cooled Hrs	LvR		L > R**	R > L**	L > R**	L = R	R > L**	R > L***
	ΔLR		-15 ± 2***	10 ± 1	-15 ± 1***	-1 ± 1	7 ± 2	6 ± 1
66-72 Cooled Hrs	LvR		L > R***		L > R**	R > L***	R > L***	R > L***
	ΔLR		-19 ± 5***		-15 ± 1***	4 ± 2	2 ± 3	8 ± 2*
Rewarm 72-78 Hrs	LvR		L > R***	L > R***	L > R***	R > L***	L > R***	R > L***
	ΔLR		-18 ± 7***	-21 ± 7***	-11 ± 6***	6 ± 1	-8 ± 3***	13 ± 5*
Rewarm 78-84 Hrs	LvR		L > R*	L > R***	L > R***	R > L***	L > R***	R > L***
	ΔLR		-6 ± 6	-12 ± 4***	-20 ± 5***	12 ± 2*	-6 ± 2**	4 ± 3
Normo-temp	LvR	L = R	L > R***	L = R	L = R	R > L***	L = R	L = R
	ΔLR	4 ± 15	-13 ± 1***	-13 ± 0*	-10 ± 1**	7 ± 4	-3 ± 2	-2 ± 3

Key:

Left and Right sides statistically similar when compared using Mann-Whitney-Wilcoxon test

Left and Right sides not statistically similar, and L>R

Left and Right sides not statistically similar, and R>L

Table N-2 Differences between sensors for Deoxy-Haemoglobin (HbR)

Time Period	CTRL	HIE13	HIE14	HIE15	HIE16	HIE17	HIE18
0-24 Cooled Hrs			R > L***	L > R		L > R***	L > R***
			2 ± 4	-4 ± 2***		-3 ± 1***	-2 ± 3***
24-48 Cooled Hrs		L > R***	R > L***	R > L***	L > R	L > R***	R > L***
		-7 ± 7***	3 ± 2	2 ± 2	-9 ± 0**	-2 ± 2***	5 ± 6**
48-54 Cooled Hrs			L > R**		L > R***	L > R**	R > L**
			-2 ± 2***		-7 ± 3***	-2 ± 1**	2 ± 0
54-60 Cooled Hrs		L > R	L > R**	L > R***	L > R***	L > R***	R > L***
		-3 ± 0***	-1 ± 2*	-7 ± 1***	-5 ± 0***	-6 ± 2***	3 ± 2
60-66 Cooled Hrs		L > R***	R > L***	L > R***		L > R	R > L***
		-5 ± 1***	4 ± 1	-5 ± 4***		-1 ± 1*	6 ± 2***
64-66 Cooled Hrs		L > R**	R > L**	L > R**	L > R*	L > R*	R > L**
		-6 ± 0***	4 ± 1	-8 ± 0***	-9 ± 1***	-1 ± 1	5 ± 0*
66-72 Cooled Hrs		L > R***		L > R***	R > L***	L = R	R > L***
		-4 ± 2***		-8 ± 1***	2 ± 1	0 ± 1	5 ± 1***
Rewarm 72-78 Hrs		L > R***	L > R***	L > R***	R > L***	L > R***	R > L***
		-3 ± 2***	-5 ± 3***	-5 ± 1***	3 ± 0	-1 ± 1**	5 ± 4**
Rewarm 78-84 Hrs		L > R***	L > R***	L > R***	R > L***	L > R***	R > L***
		-3 ± 1***	-5 ± 1***	-7 ± 2***	5 ± 2**	-1 ± 1	6 ± 1***
Normo-temp	R > L*	L > R***	L = R	L = R	R > L***	L = R	R > L***
	2 ± 5	-4 ± 0***	-5 ± 1**	-2 ± 0*	4 ± 2	0 ± 0	4 ± 0*

Key:

Left and Right sides statistically similar when compared using Mann-Whitney-Wilcoxon test

Left and Right sides not statistically similar, and L>R

Left and Right sides not statistically similar, and R>L

Table N-3 Difference between sensors for Regional Cerebral Oxygen Saturation

Time Period	CTRL	HIE13-P	HIE14-M	HIE15-P	HIE16-N	HIE17-M	HIE18-N
0-24 Cooled Hrs			L > R***	L > R		L = R	L = R
			-9 ± 6***	-6 ± 1		0 ± 1	1 ± 1*
24-48 Cooled Hrs		R > L*	L > R***	L > R***	L = R	R > L***	L > R***
		1 ± 5	-11 ± 3***	-5 ± 4***	-2 ± 0	1 ± 2**	-4 ± 3***
48-54 Cooled Hrs			L > R**		R > L***	R > L**	L = R
			-1 ± 2		3 ± 3***	7 ± 2***	0 ± 0
54-60 Cooled Hrs		R > L	L = R	R > L***	R > L***	R > L***	R > L*
		2 ± 0*	1 ± 2	7 ± 1***	11 ± 1***	9 ± 2***	2 ± 2**
60-66 Cooled Hrs		R > L***	R > L***	R > L**		R > L***	L > R***
		3 ± 1***	3 ± 2***	3 ± 3**		4 ± 2***	-3 ± 2***
64-66 Cooled Hrs		R > L**	R > L*	R > L**	R > L*	R > L**	L > R**
		3 ± 0**	3 ± 2**	6 ± 1***	15 ± 1***	4 ± 2**	-4 ± 0**
66-72 Cooled Hrs		R > L		R > L***	L > R***	R > L	L > R***
		1 ± 2		6 ± 1***	-1 ± 0	0 ± 2	-3 ± 1***
Rewarm 72-78 Hrs		L = R	L > R***	R > L***	L > R***	L > R***	L > R**
		1 ± 2	-9 ± 4***	3 ± 1***	-2 ± 1	-2 ± 1	-2 ± 3
Rewarm 78-84 Hrs		L > R***	L > R*	L = R	L > R**	L > R***	L > R***
		2 ± 1**	-2 ± 2	2 ± 2*	-3 ± 3**	-2 ± 2	-4 ± 1***
Normotemp	L = R	R > L**	L=R	L = R	L = R	L = R	L > R*
	0 ± 4	2 ± 0**	-1 ± 1	1 ± 1	-3± 2	-2 ± 1	-6 ± 1**

Key:

Left and Right sides statistically similar when compared using Mann-Whitney-Wilcoxon test

Left and Right sides not statistically similar, and L>R

Left and Right sides not statistically similar, and R>L